ISBN: 978-93-95847-68-1

PLANT SCIENCE: FROM FUNDAMENTALS TO ADVANCED RESEARCH

VOLUME II

Editors:

Dr. Prem Kumar Gautam

Dr. Niharika Dewangan

Dr. Pallavi P. Ulhe

Dr. Pravin D. Patil

BHUMI PUBLISHING, INDIA

FIRST EDITION: AUGUST 2024

Plant Science: From Fundamentals to Advanced Research Volume II

(ISBN: 978-93-95847-68-1)

Editors

Dr. Prem Kumar Gautam

Research and P.G.Dept. of Botany,

N.T.V.S'S G. T.Patil Arts, Commerce and

Science College, Nandurbar, M.S.

Dr. Niharika Dewangan

Department of Life Science,

Shri Shankaracharya Professional University,

Junwani, Bhilai. C.G.

Dr. Pallavi P. Ulhe

Department Of Botany

Narayanrao Kale Smruti Model College

(Art, Commerce and Science),

Karanja (gh.) Dist-Wardha, M.S.

Dr. Pravin D. Patil

Department of Botany,

Shankarlal Agrawal Science College,

Salekasa, Dist. Gondia, M.S.

Bhumi Publishing

August, 2024

Title: Plant Science: From Fundamentals to Advanced Research Volume II

Editors: Dr. Prem Kumar Gautam, Dr. Niharika Dewangan,

Dr. Pallavi P. Ulhe, Dr. Pravin D. Patil

First Edition: August, 2024

ISBN: 978-93-95847-68-1

Published by:

Bhumi Publishing

BHUMI PUBLISHING

Nigave Khalasa, Tal – Karveer, Dist – Kolhapur, Maharashtra, INDIA 416 207

E-mail: bhumipublishing@gmail.com

PREFACE

The study of plant science has been a cornerstone of scientific inquiry since the earliest days of human civilization. From the ancient practices of agriculture and herbal medicine to the cutting-edge biotechnological advancements of today, the exploration of the plant kingdom has continually expanded our understanding of life and its intricate systems.

"Plant Science: From Fundamentals to Advanced Research" is the first in a series that aims to bridge the gap between foundational knowledge and the latest research developments in plant biology. This volume is designed to serve as a comprehensive resource for students, educators, and researchers alike, offering insights into the fundamental principles of plant science while also delving into the most recent scientific discoveries and technological innovations.

The chapters in this volume are authored by experts in various subfields of plant science, each bringing their unique perspective and expertise to the topics covered. From the cellular and molecular mechanisms that drive plant growth and development to the ecological interactions and environmental challenges that shape plant life, this book provides a thorough exploration of the diverse and dynamic world of plants.

As the global community faces unprecedented challenges such as climate change, food security, and biodiversity loss, the importance of plant science has never been more critical. By understanding the fundamental processes that govern plant life, we can better appreciate the role that plants play in sustaining life on Earth and develop strategies to harness their potential for the benefit of humanity.

It is our hope that this volume will inspire a deeper interest in plant science and encourage further research and innovation in this vital field. Whether you are a student beginning your journey in plant biology or an experienced researcher seeking to expand your knowledge, we believe that "Plant Science: From Fundamentals to Advanced Research" will be an invaluable addition to your library.

We extend our gratitude to the contributors whose expertise and dedication have made this volume possible, and we look forward to the continued exploration and discovery that future volumes in this series will bring.

Editors

TABLE OF CONTENT

Plant Science: From Fundamentals to Advanced Research Volume II
(ISBN: 978-93-95847-68-1)

GARDENS: AN INEVITABLE WONDER IN HUMAN LIFE

D. Herin Sheeba Gracelin*[1] and P. Benjamin Jeya Rathna Kumar[2]

[1]Department of Botany

Sarah Tucker College (Autonomous), Tirunelveli - 627 007, Tamil Nadu, India.

[2]Department of Botany, Kamaraj College - 628003, Tamil Nadu, India.

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

Abstract:

Gardens are cultivated spaces that blend nature and human creativity, offering a place of beauty, tranquility, and often, utility. From small backyard plots to expansive public parks, gardens can vary greatly in size and design, reflecting the culture, climate, and preferences of those who create and maintain them. Historically, gardens have been important to civilizations worldwide, serving as places for growing food, medicinal herbs, and ornamental plants. The designs and purposes of gardens have evolved over time, influenced by different artistic movements, philosophies, and advancements in horticulture. Today, gardens can serve many purposes, such as providing fresh produce in urban areas, creating habitats for wildlife, and offering a peaceful retreat from the bustle of everyday life. They can be formal, with structured layouts and manicured plants, or informal, embracing a more natural, wild aesthetic. Gardens are also an expression of sustainability, as they can support biodiversity, promote healthy ecosystems, and offer spaces for education and community engagement. Whether for practical use, artistic expression, or simply a place to unwind, gardens are a vital part of our relationship with the natural world. Gardens come in many types, each serving different purposes and reflecting various styles and philosophies. In this review article, various types of gardens are discussed in detail.

Keywords: Biodiversity, Healthy Ecosystems, Medicinal Herbs And Ornamental Plants

Introduction:

Gardens come in a wide variety of forms, each reflecting different purposes, styles, and cultural influences. From the intimate spaces of residential gardens to the expansive layouts of public parks, each type of garden is designed to fulfill specific needs, whether for beauty, food production, relaxation, or environmental sustainability (Bisgrove and Hadley, 2002). Gardens, whether small or large, personal or public, offer numerous benefits that extend beyond their immediate space. They contribute to healthier lifestyles, stronger communities, and a more sustainable environment. The structure of a garden typically includes carefully planned elements such as pathways,

planting beds, and focal points that guide the flow and aesthetics of the space. Pathways create movement and accessibility, while planting beds are arranged to showcase a variety of plants, often grouped by height, color, or function. Focal points like fountains, sculptures, or large trees draw the eye and anchor the design. Additional features like seating areas, water features, and lighting enhance the garden's functionality and ambiance, creating a harmonious balance between natural elements and human design (Calder and Martin, 2006).

Types of Gardens

1 Residential Gardens

- **Flower Gardens:** Focused on ornamental plants and flowers, these gardens are designed for aesthetic appeal, often showcasing a variety of colors, textures, and fragrances.
- **Vegetable Gardens:** Dedicated to growing edible plants like vegetables, herbs, and sometimes fruits. These gardens are often found in backyards or community spaces.
- **Herb Gardens:** Specifically cultivated for growing herbs used in cooking, medicine, or for their aromatic qualities. They can be small and contained or integrated into larger garden spaces.
- **Water Gardens:** Feature ponds, waterfalls, or fountains, and often include aquatic plants like water lilies and fishlike koi. These gardens emphasize tranquility and reflection (Dunnett *et al.,* 2007).

2 Public and Community Gardens

- **Botanical Gardens:** Large gardens with a wide variety of plant species, often labeled with their botanical names. These gardens serve educational, research, and conservation purposes.
- **Community Gardens:** Shared spaces where members of a community grow plants, often vegetables and fruits, in individual or shared plots. They foster community engagement and provide fresh produce.
- **Park Gardens:** Integrated into public parks, these gardens can be formal or informal, designed to enhance the beauty of the park and provide recreational space for visitors (David *et al.,* 2008).

3 Specialized Gardens

- **Japanese Gardens:** Reflect traditional Japanese aesthetics, emphasizing simplicity, natural beauty, and tranquility. Elements often include rocks, water features, and carefully pruned plants.

- **Zen Gardens:** A type of Japanese garden also known as a rock or dry garden. It uses rocks, sand, and minimal plants to create a meditative space.
- **Tropical Gardens:** Feature lush, exotic plants typical of tropical climates, such as palms, ferns, and orchids. These gardens often require controlled environments in non-tropical regions.
- **Cottage Gardens:** An informal style of garden that uses a mixture of ornamental and edible plants. The design is typically dense and full, with plants allowed to grow in a natural, unstructured way.
- **Mediterranean Gardens:** Characterized by drought-resistant plants like olive trees, lavender, and rosemary. These gardens thrive in hot, dry climates and often feature gravel or stone paths.

4 Sustainable and Ecological Gardens

- **Xeriscape Gardens:** Designed for water conservation, these gardens use drought-tolerant plants and efficient irrigation methods, ideal for arid regions.
- **Wildlife Gardens:** Created to attract and support local wildlife, including birds, bees, and butterflies. They often include native plants and features like bird feeders and insect hotels.
- **Permaculture Gardens:** Based on principles of sustainability and ecological design, these gardens are self-sufficient systems that often incorporate elements like food forests, water harvesting, and natural pest control (Messervy, 2004).

5 Historical and Cultural Gardens

- **Victorian Gardens:** Reflect the ornate style of the Victorian era, often with intricate flower beds, elaborate structures like gazebos, and a mix of exotic and native plants.
- **Islamic Gardens:** Feature geometric designs, water channels, and a sense of symmetry and order, reflecting the cultural and religious values of Islamic architecture.
- **Formal Gardens:** Known for their symmetry, order, and structured design, often featuring hedges, topiaries, and carefully planned layouts. Examples include French and Italian Renaissance gardens.

6 Therapeutic Gardens

- **Healing Gardens:** Designed to promote health and well-being, often found in hospitals, nursing homes, or therapeutic centers. These gardens emphasize sensory experiences and accessibility.

- **Sensory Gardens:** Created to stimulate the senses, with plants chosen for their smell, touch, sight, sound, and even taste. These gardens are often used for therapeutic purposes, particularly for people with disabilities or sensory impairments (Santhakumar, 1996).

Benefits of Gardens

Gardens offer a wide range of benefits, impacting physical health, mental well-being, social connections, and the environment (Singh and Ram Bachan ,1976).

1. Health and Well-being

- **Physical Activity:** Gardening provides a form of low-impact exercise that can improve strength, flexibility, and cardiovascular health. Tasks like digging, planting, and weeding help keep the body active.
- **Mental Health:** Spending time in a garden can reduce stress, anxiety, and depression. The calming effects of nature and the sense of accomplishment from nurturing plants contribute to improved mental well-being.
- **Nutrition:** Growing your own fruits, vegetables, and herbs ensures access to fresh, organic produce. This can lead to healthier eating habits and a more balanced diet.

2. Environmental Benefits

- **Biodiversity:** Gardens can support local ecosystems by providing habitats for birds, insects, and other wildlife. Planting native species encourages biodiversity and helps maintain a healthy environment.
- **Climate Regulation:** Gardens, especially those with trees and shrubs, can help regulate temperatures by providing shade and reducing the urban heat island effect. They also contribute to carbon sequestration, mitigating climate change.
- **Water Management:** Gardens with proper design and plant selection can reduce soil erosion and manage stormwater runoff, helping to prevent flooding and improve water quality (Scurr and Ruth, 2022).

3. Social and Community Benefits

- **Community Building:** Community gardens bring people together, fostering a sense of belonging and cooperation. They create spaces where individuals can share knowledge, resources, and experiences.
- **Educational Opportunities:** Gardens serve as living classrooms, offering hands-on learning about biology, ecology, nutrition, and sustainability. They are valuable tools for teaching children and adults alike about the natural world.

- **Aesthetic and Cultural Value:** Well-designed gardens enhance the beauty of neighborhoods and public spaces, contributing to cultural heritage and creating environments that inspire creativity and reflection.

4. Economic Benefits

- **Cost Savings:** Growing your own food can reduce grocery bills, especially when cultivating high-value crops like herbs, tomatoes, and berries. Gardens can also reduce the need for costly landscaping services.
- **Property Value:** A well-maintained garden can increase the value of a property, making it more attractive to potential buyers. Beautiful gardens enhance curb appeal and provide a sense of pride in homeownership.
- **Job Creation:** Gardens, particularly in larger public and botanical settings, can create employment opportunities in horticulture, landscaping, and garden design.

5. Therapeutic and Healing Benefits

- **Therapeutic Spaces:** Gardens are often used in therapeutic settings to aid in recovery and rehabilitation. Healing gardens in hospitals and care facilities offer a peaceful environment that can support physical and emotional healing.
- **Sensory Stimulation:** Gardens designed for sensory engagement can help individuals with disabilities or sensory impairments by providing a rich experience through touch, sight, smell, and sound.

6. Sustainability and Food Security

- **Sustainable Practices:** Gardens promote sustainable living by encouraging composting, recycling, and the use of organic gardening methods. They help reduce the environmental footprint of food production and waste.
- **Food Security:** In urban areas, community gardens can improve food security by providing access to fresh, locally grown produce, especially in food deserts where access to grocery stores is limited.

Conclusion:

Gardens are versatile spaces that blend nature and human creativity, offering benefits for physical health, mental well-being, and the environment. They can be designed for beauty, food production, or relaxation, serving as places for community engagement, education, and cultural expression. Gardens support biodiversity, contribute to climate regulation, and promote sustainable living. Whether a small residential plot or a large public park, gardens enhance our connection to nature, improve our quality of life, and provide valuable green spaces in urban and rural areas

alike. Gardening is important to society as it enhances food security, promotes environmental stewardship, fosters social cohesion, improves health and well-being, provides educational opportunities, and contributes to economic stability. Gardens are not just personal or decorative spaces; they are essential components of a healthy, sustainable, and connected society.

References:

1. Bisgrove, R and Hadley, P (2002). Gardening in the global greenhouse: the impacts of climate change on gardens in the UK (Report). S2CID 127801132.
2. Calder and Martin (2006). Experiencing the Garden in the Eighteenth Century. Lang. p. 9. ISBN 9783039102914.
3. Dunnett and Clayden, Nigel and Andy (2007). Rain Gardens: Managing Water Sustainably in the Garden and Designed Landscape. Portland, OR: Timber Press. ISBN 978-0-88192-826-6.
4. David S.; Vince-Prue, Daphne; Gregory, Peter J (2008). Science and the Garden: The scientific basis of horticultural practice. Oxford: Blackwell. ISBN 978-1-4051-6063-6.
5. Messervy, J.M (2004). The meaning of the garden in human life. Acta Hortic. 642, 79-99
6. Santhakumar V (1996). ST Home Gardens 5.2.3\Them Doc\Paper: Biodiversity in Home gardens \Ver.3, VS.AK.VS, 10.9.01
7. Scurr and Ruth (2022). Napoleon: A Life in Gardens and Shadows. Vintage. p. 29.
8. Singh and Ram Bachan (1976). "Cities and parks in ancient India" Ekistics.42 (253): 372–376.
9. https://www.bgci.org/about/botanic-gardens-and-plant-conservation/
10. https://www.merriam-webster.com/dictionary/garden.

NUTRITIONAL AND PSYCHOLOGICAL PERSPECTIVE OF MORINGA: A SHORT REVIEW

S. M. Prasad*[1], Harikrishna M. R[2] and A. S. Sumaya[1]

[1]Department of Nutrition and Dietetics (UG and PG),

[2]Department of Psychology,

Sadakathullah Appa College (Autonomous), Rahmath Nagar,

Tirunelveli District,Tamil Nadu, Pin-627 011, India.

*Corresponding author E-mail: prasadsm33@gmail.com

Abstract:

Moringa oleifera, often referred to as the "miracle tree," is a plant with remarkable nutritional and medicinal properties that have gained widespread recognition in recent years. This short review explores the dual perspectives of Moringa's impact on both nutritional well-being and psychological health. Nutritionally, Moringa is a powerhouse, rich in essential vitamins, minerals, and antioxidants that contribute to overall health and disease prevention. Its high levels of nutrients like iron, calcium, and vitamins A, C, and E support physical vitality and immune function. From a psychological perspective, Moringa's potential role in stress reduction and cognitive enhancement is increasingly supported by emerging research. The presence of bioactive compounds, such as polyphenols and flavonoids, helps mitigate oxidative stress and inflammation, which are linked to mental health disorders like anxiety and depression. This review synthesizes current findings, emphasizing the importance of Moringa as a holistic approach to enhancing both physical and mental well-being. Further research is encouraged to fully elucidate its therapeutic potential and applications in the field of integrative health.

Keywords: Seeds, Flowers, Nutritive Value, Medicinal Value, Stress Reduction, Cognitive Function

Introduction:

Native to the Indian subcontinent, the drumstick tree (*Moringa oleifera*) grows quickly and is hardy. It is referred to as the "drumstick tree" in many places because of its long, thin pods that resemble drumsticks. Its various names, such as "horseradish tree," "ben oil tree," and "miracle tree," demonstrate its many applications and high nutritional content. As a member of the Moringaceae family, *Moringa oleifera* has long been used in traditional medicine and culinary practices. Because of its resilience and capacity to thrive in arid and semi-arid environments, it is highly valued and serves as a

vital food source in areas where food is scarce. All of the tree's edible parts, including the leaves, pods, seeds, flowers, and roots, are rich in vitamins, minerals, and essential amino acids. Particularly well-known for having high concentrations of calcium, potassium, protein, and vitamins A, C, and E, drumstick tree leaves also make a fantastic source of dietary supplements. The pods are rich in dietary fiber and vitamin C and are frequently used in cooking. When the seeds are processed, a premium oil known as "ben oil" is produced. This oil is used in cooking, cosmetics, and even as a lube. Apart from its nutritional benefits, the drumstick tree has several medical applications. It has been used to treat a variety of conditions in traditional medicine, including malnourishment, infections, inflammation, and digestive disorders. Many of these traditions are supported by recent scientific studies.

Drumstick Flowers

Matured Drumsticks

Drumstick Oil with Added Herbs

Drumstick Flowers

Nutritional Benefits:

Drumstick flowers are packed with vital nutrients, including vitamins A and C, calcium, and potassium. They also contain amino acids, making them an excellent source of protein, particularly for those following a plant-based diet. These flowers are frequently incorporated into various cooking traditions, especially in South Asian cuisine, where they are sprinkled into curries, soups, and stir-fries. Their mild taste and soft texture make them a flexible ingredient that pairs well with a broad spectrum of flavors and recipes.

Health Benefits:

In traditional medicine, drumstick flowers are valued for their health-boosting qualities. They are recognized for their anti-inflammatory, antimicrobial, and antioxidant effects. These flowers are often used in herbal remedies to address a variety

of health issues, including urinary tract infections, cold symptoms, and digestive problems. They are also thought to have a rejuvenating effect on the body, enhancing overall well-being and energy levels.

Culinary Applications:

Beyond their medicinal uses, drumstick flowers are a favored ingredient in cooking. They can be sautéed with spices, tossed into salads, or used as a topping. In South Indian cooking, they are often combined with lentils or coconut milk to create nourishing and flavorful meals. The flowers can also be dried and used to brew teas or infusions, which are believed to have a calming effect on the digestive system.

Drumstick Seeds

Researcher Collecting Dried Drumsticks

Dried Drumstick

Collected Dried Seed

Nutritional Benefits

Originating from the Moringa tree, drumstick seeds are rich in vital nutrients that support general health and wellbeing. These high-protein seeds are especially helpful for vegetarians and vegans as they offer a valuable source of plant-based protein. They also contain substantial amounts of vitamins, especially vitamin A, which is essential for keeping skin and eyes healthy, and vitamin C, which boosts immunity. Essential minerals like calcium, potassium, and iron are found in drumstick seeds. These minerals are necessary for healthy bones, strong muscles, and the body's ability to transport oxygen. The seeds are also a rich source of antioxidants, such as phenolic acids and flavonoids, which lessen inflammation and fight oxidative stress, thus reducing the risk of chronic illnesses. Their high fiber content facilitates gut health and aids in digestion. Drumstick seeds are a potent addition to a balanced diet because of their rich nutritional profile, which provides several health advantages in a tiny package.

Health Benefits

The health benefits of drumstick seeds are numerous because of their rich nutritional and medicinal properties. Drumstick seeds are derived from the Moringa tree. One of the main advantages is their high antioxidant content, which lowers the risk of chronic illnesses like cancer and heart disease by oxidative stress and helps the body combat free radicals. Additionally, the seeds have anti-inflammatory qualities that have been shown to lessen the symptoms of rheumatoid arthritis and other inflammatory diseases. Drumstick seeds are good for heart health in addition to being antioxidants and anti-inflammatory. By controlling blood pressure and cholesterol, they lower the risk of cardiovascular illnesses. In the same way that the seeds have long been used to purify water, they can also aid in the body's natural detoxification process by attaching to and removing toxins. Drumstick seeds' high fiber content facilitates digestion and helps ward off constipation, supporting digestive health. The antioxidants and anti-inflammatory properties in the seeds can help lessen acne and encourage clearer skin, so they are also advantageous for skin health. Moreover, studies have demonstrated that drumstick seeds possess antimicrobial qualities, which aid in thwarting infections and enhancing the immune system. Drumstick seeds have numerous health advantages, making them an effective natural remedy for preserving general health and wellbeing.

Culinary Applications

Drumsticks are rich in nutrients and can be harvested from the Moringa tree. There are numerous culinary uses for these seeds. Curries and stews: Drumstick seeds are commonly used in curries and stew because they absorb flavors well and add a distinctive texture. They are used in many different ways in many culinary traditions, particularly in South Asian cuisine. Indian dishes like kurma, a spicy curry, and sambar, a lentil stew, benefit greatly from their frequent use in the kitchen. Soups and Broths: Use the seeds to enhance the nutritional content and impart a mildly nutty taste to soups and broths. Often boiled until tender, they add a substantial element to the dish. Stir-Fries and Salads: After the stir-fries and salads are cooked, you can add drumstick seeds to them. They improve the flavor of dishes that contain vegetables by contributing subtle, nutty flavors and a crunchy texture. Some regions of the world use drumstick seeds for chutneys or pickles. To create a tangy, spicy condiment that pairs well with a range of dishes, the seeds are often marinated in vinegar and spices. Refreshments: Roasted drumstick seeds make a healthy snack. Slightly seasoned, they are roasted to bring out their natural flavors, making them a crunchy, healthful snack option.

Role of Moringa in Stress Reduction:

Moringa oleifera is increasingly recognized for its potential role in stress reduction. Rich in essential nutrients such as magnesium, vitamin C, and polyphenols, Moringa may help regulate the body's stress response. Magnesium plays a crucial role in calming the nervous system, while vitamin C supports adrenal gland function, which is vital for managing stress hormones like cortisol. Additionally, the antioxidants in Moringa combat oxidative stress, which is linked to anxiety and depression. Emerging studies suggest that Moringa's natural compounds can enhance mood and reduce anxiety, making it a promising supplement for stress management. However, more extensive research is needed to fully understand its efficacy and mechanisms.

Cognitive Function and Brain Health:

Moringa oleifera has garnered attention for its potential benefits on cognitive function and brain health. Rich in antioxidants such as quercetin and chlorogenic acid, Moringa helps protect brain cells from oxidative damage, which is linked to cognitive decline and neurodegenerative diseases. The anti-inflammatory properties of Moringa also contribute to reducing brain inflammation, a factor in conditions like Alzheimer's disease. Additionally, Moringa's high levels of vitamin E and C support overall brain health by enhancing memory and cognitive performance. While preliminary research is promising, further studies are needed to explore the full impact of Moringa on cognitive function and its potential as a natural remedy for maintaining brain health.

Conclusion:

Renowned for both its remarkable nutritional makeup and possible mental health advantages, *Moringa oleifera* is a potent natural remedy for improving general health. Packed with vital vitamins, minerals, and antioxidants, moringa improves nutrient intake, strengthens immunity, and fends off chronic illnesses to support physical health. Its bioactive components also appear to have potential for lowering stress, improving mental health, and improving cognitive function. Even though the available data emphasizes the many advantages of moringa, more investigation is required to completely grasp this plant's therapeutic potential. This brief review argues for the inclusion of moringa in regular diets and wellness practices by highlighting its important role as a holistic approach to both physical and psychological health.

Acknowledgement:

The author thanks Mr. Harikrishna M.R and Dr. A.S.Sumaya for collecting all the original photos and needed discussion and their timely help. Their contribution to write this article is always admirable.

References:

1. Adeyemi, O. S., & Elebiyo, T. C. (2014). *Moringa oleifera* supplemented diets prevented nickel-induced nephrotoxicity in Wistar rats. Journal of Nutrition and Metabolism, 2014, Article 958621. https://doi.org/10.1155/2014/958621
2. Amaglo, N. K., Bennett, R. N., Lo Curto, R. B., Rosa, E. A., Lo Turco, V., Giuffrida, A., & Tziboula-Clarke, A. (2010). Profiling selected phytochemicals and nutrients in different tissues of the multipurpose tree *Moringa oleifera* L., grown in Ghana. Food Chemistry, 122(4), 1047-1054. https://doi.org/10.1016/j.foodchem.2010.03.073
3. Anwar, F., Latif, S., Ashraf, M., & Gilani, A. H. (2007). *Moringa oleifera*: A food plant with multiple medicinal uses. Phytotherapy Research, 21(1), 17-25. https://doi.org/10.1002/ptr.2023
4. Azeez, O. M., & Bello, H. A. (2015). *Moringa oleifera*: A potential tree for nutrition and healthcare. Journal of Natural Sciences Research, 5(8), 29-34. https://www.iiste.org/Journals/index.php/JNSR/article/view/21928
5. Fahey, J. W. (2005). *Moringa oleifera*: A review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Part 1. Trees for Life Journal, 1(5), 1-15. https://www.tfljournal.org/article.php/20051201124931586
6. Fuglie, L. J. (2001). The Miracle Tree: *Moringa oleifera*: Natural nutrition for the tropics. Church World Service. https://www.moringanews.org/documents/miracletree.
7. Gopalakrishnan, L., Doriya, K., & Kumar, D. S. (2016). *Moringa oleifera*: A review on nutritive importance and its medicinal application. Food Science and Human Wellness, 5(2), 49-56. https://doi.org/10.1016/j.fshw.2016.04.001
8. Gupta, R., Gupta, A. K., & Ganguli, A. K. (1987). Nutritional and therapeutic values of *Moringa oleifera*. Annals of Arid Zone, 26(3-4), 239-248.
9. Leone, A., Spada, A., Battezzati, A., Schiraldi, A., Aristil, J., & Bertoli, S. (2015). *Moringa oleifera* seeds and oil: Characteristics and uses for human health. International Journal of Molecular Sciences, 16(12), 12791-12835. https://doi.org/10.3390/ijms160612791
10. Mukunzi, D., Nsor-Atindana, J., Teyssier, C., Girardon, P., Rolando, C., & Claeys-Bruno, M. (2011). Effects of storage conditions on the stability of *Moringa oleifera* leaves as a source of antioxidants. Journal of Food Composition and Analysis, 24(2), 153-159. https://doi.org/10.1016/j.jfca.2010.08.008

11. Nouman, W., Basra, S. M. A., Siddiqui, M. T., Yasmeen, A., Gull, T., & Alcayde, M. A. C. (2013). Potential of *Moringa oleifera* L. as livestock fodder crop: A review. Turkish Journal of Agriculture and Forestry, 37(1), 1-14. https://doi.org/10.3906/tar-1211-66
12. Popoola, J. O., & Obembe, O. O. (2013). Local knowledge, use pattern, and geographical distribution of *Moringa oleifera* Lam. (Moringaceae) in Nigeria. Journal of Ethnopharmacology, 150(2), 682-691. https://doi.org/10.1016/j.jep.2013.09.043
13. Razis, A. F. A., Ibrahim, M. D., & Kntayya, S. B. (2014). Health benefits of *Moringa oleifera*. Asian Pacific Journal of Cancer Prevention, 15(20), 8571-8576. https://doi.org/10.7314/APJCP.2014.15.20.8571
14. Saini, R. K., Sivanesan, I., & Keum, Y. S. (2016). Phytochemicals of *Moringa oleifera*: A review of their nutritional, therapeutic, and industrial significance. 3 Biotech, 6 (2), 203. https://doi.org/10.1007/s13205-016-0526-3
15. Tiloke, C., Phulukdaree, A., & Chuturgoon, A. A. (2013). The antiproliferative effect of *Moringa oleifera* crude aqueous leaf extract on cancerous human alveolar epithelial cells. BMC Complementary and Alternative Medicine, 13(1), 226. https://doi.org/10.1186/1472-6882-13-226

CHALLENGES AND INNOVATIONS IN ALIEN GENE INTROGRESSION FOR ENHANCING CROP TRAITS

Deepak Gupta*, Karan Sachdeva, Anita, Komal Choudhary,
Saroj Verma, Pooja Jat and Raju Yadav
Sri Karan Narendra Agriculture University, Jobner-303329
*Corresponding author E-mail: deegupta73@gmail.com

Abstract:

The twentieth century marked a transformative era in global crop production, driven by expanded cultivated areas, improved agronomic practices, and advanced crop management techniques. A key contributor to this progress has been the development of genetically enhanced crop varieties, achieved through modern plant breeding methods that introduce beneficial alleles via hybridization and genetic transformation. These advancements have led to cultivars with increased yield, stress resistance, and superior performance. Alien gene introgression, a technique that incorporates genetic material from wild or closely related species, has played a crucial role in enhancing genetic diversity, pest resistance, and overall crop quality (Feuillet *et al.,* 2008)

Definition and Significance

Alien gene introgression refers to the process of incorporating genetic material from wild relatives or closely related species into the genome of cultivated crops. This technique is pivotal in modern plant breeding as it enhances genetic diversity, which is essential for improving traits such as disease resistance, abiotic stress tolerance, and yield. Alien gene introgression allows breeders to introduce novel genes that are not present in the cultivated gene pool, thereby providing new opportunities for crop improvement (Frisch andMelchinger, 2005).

The significance of alien gene introgression is that it addresses the limitations of traditional breeding methods, especially in crops with narrow genetic bases. By utilizing the genetic resources of wild relatives, breeders can tackle challenges such as disease outbreaks, environmental stresses, and reduced productivity. This approach is essential for developing crops capable of meeting the growing demands of climate change and global food security (Jena and Khush, 2000).

Historical Background

The concept of alien gene introgression emerged in the early 20th century, with significant progress made in the 1950s when Sears successfully introduced a resistance gene from *Aegilopsumbellulata* into wheat, demonstrating the potential of using wild

relatives for crop improvement (Sears, 1956). Advancements in cytogenetics and molecular biology, including somatic hybridization and protoplast fusion, further enhanced alien gene introgression by expanding donor species and increasing efficiency (Hajjar and Hodgkin, 2007).

Key Milestones in Alien Gene Introgression

Several key milestones have marked the progress of alien gene introgression in plant breeding:

1. **1956 - Introduction of Rust Resistance in Wheat**: Sears successfully introduced a gene for resistance to leaf rust from *Aegilopsumbellulata* into wheat, setting a precedent for using wild relatives in crop improvement (Sears, 1956).
2. **1970s - Development of Hybridization Techniques**: Advances in hybridization techniques, including embryo rescue and tissue culture, enabled the successful transfer of genes between more distantly related species (Choi *et al.,* 2007).
3. **1980s - Application of Molecular Markers**: The use of molecular markers to track introgressed genes became a game-changer, allowing breeders to more efficiently select plants with the desired traits (Collard andMackill, 2008).
4. **1990s - Expansion to Other Crops**: Alien gene introgression was successfully applied to a wider range of crops, including rice, maize, and barley, resulting in improved varieties with enhanced disease resistance and stress tolerance (Jena andKhush, 2000).
5. **2000s - Integration of Gene Editing Technologies**: The advent of CRISPR-Cas9 and other gene-editing technologies revolutionized alien gene introgression by providing more precise tools for introducing and modifying genes (Zhou *et al.,* 2020).

Role in Modern Plant Breeding

In modern plant breeding, alien gene introgression plays a critical role in enhancing crop traits that are challenging to improve through conventional methods. By introducing genetic material from wild relatives or closely related species, breeders can achieve several key objectives:

- **Disease and Pest Resistance**: Alien gene introgression allows for the incorporation of resistance genes against various pathogens and pests, leading to crops with improved durability and reduced dependence on chemical controls (Hajjarand Hodgkin, 2007).

- **Abiotic Stress Tolerance**: The ability to introduce genes that confer tolerance to environmental stresses such as drought, salinity, and extreme temperatures is crucial for developing crops that can thrive under changing climatic conditions (Frisch andMelchinger, 2005).
- **Yield Improvement**: Novel genes introduced through alien gene introgression can contribute to increased yield by enhancing traits such as growth vigor, grain filling, and root development (Jena andKhush, 2000).
- **Nutritional Quality**: Improving the nutritional content of crops, such as increasing vitamin and mineral levels, can also be achieved through the introduction of beneficial genes from wild relatives (Collard andMackill, 2008).

Comparison with Traditional Breeding Methods

Alien gene introgression offers several advantages over traditional breeding methods:

- **Broader Genetic Base**: Unlike conventional breeding, which often relies on the limited genetic diversity within a single species, alien gene introgression brings in genetic material from a wider pool of wild relatives, expanding the range of available traits (Hajjarand Hodgkin, 2007).
- **Faster Trait Improvement**: Alien gene introgression can accelerate the development of new cultivars by directly introducing beneficial traits, whereas traditional breeding methods may require multiple generations to achieve similar improvements (Jena andKhush, 2000).
- **Enhanced Precision**: Modern technologies, such as marker-assisted selection and gene editing, allow for more precise introgression of specific genes, reducing the risk of undesirable traits being carried over from wild relatives (Zhou *et al.,* 2020).

However, traditional breeding methods also have their advantages, including:

- **Established Practices**: Conventional breeding methods are well-established and have been successfully used for decades to develop improved crop varieties (Collard andMackill, 2008).
- **Adaptation to Local Conditions**: Traditional breeding often involves selecting plants that are well-adapted to specific local environments, a process that may not always be fully replicated with alien gene introgression (Frisch andMelchinger, 2005)

Mechanisms of Gene Transfer

Gene transfer mechanisms are pivotal in modern plant breeding and biotechnology, facilitating the introduction of desirable traits into crop species..

(i) Natural Hybridization

Natural hybridization involves the interbreeding of different species or varieties, leading to offspring that possess genetic material from both parents. This process is a fundamental driver of genetic diversity and evolution in plants. For example, hybridization between different *Brassica* species has resulted in improved cultivars with enhanced yield and disease resistance (Schmid*et al.,* 2021). Similarly, natural hybrids of *Solanum* species have been used to introduce traits such as pest resistance and improved fruit quality (TanksleyandMcCouch, 1997). These hybrids often exhibit unique combinations of traits that can be beneficial for crop improvement.

(ii) Artificial Hybridization Techniques

Artificial hybridization involves controlled pollination between selected parent plants to produce hybrids with desired traits. Techniques such as controlled cross-pollination, hand pollination, and the use of pollen from different species are used to achieve this goal. For instance, controlled cross-pollination in maize has led to the development of high-yielding hybrids with improved disease resistance (Tuberosa, 2018). In rice, hand pollination has been employed to combine traits from different varieties, resulting in improved yield and stress tolerance (Zhang *et al.,* 2019). These artificial methods have significantly advanced crop breeding by enabling the creation of hybrids with specific, beneficial characteristics.

(iii) Somatic Hybridization

Somatic hybridization involves the fusion of somatic cells from different plant species or varieties to create hybrids with a combination of genetic traits. This technique is especially useful when sexual hybridization is not feasible. Methods such as somatic cell fusion and callus culture enable the creation of hybrids with enhanced traits. For example, somatic hybridization between *Solanum* species has produced plants with improved resistance to diseases such as late blight (Kwon *et al.,* 2015). Additionally, somatic hybrids of *Brassica*species have demonstrated increased yield and better stress tolerance (Liu *et al.,* 2018). These hybrids offer unique genetic combinations that are valuable for crop improvement.

(iv)Protoplast Fusion

Protoplast fusion involves removing cell walls from plant cells to create protoplasts, which are then fused to combine their genetic material. This method allows

for the merging of genetic material from different plant species or varieties that cannot be crossed sexually. Protoplast fusion has been used to develop new plant varieties with enhanced traits, such as disease resistance and nutritional content (Kato and Kato, 2018). Techniques such as polyethylene glycol (PEG) and electrofusion facilitate the fusion of protoplasts, leading to the regeneration of hybrid plants with valuable traits (Saito *et al.,* 2016).

(v) Horizontal Gene Transfer in Plants

Horizontal gene transfer (HGT) involves the transfer of genetic material between organisms of different species through mechanisms other than reproduction. In plants, HGT can occur through interactions with microorganisms such as bacteria and fungi. For instance, the transfer of genes from *Agrobacterium tumefaciens* to plants through natural transformation has been extensively used to introduce new traits into crops (Kempin*et al.,* 1997). HGT can result in new traits, such as pathogen resistance and stress tolerance, and contributes to the genetic diversity of plants (Molecular Plant, 2020).

Selection of Donor Species

The selection of donor species is a critical step in the alien gene introgression approach, where the goal is to introduce specific traits from wild or closely related species into cultivated crops. This process involves careful consideration of several factors to ensure the successful transfer of desirable traits. Key aspects of selecting donor species include criteria for choosing appropriate species, the significance of wild relatives, screening for desired traits, the role of gene banks and germplasm repositories, and examples of successful donor species selection.

(i) Criteria for Choosing Donor Species

Selecting suitable donor species involves evaluating several criteria to ensure that they will contribute beneficial traits to the cultivated crop. Important factors include genetic compatibility, the presence of desirable traits, and the ease of hybridization. Donor species should have traits that are lacking in the cultivated crop but are crucial for improving characteristics such as disease resistance, stress tolerance, or yield (TanksleyandMcCouch, 1997). Genetic compatibility is assessed based on the similarity between the donor species and the recipient crop, which influences the success of hybridization and introgression (Choi *et al.,* 2014).

(ii) Importance of Wild Relatives

Wild relatives of cultivated crops are valuable sources of genetic diversity and can provide traits that are not present in domesticated varieties. These traits may

include resistance to pests, diseases, and environmental stresses. Wild relatives often possess unique genetic variations that have evolved in natural environments, making them crucial for broadening the genetic base of crops (Fu and Cunningham, 2014). For instance, the use of wild *Aegilops* species has been instrumental in enhancing wheat's resistance to various pathogens and environmental conditions (Feuillet *et al.,* 2008).

(iii) Screening for Desired Traits

Screening for desired traits in donor species involves identifying and selecting individuals that possess specific characteristics beneficial for the recipient crop. This process can include phenotypic assessments, molecular marker analysis, and genetic mapping. Traits such as disease resistance, drought tolerance, and nutritional quality are evaluated to ensure that they meet the breeding objectives (Varshney*et al.,* 2016). Advanced techniques such as genome-wide association studies (GWAS) and quantitative trait loci (QTL) mapping are often used to identify and track the presence of desired traits in donor species (Huang *et al.,* 2015).

(iv) Use of Gene Banks and Germplasm Repositories

Gene banks and germplasm repositories play a vital role in preserving genetic diversity and providing access to a wide range of plant materials for breeding purposes. These facilities store seeds, tissues, and other plant materials from diverse species, including wild relatives and landraces (Guarino*et al.,* 2015). Researchers can access these resources to identify potential donor species with traits of interest. The use of gene banks facilitates the conservation of genetic diversity and supports breeding programs by providing a repository of genetic material for future crop improvement efforts.

Techniques in Alien Gene Introgression

Alien gene introgression involves integrating desirable traits from wild or closely related species into cultivated crops. Several advanced techniques are employed to achieve successful gene transfer and enhance crop varieties. These techniques include cross-breeding and hybridization, backcrossing strategies, marker-assisted selection (MAS), the use of molecular markers, and quantitative trait loci (QTL) mapping.

(i) Cross-Breeding and Hybridization

Cross-breeding and hybridization are fundamental techniques in alien gene introgression, involving the controlled mating of different plant species or varieties to produce hybrids with desirable traits. This process can introduce new genetic variations into the crop gene pool. For example, hybridization between *Brassica* species has led to the development of cultivars with improved resistance to diseases and pests

(Schmid*et al.,* 2021). Controlled cross-pollination techniques enable breeders to combine traits from donor species with those of the cultivated crop, enhancing characteristics such as yield, disease resistance, and stress tolerance (Beck *et al.,* 2016).

(ii) Backcrossing Strategies

Backcrossing is a method used to integrate specific traits from donor species into a cultivated crop while retaining the genetic background of the original crop. This technique involves crossing the hybrid progeny back with the cultivated parent over several generations. The aim is to recover a high proportion of the recipient's genetic material while retaining the introduced trait. For instance, backcrossing has been used to incorporate disease resistance genes from wild relatives into commercial wheat varieties (Choi *et al.,* 2014). Backcrossing strategies often involve rigorous phenotypic selection and molecular marker-assisted approaches to ensure the successful introgression of traits (Meyer *et al.,* 2011).

(iii) Marker-Assisted Selection (MAS)

Marker-assisted selection (MAS) is a technique that uses molecular markers to track and select for specific genetic traits in breeding programs. This approach enhances the efficiency of selecting plants with desirable traits by identifying specific genetic markers associated with these traits. MAS is particularly useful for traits that are difficult to evaluate phenotypically, such as resistance to diseases or environmental stresses (Varshney*et al.,* 2016). For example, MAS has been successfully applied to improve drought tolerance in crops by selecting for markers associated with water-use efficiency (Yan *et al.,* 2016).

(iv) Use of Molecular Markers

Molecular markers are DNA sequences used to identify specific genetic variations associated with desirable traits. These markers play a crucial role in mapping genes and facilitating the introgression of alien genes into crops. Types of molecular markers include simple sequence repeats (SSRs), single nucleotide polymorphisms (SNPs), and amplified fragment length polymorphisms (AFLPs) (Nielsen *et al.,* 2011). Molecular markers are employed to track the inheritance of traits during breeding and to ensure that the introgressed genes are retained in the progeny (He *et al.,* 2014).

(v) Quantitative Trait Loci (QTL) Mapping

Quantitative trait loci (QTL) mapping is a technique used to identify regions of the genome associated with specific quantitative traits, such as yield or disease resistance. QTL mapping involves the analysis of genetic crosses and the use of molecular markers to link phenotypic traits with specific genetic regions (Bernardo,

2008). This technique provides valuable information for breeding programs by identifying genes that contribute to complex traits and enabling their introgression into crop varieties (McMullen *et al.,* 2009).

Advances in Molecular Breeding

The field of molecular breeding has undergone significant advancements in recent years, leading to the development of innovative techniques that enhance the precision and efficiency of genetic improvements in crops. Key advances include CRISPR-Cas9 gene editing, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), cisgenesis and intragenesis, and synthetic biology approaches. These technologies are transforming crop breeding by enabling targeted modifications, introducing new traits, and improving overall crop performance.

1. CRISPR-Cas9 Gene Editing

CRISPR-Cas9 is a revolutionary gene-editing technology that allows precise modifications to the genome of organisms, including crops. This system utilizes a guide RNA (gRNA) to direct the Cas9 endonuclease to a specific location in the genome, where it creates double-strand breaks. These breaks are then repaired either by inserting or deleting genetic material, allowing for targeted gene knockouts or modifications (DoudnaandCharpentier, 2014). CRISPR-Cas9 has been used to enhance traits such as disease resistance, stress tolerance, and yield in various crops, including rice, wheat, and maize (Liu *et al.,* 2020).

2. Zinc Finger Nucleases (ZFNs)

Zinc finger nucleases (ZFNs) are artificial restriction enzymes that induce double-strand breaks at specific DNA sequences. ZFNs are composed of zinc finger DNA-binding domains fused to a DNA cleavage domain, which allows for targeted gene modifications (Carroll, 2011). These nucleases can be designed to target specific genes, facilitating the introduction of desired traits or knockout of undesirable genes. ZFNs have been utilized in various crops for trait improvement, including resistance to diseases and pests (Li *et al.,* 2015).

3. Transcription Activator-Like Effector Nucleases (TALENs)

Transcription activator-like effector nucleases (TALENs) are another class of site-specific nucleases used for genome editing. TALENs are derived from bacterial proteins that recognize specific DNA sequences via protein-DNA interactions. The DNA-binding domains of TALENs can be customized to target specific genes, and their nuclease domains induce double-strand breaks to facilitate targeted modifications

(Mahfouz *et al.,* 2011). TALENs have been applied to improve various crop traits, including stress tolerance and disease resistance (Wang *et al.,* 2019).

4. Cisgenesis and Intragenesis

Cisgenesis and intragenesis are techniques that involve the transfer of genes between organisms within the same species or closely related species, utilizing natural gene variants. Cisgenesis involves introducing genes from the same species into a recipient plant, while intragenesis involves the transfer of genes from closely related species or varieties (Haring *et al.,* 2006). These approaches are designed to introduce beneficial traits while avoiding the ethical and regulatory issues associated with transgenic methods. Cisgenesis and intragenesis have been used to develop crops with improved disease resistance and enhanced nutritional content (Haring *et al.,* 2016).

5. Synthetic Biology Approaches

Synthetic biology is an interdisciplinary field that combines biology and engineering principles to design and construct new biological parts, devices, and systems. In plant breeding, synthetic biology approaches involve creating novel genetic constructs or pathways to introduce new traits or improve existing ones. Techniques such as synthetic promoters, engineered gene circuits, and custom-designed metabolic pathways are used to achieve specific objectives (Church *et al.,* 2014). Synthetic biology has the potential to revolutionize crop breeding by enabling the design of complex traits and addressing challenges such as climate change and food security (Zhang *et al.,* 2020).

Applications in Crop Improvement

The application of advanced breeding techniques, including those discussed in previous sections, has significantly transformed crop improvement. These innovations are instrumental in enhancing various aspects of crop performance, including disease and pest resistance, abiotic stress tolerance, yield improvement, and quality traits. The following sections outline the key applications of these techniques in improving crops.

1. Disease Resistance

Disease resistance is a critical trait for maintaining crop health and productivity. Advanced breeding techniques, such as CRISPR-Cas9 gene editing and marker-assisted selection (MAS), have been used to introduce or enhance disease resistance in crops. For example, CRISPR-Cas9 has been employed to knock out susceptibility genes and enhance resistance to diseases such as wheat rust and rice blast (Wang *et al.,* 2019). Similarly, MAS has been used to incorporate resistance genes from wild relatives into cultivated crops, leading to improved resistance to various pathogens (Vivero*et al.,* 2019).

2. Pest Resistance

Pest resistance is another crucial trait that enhances crop survival and productivity. Techniques such as zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) have been utilized to develop crops with enhanced resistance to pests. ZFNs have been used to disrupt genes involved in pest susceptibility, while TALENs have enabled precise modifications in genes related to defense mechanisms (Chen *et al.,* 2018). Additionally, synthetic biology approaches have facilitated the creation of novel pest-resistant traits by engineering metabolic pathways or expressing insecticidal proteins (Kerschen*et al.,* 2004).

3. Abiotic Stress Tolerance (Drought, Salinity, etc.)

Abiotic stress tolerance, including resistance to drought, salinity, and other environmental stresses, is essential for maintaining crop productivity under adverse conditions. Advanced techniques such as CRISPR-Cas9 and cisgenesis have been used to enhance stress tolerance in crops. CRISPR-Cas9 has been employed to modify genes involved in stress response pathways, while cisgenesis has introduced stress-tolerance genes from related species into crops (Zhang *et al.,* 2020). Marker-assisted selection (MAS) has also been used to select for traits associated with improved stress tolerance (Bhatnagar-Mathur*et al.,* 2008; Kumar *et al.,* 2013).

4. Yield Improvement

Improving yield is a primary goal in crop breeding, and various advanced techniques have been applied to achieve this. Techniques such as quantitative trait loci (QTL) mapping and synthetic biology approaches have played significant roles in enhancing crop yield. QTL mapping has identified genetic regions associated with high yield, facilitating the selection of superior genotypes (Bernardo, 2008; McMullen *et al.,* 2009). Synthetic biology approaches have enabled the design of novel genetic circuits and pathways to boost yield (Church *et al.,* 2014; Khalil and Collins, 2010).

5. Quality Traits (Nutritional Content, Taste, etc.)

Enhancing quality traits such as nutritional content and taste is essential for meeting consumer preferences and improving the nutritional value of crops. Techniques like cisgenesis, CRISPR-Cas9, and synthetic biology have been utilized to achieve these improvements. Cisgenesis allows the introduction of beneficial genes related to nutritional content, while CRISPR-Cas9 can be used to modify genes involved in flavor and nutrient composition (Schouten *et al.,* 2011; Zhang *et al.,* 2018). Synthetic biology approaches have also been applied to engineer metabolic pathways to enhance the nutritional profile of crops (Endy, 2005; Zhang *et al.,* 2020).

References:

1. Beck, C., Wurst, M., Knapp, S. andRoder, M. S. (2016). Cross-breeding and hybridization in Brassica species: Strategies for improvement. *Plant Breeding*, 135(4): 354-361.
2. Bernardo, R. (2008). Molecular markers and selection for complex traits in plants: Learning from the last 20 years. *Crop Science*, 48(5): 1649-1664.
3. Bhatnagar-Mathur, P., Vadez, V.and Sharma, K. K. (2008). Transgenic approaches for abiotic stress tolerance in plants: Retrospect and prospects. *Plant Cell Reports*, 27(3): 411-424.
4. Carroll, D. (2011). Genome engineering with zinc-finger nucleases. *Genetics*, 188(4), 773-782.
5. Choi, S. M., Lim, Y. P., Lee, J. S. and Shin, J. S. (2007). Genetic introgression of alien chromosomes into wheat through embryo rescue and its application to crop improvement. *Euphytica*, 156(1-2): 195-208.
6. Choi, Y. J., Kwon, S. J., Kim, B. G. and Kim, J. H. (2014). Backcrossing strategies in wheat breeding programs: Implications for improving resistance traits. *Journal of Plant Breeding and Genetics*, 2(1): 35-44.
7. Church, G. M., Elowitz, M. B., Smolke, C. D., Voigt, C. A. and Weiss, R. (2014). Realizing the potential of synthetic biology. *Nature Reviews Molecular Cell Biology*, 15(4): 289-294.
8. Collard, B. C. Y. andMackill, D. J. (2008). Marker-assisted selection: An approach for precision plant breeding in the twenty-first century. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 363(1491): 557-572.
9. Doudna, J. A. and Charpentier, E. (2014). The new frontier of genome engineering with CRISPR-Cas9. *Science*, 346(6213): 1258096.
10. Endy, D. (2005). Foundations for engineering biology. *Nature*, 438(7067): 449-453.
11. Feuillet, C., Langridge, P. and Waugh, R. (2008). Cereal breeding takes a walk on the wild side. *Trends in Genetics*, 24(1): 24-32.
12. Frisch, M. and Melchinger, A. E. (2005). Selection theory for marker-assisted backcrossing. *Genetics*, 170(2): 909-917.
13. Fu, Y. B. and Cunningham, G. (2014). Genetic diversity and genetic erosion of crops in the agroecosystem: Two sides of the same coin. *Biodiversity and Conservation*, 23(6); 1345-1360.

14. Guarino, L., Ramanatha Rao, V. and Goldberg, E. (2015). Collecting Plant Genetic Diversity: Technical Guidelines. *Bioversity International*.
15. Hajjar, R. and Hodgkin, T. (2007). The use of wild relatives in crop improvement: A survey of developments over the last 20 years. *Euphytica*, 156(1-2): 1-13.
16. Haring, M. A., Rybel, B. V. andOffringa, R. (2016). Cisgenesis and intragenesis: Natural resistance to traditional concerns. *Plant Biotechnology Journal*, 14(3): 496-502.
17. He, Y., Tian, Z., Li, X. and Zhang, M. (2014). A review of molecular markers for genetic mapping and introgression of traits in crop species. *Journal of Plant Breeding and Genetics*, 2(1): 1-14.
18. Huang, X., Kurata, N., Wei, X., Wang, Z. X., Wang, A., Zhao, Q. and Han, B. (2015). A map of rice genome variation reveals the origin of cultivated rice. *Nature*, 490(7421): 497-501.
19. Jena, K. K. andKhush, G. S. (2000). Introgression of genes from Oryza officinalis Well ex Watt to cultivated rice, O. sativa L. *Theoretical and Applied Genetics*, 101(2-3): 294-299.
20. Kato, K. and Kato, H. (2018). Application of protoplast fusion technology to plant breeding: Progress and prospects. *Journal of Plant Breeding and Crop Science*, 10(7): 149-158.
21. Kempin, S. A., Liljegren, S. J., Block, L. M., Rounsley, S. D., Yanofsky, M. F. and Lam, E. (1997). Targeted disruption in Arabidopsis. *Nature*, 389(6650): 802-803.
22. Khalil, A. S. and Collins, J. J. (2010). Synthetic biology: Applications come of age. *Nature Reviews Genetics*, 11(5): 367-379.
23. Kwon, S. J., Sim, S. C. and Lee, J. S. (2015). Somatic hybridization in Solanum species: Achievements and prospects for crop improvement. *Plant Breeding*, 134(4): 398-407.
24. Liu, Y., Duan, M., Zhang, Y., Wang, Q., Li, W., Zhang, C. and Li, C. (2018). Somatic hybridization between Brassica napus and B. oleracea: Achievements and prospects. *Plant Breeding*, 137(5): 757-766.
25. Liu, Y., Zeng, X., An, S. and Zhang, W. (2020). Application of CRISPR-Cas9 technology in wheat (Triticumaestivum L.) improvement: Progress and prospects. *Journal of Integrative Agriculture*, 19(2): 226-238.
26. Mahfouz, M. M., Li, L., Shamimuzzaman, M., Wibowo, A., Fang, X. and Zhu, J. K. (2011). TALEN-based targeting of a plant genome. *Nature Protocols*, 6(3): 269-279.

27. McMullen, M. D., Kresovich, S., Villeda, H. S., Bradbury, P., Li, H., Sun, Q. and Buckler, E. S. (2009). Genetic properties of the maize nested association mapping population. *Science*, 325(5941): 737-740.
28. Meyer, R. S., DuVal, A. E. and Jensen, H. R. (2011). Patterns and processes in crop domestication: An historical review and quantitative analysis of 203 global food crops. *New Phytologist*, 190(1): 5-28.
29. Molecular Plant. (2020). Horizontal gene transfer in plants. *Molecular Plant*, 13(6): 762-764.
30. Nielsen, R., Paul, J. S., Albrechtsen, A. and Song, Y. S. (2011). Genotype and SNP calling from next-generation sequencing data. *Nature Reviews Genetics*, 12(6): 443-451.
31. Saito, H., Kurihara, Y. and Tateno, Y. (2016). Protoplast fusion in plants: From bench to field. *Plant Cell Reports*, 35(5): 1043-1054.
32. Schmid, M., Davison, S., Zicola, J., Keizer, M. andFedorova, V. (2021). Advances in hybridization techniques in Brassica crops. *Plant Breeding*, 140(1): 13-24.
33. Schouten, H. J., Krens, F. A. and Jacobsen, E. (2011). Cisgenesis and intragenesis in crop improvement: Implications for developing new varieties. *Plant Breeding*, 130(5): 456-464.
34. Sears, E. R. (1956). The transfer of leaf-rust resistance from Aegilopsumbellulata to wheat. *Brookhaven Symposia in Biology*, 9: 1-22.
35. Tanksley, S. D. andMcCouch, S. R. (1997). Seed banks and molecular maps: Unlocking genetic potential from the wild. *Science*, 277(5329): 1063-1066.
36. Tuberosa, R. (2018). Phenotyping for drought tolerance of crops in the genomics era. *Frontiers in Plant Science*, 9: 1195.
37. Varshney, R. K., Terauchi, R. andMcCouch, S. R. (2016). Harvesting the promising fruits of genomics: Applying genome sequencing technologies to crop breeding. *PLOS Biology*, 12(6): e1001883.
38. Vollbrecht, E. andSigmon, B. (2005). Amazing grass: Developmental genetics of maize domestication. *Biochemistry and Molecular Biology Education*, 33(4): 317-325.
39. Von Bothmer, R., Seberg, O. and Jacobsen, N. (1992). Genetic resources in the Triticeae. *Hereditas*, 116(2): 141-150.
40. Yang, S. J., Lee, Y. H. and Bae, S. H. (2013). Somatic hybridization for crop improvement: Current status and future prospects. *Plant Breeding*, 132(2): 109-116.

MUTATION BREEDING

Jayshree Subhash Nayka* and Prem Kumar Gautam

Department of Botany,

NTVS'S G.T. Patil Arts, Commerce and Science College, Nandurbar – 425412, M.S.

Affliated to Kavyitri Bahinabai Chaudhari North Maharashtra University, Jalgaon,

*Corresponding author E-mail: jayshreenayak1994@gmail.com

Introduction:

It is a technique used to induce genetic mutations in plants to create new crop varieties with desirable traits. This method involves the use of physical and chemical agents to induce genetic mutations in plants, which can lead to the development of novel crop varieties with improved characteristics**.**

Mutation breeding is a sustainable and ecologically sound method that plays a key role in increasing food production and providing a sustainable diet for this growing world (Pandit, Rishav *et al.,* 2021). Through breeding, scientists can develop new plant varieties with improved characteristics such as climate tolerance, yield, maturity time, resistance to pests and diseases, etc. There are many plant breeding techniques. Mutation breeding is one of the techniques used to improve crops. (Udage, Ashan 2021).

Over the past 50 years, mutation breeding has primarily focused on improving yields and reducing height, especially in wheat and rice. However, today's challenges are environmental pollution and its associated impacts, as well as the motivation for climate-friendly agriculture to ensure food supply (Saima Mir *et al.,* 2021). Among several plant breeding methods, mutation breeding has shown remarkable success in improving crops at a much faster rate than conventional breeding.

Mutation breeding generally has been more successful in self-fertilized and asexually propagated than in cross-fertilized crops because of the recessive nature of most mutations. Mutations are more easily recognized in diploid than in polyploid species, but examples of success with hexaploid wheat and oat crops are found. Verticillium wilt resistance, for example, was obtained int polyploid peppermint (*Mentha piperita* L.) following screening of over 100,000 stolon's (Murray, 1969).

Definition and types of Mutation

Mutations are changes to the DNA sequence and can be classified in several ways based on their effects, origin, or molecular nature. Here are some common classifications of mutations.

1. By Effect on Protein Function:

- **Missense Mutation:** A single nucleotide changes results in a codon that codes for a different amino acid, potentially altering protein function.
- **Nonsense Mutation:** A single nucleotide change creates a stop codon, leading to premature termination of protein synthesis.
- **Silent Mutation:** Changes in the DNA sequence do not affect the amino acid sequence of the protein.

2. By Origin:

- **Spontaneous Mutations:** Occur naturally without external influence, often as a result of errors during DNA replication.
- **Induced Mutations:** Result from external factors, such as radiation, chemicals, or viruses.

3. By Molecular Nature:

- **Point Mutation:** A change in a single nucleotide pair in the DNA sequence.
- **Insertions:** Addition of one or more nucleotide pairs into the DNA sequence.
- **Deletions:** Removal of one or more nucleotide pairs from the DNA sequence.
- **Duplication:** The DNA segment is copied and inserted into the genome.
- **Frameshift Mutations:** Insertions or deletions that alter the reading frame of the gene.

4. By Consequence on Organism:

- **Beneficial Mutation:** Confers an advantage to the organism.
- **Neutral Mutation:** Has no significant effect on the organism's fitness.
- **Deleterious Mutation:** Harms the organism or reduces its fitness.

5. By Location:

- **Germline Mutation:** They occur in reproductive cells and can be passed on to offspring.
- **Somatic Mutation:** Occurs in non-germinal cells and cannot be inherited.
- Each variant has different effects on genetic diversity, disease, and evolution.

History of Mutation Breeding:

Freisleben and Lein (1944) coined the term crossbreeding to refer to the introduction and development of mutant lines for plant improvement. The term also has a broader meaning, including the use of natural and spontaneous mutants, as well as their evolution a known mutation from whatever source.

In the late 1920 researchers discovered that they could greatly increase the number of these variations or mutations by exposing plants to X-rays and chemicals. After the

Second World War, through the widespread use of nuclear technology, the "development of changes" accelerated. Expose plants to gamma rays, protons, neutrons, alpha and beta particles to see if they induce beneficial changes. Chemicals, too, such as sodium azide and ethyl methane sulphonate, were used to cause mutations. Mutation breeding efforts continue around the world today. Of the 2,252 officially issued variants, 1,019, or nearly half, have been issued in the past 15 years. Crops that come from cross breeding include wheat, barley, rice, potatoes, soybeans, onions, and others.

The history of plant mutation can be traced back to 300 BC, with reports of mutant plants in China. (Oladosu, Y. *et al* (2015). Hugo de Vries (a Dutch botanist), at the turn of nineteenth century to twentieth century, gave the term "mutations" to "sudden heritable changes" or "discontinuous variations" (called as "sports" by Darwin), According to de Vries, mutations play a significant role in evolution. However, the rates of spontaneous mutations are so low that researchers many times ignore the naturally occurring mutations as a prominent cause of change in the variability of populations. But the discovery of artificial, radiation induced mutagenesis by Muller (1927) in Drosophila through X-rays and in 1928, Lewis Stadler published the first result of mutation induction in crop plants, such as barley, maize, wheat, and oat. However, he is skeptical about the use of persuasive modifications to increase productivity.

While experimenting on the 'rediscovery' of Mendel's laws of inheritance. (Oladosu *et al* 2015). In this way, a whole new process entered traditional agriculture. Before 1927, plant mutations occurred naturally, not artificially. It wasn't until the time of Müller and Stadler that scientists began using radiation to induce change. After 1944, it was discovered that chemicals and radiation could be used to produce artificial changes in plants and seeds.

In 1946, Auerbach and Robson discovered that mustard gas has clear mutagenic effects. Chemical mutagens are highly capable of inducing true gene mutations, but still, the question remained whether the chemical mutagens are capable of inducing mutations in the same frequency as physical mutagens. Auerbach and Robson (1946) answered this question experimentally and found that chemical mutagens were as effective as physical mutagens. (Udage, Ashan, 2021). Mutation induction using chemicals was tried by many scientists for decades in the past century. The First convincing result of mutagenesis using chemicals was recorded in 1939 when Thom and Steinberger induced mutations in Aspergillus using nitrous acid. (Udage, Ashan 2021).

Definition of Mutation Breeding

Mutation is sudden heritable change in the genetic material not caused by recombination or segregation. The use of induced mutations with the aim of improving plants is called breeding. (Pandit, Rishav *et al* 2021). Mutation breeding is also known as variation breeding (Saima Mir, *et al* 2021). In other words, genetic breeding is the process of exposing seeds to chemicals or radiation to produce mutants with traits needed for breeding with different species.

- Mutation Breeding treating a biological material with a mutagen in order to induce mutations is known as mutagenesis.
- Exposure of a biological material to a radiation like Xray's, gamma- rays, etc. is known as irradiation.
- When mutations are induced and aimed at plant improvement, the total introduction and isolation of mutants is called breeding.
- Commonly used in self-pollinated & asexually propagated species while, rarely used in cross pollinated species
- Radiological breeding programs must be well planned and of sufficient size and facilities to effectively screen large populations.

Types of Mutation Breeding:

- **Chemical mutation breeding:** This method involves the use of chemical mutagens, such as EMS (ethyl methane sulfonate), NaN3 (sodium aside), and MMS (methyl methane sulfonate), to induce genetic mutations in plants.
- **Physical mutation breeding:** This method involves the use of physical agents, such as gamma rays, X-rays, and ultraviolet light, to induce genetic mutations in plants.
- **Biological mutation breeding:** This method involves the use of biological agents, such as viruses and bacteria, to induce genetic mutations in plants.

The technique of mutation breeding should be used only under specific circumstances like:

If the crop to be improved is a vegetatively propagated one, mutation breeding can be preferably (used to remove some specific defects. In these crops, unspecified and genetically unstable mutants can be maintained indefinitely.

- In fruit trees and several other fruit crops which have lengthy life cycle, the use of conventional breeding methods is not easy. In these crops, mutation breeding can be used to improve some specific characters while retaining all the desirable characteristics of the old variety (like colour, flavour and taste of fruits).

- Mutation breeding has a good scope when a desired gene is tightly linked with undesirable (genes).
- When the breeder is willing to develop chimeras in an ornamental plant species, induced mutagenesis can be of great help in this regard. The development of chimeras in ornamentals is perhaps the most significant contribution made by mutation breeding.
- When the breeder desires to change some biochemical pathway in any crop species, mutation breeding can make significant contribution.
- When there is negative association between disease resistance and some quality trait, one can resort to mutation breeding for example, level of disease resistance in sugarcane is regatively associated with juice percentage. Similarly, in some varieties of wheat, desirable resistance is associated with dark red grain colour.

Advantages of Mutation Breeding:

- Mutation breeding is more effective for the improvement of oligogenic characters like disease resistance than polygenic characters.
- Mutation breeding is the simple, quick and best way when a new trait is to be induced in vegetatively propagated crops.
- Induced mutagenesis is utilized for the induction of cytoplasmic male sterility. Ethidium bromide (EB) has been utilized for induction of cytoplasmic male sterility in pearl millet and barley**.**
- Mutation breeding is an affordable and swift technique for creating new types, unlike backcross, pedigree, and bulk breeding approaches.

Limitations of Mutation Breeding:

- Most of the mutations are deleterious and undesirable.
- Mutation breeding has limited scope for the genetic improvement of oligogenic or polygenic characters.
- Identification of micro-mutations, which are more useful to a plant breeder is generally very difficult.
- Because useful mutations are produced at a very low frequency (0.1%), a very large plant population has to be screened to identify and isolate desirable mutants. Therefore, efficient, rapid and inexpensive screening methods are needed to screen large populations.
- Most of the mutations are recessive.
- Desirable mutation are commonly associated with undesirable side effects due to other mutations, chromosomal aberrations etc.

Use of Mutation in Plant Breeding

Mutagenesis, the act of manipulating the genome of an organism, has been used in plant breeding since Muller discovered the mutagenic effects of X rays in fruit flies (Muller, 1927). The first plant species to be genetically modified was barley by LJ Stadler, who pioneered the use of X-rays to induce specific Muller mutations. (Stadler, 1928), although he published his first paper on the subject the following year. These early mutation experiments were designed mainly to discern genetic truths of inheritance and chromosomal theory. Recall that at this point Watson and Crick's double-helix DNA model had still not been arrived at.

Dose, Rate, Species/Genotypes, Conditions of application, etc. Much of the early research using ionizing radiation and chemical mutagens was aimed at determining the effective dose and exposure of various chemicals to achieve a high percentage of mutations without causing lethality. The researchers noticed that the rates and doses varied tremendously for species, genotype, ploidy level, and the conditions in which treatment were conducted.

When determining the most efficient dose for one's crop species, it is important to first consult the literature for any information on the mutagenic agent to be used and the crop it will be used on. Chances are good that someone has already used the particular mutagenic agent on your crop. If not, it is important to consider several factors.

- The first of course is safety issues regarding the mutagen. A researcher needs to know what sort of certifications, licenses, and precautions must be taken while carrying out the experiment and handling the plant material after the experiment is complete.
- Next is the mutagen's type and mechanism. Understanding the effect of mutagens on test tissue not only improves safety, but also helps to make better decisions about conducting tests. For instance, if using X-rays, distance from the source of radiation reduces dose. It would be unwise to place a bucket of seed in front of an X-ray source and assume all the seeds in the bucket received equivalent doses of radiation. Similarly, EMS mutagenic substances require very rigorous testing procedures to produce mutations.
- Tissue specific reactions. Seeds react differently than stem cuttings, meristem tissues or callous tissue to the same level of radiation.
- Species and ploidy level affect mutational response to the mutagen a great deal. Some types of plants can withstand different types of stress than others. They can improve DNA damage. The ploidy level of the target species also influences the mutational response, as discussed in the next section.

Mutation Breeding in Self Fertilizing Species

Breeding mutant traits into plants capable of self-fertilization is relatively easy. Because many mutations occur, after the mutagenesis treatment, the material must be fertilized and developed to at least M2 before the characterization test. At this time, the plant isolates for the recessive mutant form. Identification of good mutants should be kept for future selection. Because mutagens act randomly upon the genome, it is important to collect as many positive mutants as possible. This gives the creators a wide variety of characters to choose from based on their work in addition to the mutant characters.

Mutation Breeding in Cross Fertilizing Species

Cross-fertilizing species raise some difficulties. Because species which are predominantly cross-fertilizing typically exhibit significant inbreeding depression, the necessary self-fertilizations to identify mutants in the population result in reduced plant vigor due to the genetic background and not necessarily the mutations. This compounds the difficulty of successfully identifying mutations. This increases the difficulty of successfully identifying changes. Significant changes can be identified, but this is rare. Different types of plants and auto sterile machines are very difficult to use different breeding methods without an established hybrid program. However, the amount required to achieve this may be impractical or non-existent.

Mutation Bbreeding in Vegetatively Propagated Species

When attempting to effect mutation in vegetatively propagated species such as sugarcane or banana, it is important to note the chimeric nature of mutagenic treatment. Not all cells affected by mutagens undergo mutations, but cells that undergo mutations produce cells that undergo mutations. For this reason, it is important to treat parts of the plant that will give rise to either seed or vegetative propagules. For many plants breeding, it is difficult to identify and grow plants in a large number of plants in order to identify the mutant, however, when one of the mutants is found, the mutation is stable. in closed seed. Plant species are in vitro techniques that can be used to transform plant material that can regenerate seeds in large numbers. This system is suitable for all types of seed propagation and propagation.

Mutation Breeding in Seed Propagated Species

Seeds treated with mutagens produce chimeric plants. Chimeric plants produce both mutant and non-mutant seeds. This can be problematic; however, seeds must be replanted to find the desired mutant. As long as an effective screening method is used, there are no major obstacles. Seed mutagenesis is the most popular technique in genetic breeding programs.

The Future of Mutation Breeding

Latest developments in genomics technology have caused a widespread adoption of genomic methods in general breeding and specifically in mutational breeding. Technologies such as high throughput sequencing has allowed for the relatively cheap and fast genome sequencing of plants. Methods such as TILLING (Targeting Induced Local Lesions in Genomes), Zinc finger nuclease mediated mutagenesis, and the use of mega nucleases, has allowed us to produce targeted mutations in crop plants to delineate gene function as well as improve cultivars. These new and more specific methods are very promising.

TILLING relies on high throughput sequencing to assemble an array of mutants for a particular target sequence. Plant materials are mutagenized, the DNA is extracted and the target sequence PCR amplified and sequenced to identify mutants and locate the polymorphisms (Mc Callum *et al.,* 2000). Although the mutations are induced randomly across the plant genome, they are detected only in the gene of interest. This allows the researcher to keep only those plants with mutations in the desired region. A similar process, Eco TILLING, screens for the spontaneous mutations present due to natural variation within a population.

Zinc finger nucleases (ZFN) and Mega nucleases (MN) present a more targeted approach to induced mutation. ZFNs can be tailored to bind to specific recognition sites associated with the desired sequence. Once dimerized, the target DNA is cleaved, and a donor sequence introduced (Bibikova *et al.,* 2003). The donor sequence typically exhibits desired mutations or it can be used to introduce new transgenes altogether into the target genome. Mega nucleases have a similarly specific mode of action, and a great deal of research is going into both of these promising techniques for targeted mutagenesis as well as plant transformation.

References:

1. Saima Mir, A., Maria, M., Muhammad, S., & Mahboob Ali, S. (2021). Potential of Mutation Breeding to Sustain Food Security. IntechOpen. doi: 10.5772/intechopen.94087.
2. Udage, Ashan. (2021). Introduction to Plant Mutation Breeding: Different Approaches and Mutagenic Agents. Journal of Agricultural Sciences – Sri Lanka. 16. 466. 10.4038/jas.v16i03.9472.
3. Plant Mutation Breeding and Biotechnology Edited by Q.Y. Shu, B.P. Forster, H. Nakagawa Plant Breeding and Genetics Section Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture International Atomic Energy Agency, Vienna, Austria.

4. Pandit, Rishav & Bhusal, Bishnu & Regmi, Rashmi & Neupane, Pritika & Bhattarai, Kushal & Maharjan, Binju & Acharya, Suprava & K.C., Bigyan & Poudel, Mukti. (2021). Mutation Breeding for Crop Improvement: A Review. Reviews in Food and Agriculture. 02. 31-35. 10.26480/rfna.01.2021.31.35.
5. (Oladosu, Y., Rafii, M. Y., Abdullah, N., Hussin, G., Ramli, A., Rahim, H. A., … Usman, M. (2015). Principle and application of plant mutagenesis in crop improvement: a review. Biotechnology & Biotechnological Equipment, 30(1), 1–16. https://doi.org/10.1080/13102818.2015.1087333).
6. Neal C. Stoskopf with Dwight T. Thomes and B.R. Plant breeding theory and practice, Christie, ISBN: 81-7233-202-6 By Scientific Publishers (India).
7. Sultan Singh I.S. Pawar, Genetic Basis and Methods of PLANT BREEDING, ISBN: 8123913001, New Delhi: CBS Publishers, 2019).
8. B.D. Singh, Plant breeding, ISBN 81-7036-184-X , Kalyani Publishers, New Delhi.
9. A.M. van Harten, Mutation breeding theory and practical application, ISBN-0531470749 Cambridge University Press 1998).
10. Sanjay Kumar Singh 2005 Plant Breeding , ISBN 81-8030-083-8,)
11. https://plantlet.org/mutation-breeding-definition-history-types-of-mutation/
12. https://www.isaaa.org/kc/inforesources/publications/biotechinagriculture/Conventional_Plant_Breeding.htm
13. https://plantlet.org/mutation-breeding-definition-history-types-of-mutation/ .
14. https://books.google.co.in/books?hl=en&lr=&id=rXuj5R0pW_QC&oi=fnd&pg=PR13&ots=9bk4Ux6yvh&sig=ocPIdPQCVOSNZSkkbv1oScb5ESA&redir_esc=y#v=onepage&q&f=false

ISOLATION, BIOCHEMICAL CHARACTERISATION, POT ASSAY AND QUALITATIVE MEASUREMENT OF PHOSPHATE SOLUBILIZATION ISOLATED FROM AGRICULTURAL SOIL

Rahul More*[1], Diksha Landge[1], Mahesh Karale[1], Shreyas Mahurkar[2], Govind Sanap[3], Kailash Sontakke[4], Yuvaraj Sarnikar[2] and Santosh Chobe[5]

[1]Department of Microbiology, Dayanand Science College, Latur- 413512 (India)

[2] Department of Chemistry, Dayanand Science College, Latur-413512 (India)

[3] Department of Zoology, Khare Dhere Bhosale College, Guhagar-415703(India)

[4]Department of Botany, Gopikabai Sitaram Gawande Mahavidyalaya Umarkhed-445206

[5]Department of Chemistry, M.G. V's Loknete Vyankatrao Hiray, Arts, Science and Commerce College, Panchavati, Nashik-422001 (India)

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

Abstract:

The Biological Approaches used instead of chemicals to improve agricultural production has area of interest. For this purpose, The Phosphorus which is a second most important macronutrient required by plants for its growth and development. It required by plants in soluble form. For this chemical fertilizer used which badly affect on soil fertility. Here The Role of Phosphate Solubilising Bacteria (PSB) play an important role. It converts Insoluble phosphate to soluble form which is assimilated by plants. PSB fulfill the Phosphorus requirement of plants. It is used as Phosphate Biofertilizer. This pivotal role played by PSB .For Isolation of Phosphate Solubilising Bacteria Pikovskaya's Medium was used. Isolation of PSB from the Rhizopheric soil sample of different Crops that were Sugarcane, Gram, Tomato, Spinach and Onion carried and six PSBs Isolates were Isolated and their biochemical characterization were performed, And Then Analysis of Phosphate Solubilization was carried out by means of Qualitative Measurement of Phosphate Solubilization and Pot Experiment were performed.

Keywords: Phosphate Solubilization, phosphorus, Phosphate Solubilizing Bacteria (PSB).

Introduction:

Phosphorus is second most essential macronutrient required for plant for its growth and development phosphorus a major macronutrient which is responsible for limiting the growth of plant unlike nitrogen, phosphorus is not abundant in atmosphere. [1] Phosphorus plays an important role in physiological activities of plants that is cell division, photosynthesis etc. phosphorous deficiency leads to stunted growth, small

leaves, slow developments of plants. All phosphorous present in soil is in insoluble form but plant can absorb phosphate only in soluble form. Here the role of microbes present in soil is important .the transformation of insoluble phosphate into soluble form of phosphate which is easily assimilated by plants is carried out by number of microorganisms 1n the soil. This soluble form of phosphate is made available by phosphate solubilizing bacteria (PSB). PSB have shown to enhance the Solubalization of insoluble Phosphorous compound to soluble phosphorous form. this conversion is carried out by PSB by release of organic acids and phosphates enzyme [2] therefore 1t is very pivotal to use the accumulated phosphate in soil for improving the plant growth and productivity. Hence the interest to isolating phosphate solubilizing bacteria PSB.

Phosphate solubilizing bacteria those belonging to the different genera are Pseudomonas, Arthrobacter, Azotobacter, Bacillus, Enterobacter, Flavobacterium these are the are most common one and remaining some others have the ability to bring insoluble phosphate in the soil into soluble form by releasing different organic acid such as formic acid, acetic acid, propionic acid, lactic acid, glycolic, fumaric and succinic acids. Higher number of these microorganisms is always found in plant rhizosphere sometimes they are called Rhizobacteria.

The isolation of efficient PSBs is pivotal because they provide Phosphorus to plants in soluble form which is only form assimilated by plant. For increase the strength of cereal straw, fruit production, promotes flower formation, stimulates the developments of roots and one of the important is for seed formation and adequate phosphorus fertilization may improves plants growth and development also fruits, vegetables quality and raises the resistance to diseases these PSB also called plant growth promoting Rhizobacteria. The most important use of PSB is they used as Biofertilizer. Here involvement of microorganism's insoluble form of phosphorus / phosphate compound includes tricalcium phosphate, dicalcium phosphate, hydroxyapatite and rock phosphate.

Materials and Methods:

Collection of Soil Sample- Soil samples were collected From the Rhizosphere of Five different Crop that are Tomato, gram, Onion, Spinach, And Sugarcane. This Soil sample were collected From Latur District. Soil sample taken from depth of 15 cm. The Soil Sample was collected in disposable, autoclavable bags and transported this sample in Dayanand Science College. Research Laboratory. Then this sample stored at 4 c before use

Isolation of PSB- Bacterial Strain were isolated using lo-fold serial dilutions and spread plate method. 1 gm of soil sample was dispersed in 9ml of autoclaved distilled water aseptically and shaken thoroughly. 1 ml of the above solution was again transfected to 9

ml of sterile distilled similarly 10, 10, 10, 10. Serial were made for each soil sample. 0.1ml was spread on Pikovskaya's agar medium (PVK) containing insoluble Tricalcium Phosphate and incubated at 20-30^{0}c for 2-3days, Colonies showing zones were picked and purified on fresh PVK agar plate. Then appearance of zones was used for confirmation of the presence of PSB, Single Colonies with Clear zone around them were picked up and streaked on new plates of same medium to obtain pure cultures.

Characterization-Morphological Characterization/ Colony Morphology- The PSB Colony was streaked on plate count agar then incubated at 28 ^{0}c for 2 days. This is done to study the Morphological characters of PSB Colonies.

Gram Staining-The standard Procedure were followed for Gram Staining of Isolates.

Biochemical Characterization: - biochemical analysis has done by Indole test, MR-VP Test, Citrate test, Carbohydrate Fermentation test as well as Starch Hydrolysis.

Analysis of Phosphate Solubilizing Activity-

Qualitative Measurement of Phosphate Solubilization - Bacterial isolates were screened for their ability to solubilizing tri-calcium phosphate (TCP) on Pikovaskaya's agar plate this done by plate screening Method. For qualitative measurements isolates were spot inoculated on the center of pikovaskaya's agar (PVK) plate [3, 4]. Plate was then incubated at 28 — 30^{0}c for 3 days. A Clear Zone around a Growing Colony indicated phosphate solubilization and measured as phosphate solubilization index calculated by formula,

Formula- Solubilizing index - Colony Diameter * Clear zone

Pot Experiment- The pot Experiment was carried out to study the Efficiency of Phosphate solubilizing Bacteria PSB and to study the growth promoting effect of PSBs on Fenugreek. The Isolates were inoculated in pikovskaya's broth medium .and incubated up to 7 days. Then these inoculums were used as phosphate biofertilizer. The pot filled with soil and pot irrigated with tap water. Then the seeds of Fenugreek had sown in pot and control also prepared with same number of Seeds and Fenugreek seeds were allowed to grow in two sets one with biofertilizer and another without biofertilizer. Pots were allowed to grow in sunlight about 3-4 week and after sufficient growth measured number of germinated seeds in both sets. By using PSB the lab scale biofertilizer is prepared to check the efficacy of PSBs [5, 6].

Result and Discussion:

Bacterial Isolates were identified as phosphate solubilizing bacteria on the basis of their ability to solubilize tri-calcium phosphate with forming the clear zone on pikovskaya's agar. The 6 Isolates that are Ps-1, Ps-2, Ps-3, Ps-4, Ps—5, Ps-6 were Isolated from rhizosphere soil samples of crops that was Sugarcane, Gram, Tomato,

Spinach and Onion. The gram staining and biochemical characterization were performed and colony morphology noted for all isolates.

The analysis of phosphate solubilization performed by qualitative measurement of phosphate solubilization by spot inoculation. On the basis of Solubilization Index the PS-2 Isolate showed highest phosphate solubilization activity. PS-2 among others is most efficient PSB.

The pot experiments were performed in which the PSBs inoculums used as phosphate biofertilizer and showed growth of fenugreek and number of fenugreek seed germinated twice of fenugreek without PSB s inoculums.

Isolation of PSB on Pikovskaya's Agar Plate-

Figure 1: Isolation of phosphate solubilizing bacteria on pikovskayas agar plate

Figure 2: Preserved isolates of PSB

Cultural Characteristics-

Table 1: Showing cultural characteristics of bacterial isolates

Colony Character	PS -1	PS-2	PS-3	PS-4	PS -5	PS-6
Size	2 mm	4 mm	3 mm	4mm	3 mm	3 mm
Shape	Irregular	Circular	Circular	Circular	Circular	Irregular
Margin	Undulate	Entire	Entire	Entire	Undulate	Undulatte
Elevation	Flat	Raised	Raised	Raised	Flat	Flat
Consistency	Non-sticky	Sticky	Sticky	Sticky	Sticky	Sticky
Opacity	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque
Color	Cream	Yellowish white	Cream	Cream	Cream	Fuzzy white
Surface	Smooth	Smooth	Smooth	Smooth	Rough	Rough
Gram's nature	Gram Positive	Gram Negative	Gram Negative	Gram Positive	Gram Positive	Gram Positive

Biochemical Characterization-

Table 2. Showing biochemical characteristics of bacterial isolates.

Test	PS-1	Ps-2	PS -3	PS-4	PS-5	PS-6
Indole	-	-	-	-	-	-
Methyl red test	-	-	-	-	-	-
Vogue proskruate	-	+	-	-	-	-
Citrate	+	+	+	-	-	-
Starch Hydrolysis	-	-	-	+	-	+
Catalase	-	-	+	+	-	-
Oxidase	-	-	-	+	+	-
Sugar test						
Glucose	A+ G-	A+ G+	A+ G-	A- G-	A+ G-	A- G-
Sucrose	A+ G-	A+ G+	A+ G-	A- G-	A+ G-	A- G-
Lactose	A- G-	A+ G+	A+ G-	A- G-	A+ G-	A- G-

Qualitative Measurement of Phosphate Solubilization:

The phosphate solubilization index is calculated by formula and the PS 2nd Isolate show maximum solubilization index.

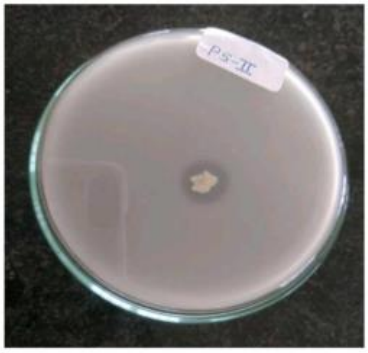


Figure 3: PS 2nd Isolate show maximum solubilization index

Pot Experiment-

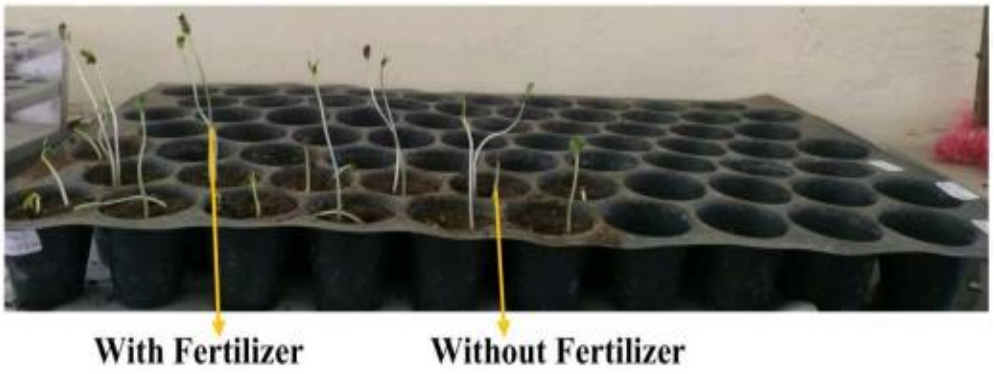


Figure 4: Showing the difference in results with and without biofertilizer

Table 3: Rate of germination of seeds with and without biofertilizer

	Soil sample without phosphate biofertilizer	Soil sample with phosphate biofertilizer
Number of seeds germinated in pot	8	16
Percentage of germination of seeds	40	80

Discussion:

The phosphates Solubilising Bacteria PSBs have wide scope in agriculture. Now because of chemicals fertilizer fertility of soil decreases .so the alternative that is Biofertilizer have great importance because it is natural contain formulation of Microorganisms. Mainly The Phosphorus which is Macronutrient required by plants for growth and development it's deficiencies leads to diseases in plants. The Isolation of Efficient Strain of Phosphate Solubilizing Bacteria becomes a key for sustainable agriculture.

Conclusion:

The findings of this investigation showed that PSB from Rhizopheric soil samples could be easily isolated and exploited for providing phosphate Requirements of plants for its growth and development. From the results it is concluded that The PS -2 is most efficient Phosphate Solubilizing Bacteria. And the laboratory scale Biofertilizer of these isolates were used and its role in plant growth and development studied successful by performing pot Experiment.

References:

1. Hafiza M. S., G. Z. Jahangir, I. A. Nasir, M. I. and M. Iqbal (2013), Biotechnology and Biotechnology Equipment. LLC 2013
2. S. Sharma, V. Kumar and R. B. Tripathi (2011) J. Microbiol . Biotech.Res. 1(2):90-95
3. S. Aarab', F. J. Ollero, M. M. A. Laglaoui', (2013) Moroccan Journal of Biology N 10
4. V. W. and S. Shukla, (2017) Environment conservation Journal 18(3) 75-78
5. T. Karpagam and P. K. Nagalakshmi, (2014) International Journal f Cur Microbio and Applied Sci 3(3): 601-614.
6. M. Satyaprakash, T. Nikitha, E.U.B. Reddi', B. Sadhana and S. Satya Vani, International Jrnl of Cur Microbio and Applied Sci 29(5) 654.

STATISTICAL METHODS IN PLANT SCIENCE RESEARCH

Yogesh Rajendra Yewale
Department of Statistics,
Ahmednagar College, Ahmednagar
Corresponding author E-mail: yryewale@gmail.com

Abstract:

Statistical methods are useful at all stages of plant research. It is applied in designing the experiments, determining the sample size, conducting data analysis, and determining the conclusion. The first section of the chapter discusses how various experimental designs like completely randomized design (CRD), randomized block design (RBD), Latin square design (LSD), factorial designs (FD), and split-plot designs(SPD) can be used to design the experiments. The next section of the chapter discusses the analysis of the data obtained through the statistically designed experiments. This includes descriptive statistics, hypothesis testing (t-test, Chi-Square test), analysis of variance (ANOVA), post-hoc analysis, regression analysis (RA), multivariate analysis methods like principal component analysis (PCA), cluster analysis (CA), and discriminant analysis (DA), etc. It then discusses the use of advanced statistical tools like mixed effect model, random effect model, meta-analysis, Bayesian analysis, etc. The next section discusses the open-source statistical software namely R software to be used for the analysis of the data. The last section discusses the case study with the application of the statistical methods, along with packages and code in R-software. This chapter can be helpful to students doing research in the areas of plant science, agriculture science, ecology, etc.

Keywords: Analysis Of Variance, Post-Hoc Analysis, Principal Component Analysis, Discriminant Analysis, R-Software, etc.

Introduction:

Statistical methods play an important role in the analysis of experimental data collected for research purposes. However, the application of statistical methods is not restricted to just analyzing the data, they are useful in the pre-analysis part of the experiment like determination of the sample size, designing the experiment, analyzing the data generated out of it, and drawing the conclusion. In Plant Research, the use of statistical methods is very wide, some of the examples include the study of the impact of different treatments on crop yield, identification of the regions of the genome associated with specific traits such as disease resistance, prediction of the genetic value of different

plant lines based on observed data and pedigree information, to model the relationship between environmental variables (such as temperature, soil type, or precipitation) and the distribution of plant species, and many more.

The various stages in the Plant research include the design of experiments which is useful for planning the experiments, followed by the analysis of the data collected after the experimentation using various statistical tools such as test procedures, analysis of variance (ANOVA), regression analysis, multivariate analysis, and many more, then concluding based on the Statistical analysis. The experiments in Plant research can be designed using the completely randomized design("Assessing the Tolerance of Sagittaria Lancifolia (Spear Plant) to Linear Alkylbenzene Sulfonates (LAS) Exposure," 2023; Chamma *et al.,* 2023; Hairuddin *et al.,* 2023), randomized block design(Meiriani *et al.,* 2023; Teshale Beyene, 2024; Zulia & Lana Reni Gustianty, 2023), Latin square design(Akhsan Akib *et al.,* 2022), factorial design(Mohammed *et al.,* 2022). The statistical analysis can be performed using simple linear regression(Panday *et al.,* 2020), multiple linear regression(Julian Santosa *et al.,* 2024), logistic regression(Arief Subchan & Andayani, 2021; Bg & E, 2022), principal component analysis(Albert *et al.,* 2023; R Sasikala *et al.,* 2020; Sivabharathi *et al.,* 2023), cluster analysis(Bibi *et al.,* 2022; Edwards & Cavalli-Sforza, 1965; Sivabharathi *et al.,* 2023), machine learning methods(Rohit Kumar Awasthi & Srikant Singh, 2023; Xie *et al.,* 2023), etc.

This paper attempts to provide an overview of how the various statistical methods are applied at different stages of the experiments along with the necessary assumptions and one example. The first section discusses how an experiment can be designed with the use of various statistical designs, the second section discusses the various Statistical methods for analyzing the data, and the last section, a case study with its analysis performed in R software version 4.1.1 provided.

This chapter may help the researcher to select the appropriate design, for designing the experiment and statistical tool for analyzing the data.

Experimental Designs

Completely Randomized Design (CRD):

This design is applied when the experimental units are homogeneous. The principles of the experiment design used in this design are randomization and replication. In this design, the treatments are randomly assigned to the experimental units.

Example Situation: To compare the different varieties of plants by studying the growth of plants (response variable) in a Greenhouse experiment.

Randomized Block Design (RBD):

This design is used in a situation where experimental units are heterogeneous and can be divided into homogeneous groups called blocks. This design uses all the principles of the design of experiments viz. randomization, replication, and local control. Treatments are assigned randomly to each element in the block.

Example Situation: To study the effect of temperature variation (blocks) and varieties of plants (Treatments) on the growth of plants (response variable) in a Greenhouse experiment.

Latin Square Design (LSD):

This design is an extension of the randomized block design which uses two blocking factors rather than one. The principles of the design of experiments it uses are randomization, replication, and local control. This design is used when

- It is required to control two sources of variation generally known as rows and columns in addition to the treatments being tested.
- The experimental units are arranged in a squared layout with the condition of an equal number of treatments, rows, and columns.
- The factors rows and columns are independent.

The treatments are applied in such a way that each treatment appears only once in a row or in a column

Example Situation: Consider a situation of studying the impact of light and temperature conditions on the growth of plants in Greenhouse experiments. The Latin Square Design can be used in this situation with sources of variation intensity of light as rows, temperature as columns, and treatments as different varieties of plants with the growth variable as a response variable or study variable.

Factorial Design (FD):

In this design individual effects of factors (main effect) and their combined effect (interaction effect) are also tested.

If the study aims to investigate the effect of 'm' levels of factor A and 'n' levels of factor B on the study variable then it is called $m \times n$ factorial experiment.

Example: A study of the effect of different levels of irrigation (low, medium, and high) and fertilizers (low, medium, and high) on the yield of a crop. In this experiment, three levels of factor A and 3 levels of factor B are considered, thus it is called a 3×3 factorial experiment.

Split Plot Design (SPD)

This design is used when applying one factor is more difficult or costly across all experimental units and applying another factor using randomization is relatively easier. The difficult factor is applied to the large experimental units (main plot) whereas the easier factor is applied to smaller experimental units (subplots) within each whole plot.

Example: A study investigating the effect of irrigation methods (drip, sprinkler) and fertilizer types (organic, chemical) on crop yield. In this experiment, the irrigation method is an expensive factor and thus is applied to the main plot whereas the fertilizer type is cheaper and hence applied within the main plots (subplots)

Statistical Methods of Data Analysis

Descriptive Statistics

Descriptive statistics is computed to get an idea about the distribution of the data. This includes measures of central tendency, measures of dispersion, coefficient of skewness, and coefficient of kurtosis.

- The common measures of central tendency are mean, median, and mode. The common measure of dispersion is variance, standard deviation, etc.
- The common measure of skewness is Karl Pearson's coefficient of skewness S_k, Bowley's coefficient of Skewness S_B, and Pearsonian coefficient of skewnessγ_1. A positive value of these coefficients indicates that data is positively skewed meaning that smaller values appear more frequently than large values, whereas the negative value of the coefficient indicates that data is negatively skewed meaning that larger values occur more frequently than small values.
- The common measure of kurtosis is the Pearsonian coefficient of kurtosis (γ_2). A positive value indicates that data is leptokurtic meaning that the frequency curve will be sharper than the normal curve while a negative value indicates that data is platykurtic meaning that the frequency curve is flatter than the normal curve.

Hypothesis Testing

Hypothesis testing is a statistical method used to draw inferences about the population based on the sample data. In Plant research, it is used to answer questions like, whether the differences in plant characteristics are due to chance. The first step in the testing of the hypothesis process is to set up the null (H_0) and alternative (H_1) hypotheses, followed by the computation of test statistics, comparison of the value of the test statistics under the null hypothesis with the table value at a predetermined level of significance, then in the last step concluding whether the null hypothesis is rejected or not. The hypothesis testing procedure can be parametric or non-parametric.

The parametric tests make some assumptions about the population from which the sample is taken while the non-parametric tests do not make such assumptions

t-test

This is a parametric test used to test whether the mean of the two populations is equal against the hypothesis that they are not. The t-test is helpful to the researchers to evaluate whether observed differences between groups are likely due to the treatment or condition applied, or if they could have occurred by chance. There are two types of t-tests, the first is a t-test for independent samples while the second one is a paired t-test for dependent samples.

As it is a parametric test it needs to satisfy the following assumptions.

i) The observations are taken from a normal distribution and they are small in size.

ii) The variance of the two groups is equal. If they are not then Welch's t-test is used.

Example Situation:

In assessing the impact of two fertilizers (say fertilizer A and fertilizer B) on the height of the plant t-test can be applied. Fertilizer A will be applied to the plants in group one, while fertilizer B will be applied to the plants in group 2. In this case, the null hypothesis would be that there is no significant difference between the heights of the plants of two groups while the alternative hypothesis would be that there is a significant difference between them. If the null hypothesis (H_0) is rejected then it can be concluded that there is a significant between the heights of the plants in the two groups. However, if the null hypothesis (H_0) is not rejected then it can be concluded that there is no significant difference in the heights of the plants of the two groups.

Chi-square test

The chi-square test is non-parametric. There are two types of chi-square tests used in Plant research. The first one is the chi-square test of independence which is used to check whether the two categorical variables are associated or not, the second one is the chi-square test of goodness of fit which is used to test whether the observed and theoretical values agree or not.

Example Situation:

- To study the association between the soil type (Sandy, Loamy) and the incidence of a particular disease (Diseased, Healthy), a chi-square test of independence may be used.
- To test whether the observed segregation ratio of the trait in offspring fits the expected Mendelian ratio (e.g., 3:1 for dominant/recessive traits).

Analysis of Variance (ANOVA)

Analysis of Variance (ANOVA) is a statistical tool used to compare the means of several populations (more than three). It is considered as an extension of the t-test for more than two populations. When the researcher wants to test the effectiveness of treatments on the growth, and yield of plants, ANOVA can be used. In Plant research, there are several types of ANOVA used.

- One-way ANOVA: To compare the means of three or more groups based on one independent variable (e.g., different fertilizer types). It is applied in experiments conducted in completely randomized design(CRD)/
- Two-way ANOVA: To compare the mean of the three or more groups simultaneously based on two independent variables and assess interactions between them (e.g., different fertilizer types and irrigation levels). It is applied in experiments conducted in randomized block design (RBD)
- Factorial ANOVA: To study the significance of factors at different levels individually (main) as well as simultaneously (interaction).

Some assumptions are to be satisfied before applying ANOVA. These are

- Observations are independent. This is achieved by the random allocation of treatments to the experimental units
- Normality of residuals. This is checked by drawing normal probability plot, quantile, quantile plot(Q-Q) plot
- Equality of Variance: Variance of the residuals should be the same across all groups. This can be checked by Levene's or Bartlett's test.
- Additivity: The total effect of multiple factors is the sum of individual effects. This is tested by adding interaction terms in the model if they are found to be insignificant then the assumption of additivity of different factors is said to be true.

The following are the important steps in performing ANOVA.

i) Set up Null hypothesis (There is no significant difference between the population mean of all groups) and alternative hypothesis(There is a significant difference between the population mean of all groups)
ii) Check the assumptions of the model
iii) Compute the value of the test statistic under the null hypothesis
iv) Compute the p-value

v) Conclude. If the p-value is less than the level of significance then H_0 is rejected (treatments differ significantly) else H_0 is not rejected (treatments do not differ significantly).

vi) Post-hoc Analysis: If H_0 is rejected (treatments differ significantly) then which pairs of treatments differ significantly can be identified by Post-hoc analysis. Tuckey's test is generally used to perform this analysis.

Example situation: ANOVA can be applied to all the examples discussed above in the experimental design section.

Regression Analysis

Simple Linear Regression

The simple linear regression method studies the linear relationship between two variables. One variable is the independent variable (regressor variable) while the other is called a dependent variable (study variable or response variable). The regression model requires the assumption about the error term to be satisfied. This method is to be used when the dependent variable is linearly related to the independent variable. Thus the first step in modeling the data by simple linear regression is to plot the scatter diagram. Then the next step is the estimation of the parameters involved in the model, which is followed by testing the significance of parameters. Lastly, the model adequacy check is performed. If estimated parameters are found to be statistically significant and all the assumptions about the model are satisfied then this model is used for the prediction.

Example Situation: Consider a situation where water usage is predicted based on soil moisture content. In this example, the independent variable is soil moisture content while the dependent variable is water usage.

Multiple Linear Regression (MLR)

It is used to model the relationship between one dependent variable and more than one independent variable. This model requires certain assumptions to be satisfied which include, error terms to be independent, linearity between independent variable and dependent variable, equality of variance of residuals (homoscedasticity), and normality of residuals.

Example Situation: To study the effect of factors like soil nitrogen content, soil moisture level, and average temperature on crop yield. In this case, crop yield is a dependent variable whereas soil nitrogen content, soil moisture level, and average temperature are the independent variables.

Logistic Regression

It is used when the dependent variable is binary. It models the probability which can be used to classify the observations into two categories.

Example situations:

- To study the effect of Humidity, temperature, and soil pH on the incidence of disease. In this example, disease incidence (1 for diseased, 0 for healthy) is the dependent variable whereas humidity, temperature, and soil pH are the independent variables.
- To study the effect of different treatments like soil moisture, and temperature on the survival of seedlings (1 for survived, 0 for not survived). In this example, soil moisture, and temperature are independent variables whereas survival of seedlings (1 for survived, 0 for not survived) is a dependent variable.

Multivariate Analysis

Principal component analysis

Principal Component Analysis (PCA) is used to reduce the dimensionality of large datasets by transforming them into a new set of variables called principal components. The PCA simplifies the dataset while retaining as much of the original variance as possible.

Example Situation: To analyze genetic markers from different plant varieties to understand their genetic relationships and classify them into distinct groups.

PCA will reduce the dimensionality of the genetic data and visualize the genetic relationships between varieties in a two- or three-dimensional space. The principal components will reveal clusters of varieties with similar genetic profiles, helping to identify genetically distinct groups or populations.

Cluster Analysis

This technique is used to group similar objects or observations into clusters based on specific characteristics. This technique is particularly useful when dealing with large datasets where the goal is to explore patterns, classify plants, or identify relationships among variables.

Example situation: To study the morphological traits of several plant varieties to classify them into distinct groups. Variables such as leaf size, flower color, plant height, and seed shape may be used to group the plant varieties. The cluster analysis will provide the groups of plant varieties with similar morphological characteristics.

Case Study

Objective

To study the effect of Fertilizer Treatments on Plant Growth

Data

Dependent Variable: Plant Height (cm)

Independent Variables: Fertilizer Treatment, Soil pH, Watering Frequency.

A dummy data is generated in R software.

R Code

```
set.seed(123)
data <- data.frame(
  Plant_Height = rnorm(40, mean = 50, sd = 10),
  Fertilizer_Treatment = factor(rep(c("Treatment1", "Treatment2", "Treatment3", "Control"), each = 10)),
  Soil_pH = rnorm(40, mean = 6.5, sd = 0.5),
  Watering_Frequency = rnorm(40, mean = 3, sd = 1)
)
```

Output:

```
> head(data)
  Plant_Height Fertilizer_Treatment  Soil_pH Watering_Frequency
1     44.39524           Treatment1 6.152647           3.00576
2     47.69823           Treatment1 6.396041           3.38528
3     65.58708           Treatment1 5.867302           2.62934
4     50.70508           Treatment1 7.584478           3.64437
5     51.29288           Treatment1 7.103981           2.77951
6     67.15065           Treatment1 5.938446           3.33178
```

Descriptive Statistics

Calculate the basic statistics to get an idea about the distribution of the data.

```
# Descriptive Statistics
summary(data)
```

```
> summary(data)
  Plant_Height    Fertilizer_Treatment    Soil_pH      Watering_Frequency
 Min.   :30.33   Control   :10        Min.   :5.345   Min.   :1.332
 1st Qu.:44.43   Treatment1:10        1st Qu.:6.226   1st Qu.:2.393
 Median :50.91   Treatment2:10        Median :6.468   Median :2.950
 Mean   :50.45   Treatment3:10        Mean   :6.497   Mean   :3.008
 3rd Qu.:56.92                        3rd Qu.:6.741   3rd Qu.:3.527
 Max.   :67.87                        Max.   :7.584   Max.   :5.187
```

Analysis of Variance (ANOVA)

Test if there are significant differences in plant height between different fertilizer treatments.

R Code:

```
# ANOVA
anova_model <- aov(Plant_Height ~ Fertilizer_Treatment, data = data)
summary(anova_model)
```

Output

```
> anova_model <- aov(Plant_Height ~ Fertilizer_Treatment, data = data)
> summary(anova_model)
                    Df Sum Sq Mean Sq F value Pr(>F)
Fertilizer_Treatment  3  324.9  108.30   1.383  0.264
Residuals            36 2818.6   78.29
```

Multiple Linear Regression

Build a regression model to understand how plant height is influenced by fertilizer treatment, soil pH, and watering frequency.

R code

```
regression_model <- lm(Plant_Height ~ Fertilizer_Treatment + Soil_pH + Watering_Frequency, data = data)
summary(regression_model)
```

Output:

```
> # Multiple Linear Regression
> regression_model <- lm(Plant_Height ~ Fertilizer_Treatment + Soil_pH + Wateri
> summary(regression_model)

Call:
lm(formula = Plant_Height ~ Fertilizer_Treatment + Soil_pH +
    Watering_Frequency, data = data)

Residuals:
    Min      1Q  Median      3Q     Max
-21.163  -6.211  -1.082   4.785  17.244

Coefficients:
                              Estimate Std. Error t value Pr(>|t|)
(Intercept)                    45.9650    22.0152   2.088   0.0444 *
Fertilizer_TreatmentTreatment1 -2.2383     4.3042  -0.520   0.6064
Fertilizer_TreatmentTreatment2 -0.9718     4.4759  -0.217   0.8294
Fertilizer_TreatmentTreatment3 -7.8828     4.1132  -1.916   0.0637 .
Soil_pH                         1.4800     3.1783   0.466   0.6444
Watering_Frequency             -0.7830     1.9614  -0.399   0.6923
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 9.041 on 34 degrees of freedom
Multiple R-squared:  0.1158,    Adjusted R-squared:  -0.01422
F-statistic: 0.8907 on 5 and 34 DF,  p-value: 0.4982
```

Principal Component Analysis (PCA)

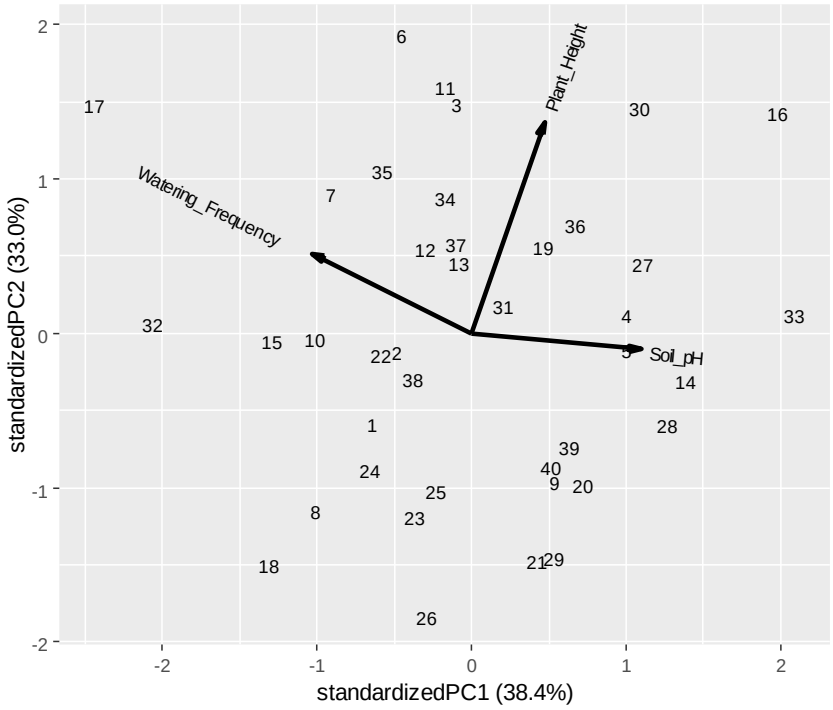
Perform PCA to reduce the dimensionality of the dataset and visualize key components.

R code

```
install.packages("ggbiplot")
library(ggbiplot)
pca_data <- data[, c("Plant_Height", "Soil_pH", "Watering_Frequency")]
pca_result <- prcomp(pca_data, center = TRUE, scale. = TRUE)
summary(pca_result)
ggbiplot(pca_result, labels = rownames(data), ellipse = TRUE, circle = TRUE)
```

Output:

```
> library(ggbiplot)
Loading required package: ggplot2
> pca_data <- data[, c("Plant_Height", "Soil_pH", "Watering_Frequency")]
> pca_result <- prcomp(pca_data, center = TRUE, scale. = TRUE)
> summary(pca_result)
Importance of components:
                          PC1    PC2    PC3
Standard deviation     1.0731 0.9953 0.9261
Proportion of Variance 0.3839 0.3302 0.2859
Cumulative Proportion  0.3839 0.7141 1.0000
> ggbiplot(pca_result, labels = rownames(data), ellipse = TRUE, circle = TRUE)
>
```



Cluster Analysis

Apply K-means clustering to group plants based on their growth characteristics.

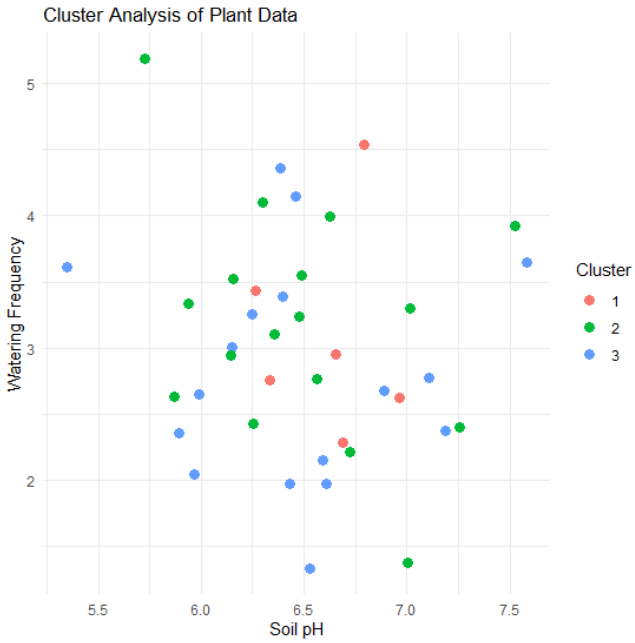
```
# K-means Clustering
set.seed(456)
kmeans_result <- kmeans(pca_data, centers = 3, nstart = 20)

# Add cluster information to the original data
data$Cluster <- as.factor(kmeans_result$cluster)

# Visualize Clusters
library(ggplot2)

ggplot(data, aes(x = Soil_pH, y = Watering_Frequency, color = Cluster)) +
  geom_point(size = 3) +
  labs(title = "Cluster Analysis of Plant Data",
       x = "Soil pH",
       y = "Watering Frequency") +
  theme_minimal()
```

Output



Conclusion:

Statistical methods play an important role in Plant science research. This chapter attempts to provide an overview of the Statistical part required in every research study. A case study provides an idea about how these methods can be applied to the experimental data. The code used for the analysis is also provided to give an overview of how R software can be used for the analysis. Also, the packages to be used for the analysis of these methods are listed. With the application of these Statistical methods, a researcher can effectively make an impact on the quality of his research work

References:

1. Akhsan Akib, Muh., S, S., & Prayudyaningsih, R. (2022). Plant growth analysis and arbuscular mycorrhizal colonization in Amaranthus tricolor L. *International Journal of Current Research in Biosciences and Plant Biology*, *9*(7), 1–7. https://doi.org/10.20546/ijcrbp.2022.907.001
2. Albert, E. T. A., Bille, N. H., & Leonard, N. M. E. (2023). Application of Principal Component Analysis to advancing digital phenotyping of plant disease in the context of limited memory for training data storage. *Journal of Scientific Agriculture*, 34–43. https://doi.org/10.25081/jsa.2023.v7.8327
3. Arief Subchan, M., & Andayani, N. N. (2021). Classification of maize genotype using logistic regression. *IOP Conference Series: Earth and Environmental Science*, *911*(1), 012017. https://doi.org/10.1088/1755-1315/911/1/012017

4. Assessing the Tolerance of Sagittaria Lancifolia (Spear Plant) to Linear Alkylbenzene Sulfonates (LAS) Exposure. (2023). *Indonesian Journal of Biology Education*, *6*(1). https://doi.org/10.31002/ijobe.v6i1.583
5. Bg, A., & E, A. (2022). Liquid Waste Management Practices and the Role of Communal Treatment Plant in the Eastern Industrial Park of Dukem town, Ethiopia. *International Journal of Water and Wastewater Treatment*, *8*(2). https://doi.org/10.16966/2381-5299.184
6. Bibi, A., Iqbal, Z., Shah, G. M., & Nawaz, S. (2022). CLASSIFICATION OF VEGETATION OF LOWER TANAWAL, PAKISTAN BY CLUSTER ANALYSIS ON THE BASIS OF ALTITUDE. *International Journal of Research -GRANTHAALAYAH*, *10*(1), 193–208. https://doi.org/10.29121/granthaalayah.v10.i1.2022.4454
7. Chamma, L., Silva, G. F. D., Perissato, S. M., Alievi, C., Chaves, P. P. N., Giandoni, V. C. R., Calonego, J. C., & Silva, E. A. A. D. (2023). Does Forced Plant Maturation by Applying Herbicide with Desiccant Action Influence Seed Longevity in Soybean? *Plants*, *12*(15), 2769. https://doi.org/10.3390/plants12152769
8. Edwards, A. W. F., & Cavalli-Sforza, L. L. (1965). A Method for Cluster Analysis. *Biometrics*, *21*(2), 362–375. JSTOR. https://doi.org/10.2307/2528096
9. Hairuddin, R., Idris, M. Y., & Nur, K. (2023). Organogenesis of corn plants (Zea mays L.) at various concentrations of auxin and cytokinin plant growth regulators in vitro. *Asian Journal of Agriculture and Rural Development*, *13*(1), 91–97. https://doi.org/10.55493/5005.v13i1.4770
10. Julian Santosa, F., Padmaningrum, D., Widiyanto, Purwanto, D., & Roro Ilma Kusuma Wardani, R. (2024). The economic impact of agroforestry practice in production forest areas, Central Java province, Indonesia. *Scientific Horizons*, *27*(4), 141–153. https://doi.org/10.48077/scihor4.2024.141
11. Meiriani, Lahay, R. R., & Sutra, M. R. (2023). Enhanced production of pruned okra plant (Abelmoschus esculentus L. Moench) by applying IAA and Urea fertilizer. *IOP Conference Series: Earth and Environmental Science*, *1241*(1), 012021. https://doi.org/10.1088/1755-1315/1241/1/012021
12. Mohammed, S. S. A., Abu Amr, S. S., & Abujazar, M. S. (2022). Factorial Design and Optimization of Date Stone as A Natural Coagulant for Organic and Heavy Metals Removal from Industrial Wastewater. *Global NEST Journal*. https://doi.org/10.30955/gnj.004486
13. Panday, U. S., Shrestha, N., Maharjan, S., Pratihast, A. K., Shahnawaz, Shrestha, K. L., & Aryal, J. (2020). Correlating the Plant Height of Wheat with Above-Ground

Biomass and Crop Yield Using Drone Imagery and Crop Surface Model, A Case Study from Nepal. *Drones*, *4*(3), 28. https://doi.org/10.3390/drones4030028

14. R Sasikala, P L Viswanathan, & S Manonmani. (2020). Principal component analysis for yield attributing traits of sunflower (Helianthus annuus L.) genotypes: Principal component analysis for yield attributing traits of sunflower (Helianthus annuus L.) genotypes. *Journal of Oilseeds Research*, *37*(Specialissue). https://doi.org/10.56739/jor.v37iSpecialissue.139687
15. Rohit Kumar Awasthi & Srikant Singh. (2023). An Overview of Machine Learning Methods for the Detection of Diseases in Rice Plants in Agricultural Research. *International Journal of Scientific Research in Science and Technology*, 837–846. https://doi.org/10.32628/IJSRST523103150
16. Sivabharathi, R. C., Muthuswamy, A., Anandhi, K., & Karthiba, L. (2023). Genetic Diversity Studies of Soybean [Glycine max (L.) Merrill] Germplasm Accessions using Cluster and Principal Component Analysis. *LEGUME RESEARCH - AN INTERNATIONAL JOURNAL*, *Of*. https://doi.org/10.18805/LR-5071
17. Teshale Beyene. (2024). Effect of Plant Spacing and Harvesting Stage on Morphological Characteristics and Yield of Desho Grass (Pennisetum Glaucifolium L) at Adola sub-site of Bore Agricultural Research Center, Oromia Region, Ethiopia. *International Journal of Scientific Multidisciplinary Research*, *2*(7), 687–708. https://doi.org/10.55927/ijsmr.v2i7.9719
18. Xie, J., Chen, Y., Yu, Z., Wang, J., Liang, G., Gao, P., Sun, D., Wang, W., Shu, Z., Yin, D., & Li, J. (2023). Estimating stomatal conductance of citrus under water stress based on multispectral imagery and machine learning methods. *Frontiers in Plant Science*, *14*, 1054587. https://doi.org/10.3389/fpls.2023.1054587
19. Zulia, C. & Lana Reni Gustianty. (2023). Response Of Natural Zpt Generation Of Green Bean Sprouting Extract And Plant Media On The Growth Of Lemon Orange (Citrus Limon L.) Plant Cuttings. *Journal of Scientific Research, Education, and Technology (JSRET)*, *2*(1), 369–377. https://doi.org/10.58526/jsret.v2i1.37

HERBAL APPROACHES IN THE MANAGEMENT OF POLYCYSTIC OVARY SYNDROME: EFFICACY, MECHANISMS, AND CLINICAL APPLICATIONS

Cyril Sajan

Department of Pharmacy,

Sumandeep Vidyapeeth Deemed to be University, Piparia, Vadodara, Gujarat, India

Corresponding author E-mail: cyrilsajan97@gmail.com

Abstract:

Polycystic Ovary Syndrome (PCOS) is a common hormonal disorder among women of reproductive age, marked by symptoms like irregular menstrual cycles, excess androgen levels, and the presence of multiple ovarian cysts. Standard treatments usually include hormonal therapies and lifestyle changes; however, a growing number of women are turning to alternative and complementary methods, including herbal remedies, to manage their symptoms. This review examines various herbs used to manage PCOS, including spearmint, cinnamon, saw palmetto, maca root, holy basil, licorice root, *Tribulus terrestris*, vitex, and flaxseed. It discusses the potential mechanisms, effectiveness, and safety of these herbs, emphasizing their roles in regulating hormones, improving insulin sensitivity, and alleviating symptoms. The review highlights the need to combine herbal treatments with conventional therapies under medical guidance to achieve the best outcomes for women with PCOS.

Keywords: Polycystic Ovary Syndrome (PCOS), Herbal treatment, Alternative medicine, Hormone regulation, Insulin sensitivity, Spearmint, Cinnamon, Saw palmetto

Introduction:

Polycystic Ovary Syndrome (PCOS) is a multifaceted endocrine disorder affecting a significant number of women of reproductive age. It presents a range of symptoms, including irregular menstrual cycles, elevated androgen levels, polycystic ovaries, insulin resistance, and obesity. Conventional medical treatments typically involve hormonal contraceptives, anti-androgens, and insulin-sensitizing drugs aimed at reducing symptoms and long-term health risks. Due to the side effects and limitations of these treatments, many women are turning to alternative and complementary therapies. Herbal treatments have gained popularity for their potential to naturally regulate hormones, enhance insulin sensitivity, and address various PCOS symptoms. This chapter delves into the use of several key herbs in managing PCOS, discussing their mechanisms, effectiveness, and safety profiles.[1]

Spearmint (*Mentha spicata*)

Spearmint tea has garnered interest as a possible herbal remedy for managing symptoms of Polycystic Ovary Syndrome (PCOS). Recognized for its anti-androgenic properties, spearmint tea may help reduce symptoms like hirsutism and acne, which are prevalent in women with PCOS. This section offers an in-depth look at spearmint tea, covering its mechanisms of action, benefits, recommended usage and dosage, and safety considerations.

Mechanism and Benefits

Mechanism: Spearmint contains compounds that demonstrate anti-androgenic effects by lowering free testosterone levels in the body. This reduction in testosterone can help manage symptoms such as hirsutism (excess hair growth) and acne, which are frequently caused by elevated androgen levels in women with PCOS.

Benefits:

Reduction in Hirsutism: Regular consumption of spearmint tea can significantly decrease androgen levels, leading to reduced unwanted hair growth.

Improved Skin Health: Lower androgen levels can also help clear up acne, resulting in healthier skin.

Hormonal Balance: Spearmint tea may aid in balancing overall hormone levels, contributing to more regular menstrual cycles.

Usage and Dosage

Usage: Spearmint tea is simple to add to your daily routine. While commonly consumed hot, it can also be enjoyed cold. To prepare spearmint tea:

1. Boil water and pour it over a teaspoon of dried spearmint leaves or a spearmint tea bag.
2. Allow it to steep for 5-10 minutes.
3. Strain the loose leaves if used, and then enjoy your tea.

Dosage:

Standard Dosage: It is generally advised to drink 1 to 2 cups of spearmint tea daily.

Duration**:** Regular consumption over several weeks is typically required to see noticeable effects on hormone levels and symptoms.

Safety and Precautions

Safety: Spearmint tea is generally regarded as safe for most people when consumed in moderate amounts.

Precautions:

- **Gastrointestinal Discomfort:** Excessive intake of spearmint tea may lead to gastrointestinal issues, such as nausea and stomach upset.

- **Acid Reflux:** Those prone to acid reflux might find that spearmint worsens their symptoms.
- **Pregnancy and Breastfeeding:** Pregnant or breastfeeding women should consult a healthcare provider before adding spearmint tea to their diet.

General Advice: As with any herbal remedy, it's important to begin with a lower dose to gauge your tolerance and gradually increase it if needed. Consulting a healthcare provider before adding cinnamon to your routine is recommended, particularly if you are taking other medications or have existing health conditions.

Conclusion: Spearmint tea offers a natural and convenient approach for managing specific PCOS symptoms, particularly those associated with high androgen levels. By reducing free testosterone, it can help alleviate hirsutism and acne, potentially improving the quality of life for many women with PCOS. While generally safe, it's essential to use spearmint tea in moderation and seek advice from a healthcare professional to ensure optimal outcomes.[2]

Cinnamon (*Cinnamomum verum*)

Cinnamon, sourced from the inner bark of *Cinnamomum verum* trees, has been esteemed for its medicinal benefits for a long time. Recent studies have highlighted its potential benefits in managing Polycystic Ovary Syndrome (PCOS), particularly due to its ability to enhance insulin sensitivity and lower blood glucose levels. This section examines the mechanisms, advantages, recommended usage, dosage, and safety considerations of using cinnamon for PCOS management.[3]

Mechanism and Benefits

Mechanism: Cinnamon contains bioactive compounds like cinnamaldehyde, which have been demonstrated to improve insulin sensitivity and glucose metabolism. These effects are especially advantageous for women with PCOS, as many of them face insulin resistance, a condition that can worsen other PCOS symptoms.

Benefits:

Improved Insulin Sensitivity: Cinnamon enhances the body's response to insulin, leading to lower blood sugar levels and better metabolic health.

Hormonal Balance: Stabilizing blood sugar levels with cinnamon can indirectly aid in hormonal balance, potentially helping to regulate menstrual cycles.

Anti-inflammatory Properties: Cinnamon's anti-inflammatory effects may help reduce chronic inflammation commonly associated with PCOS.

Antioxidant Effects: Rich in antioxidants, cinnamon helps combat oxidative stress and supports overall health.

Usage and Dosage:

Usage: Cinnamon can be included in the diet in several ways:

- **Ground Cinnamon:** Can be added to foods and beverages like oatmeal, smoothies, yogurt, and tea.
- **Cinnamon Supplements:** Available in capsule or tablet form for those who prefer a more precise dosage.

Dosage:

Ground Cinnamon: A typical dosage for enhancing insulin sensitivity is 1 to 2 grams (approximately 1/2 to 1 teaspoon) per day.

CINNAMON SUPPLEMENTS: The usual dosage ranges from 500 mg to 1000 mg per day, following the directions on the supplement label.

Safety and Precautions:

Safety: Cinnamon is generally safe for most people when consumed in appropriate amounts.

Precautions:

Coumarin Content: Ceylon cinnamon (*Cinnamomum verum*) is preferred over Cassia cinnamon (Cinnamomum cassia) due to its lower coumarin levels, a compound that can cause liver damage in large amounts.

Allergic Reactions: Some people may experience allergic reactions to cinnamon, such as skin irritation or gastrointestinal discomfort.

Blood Sugar Levels: Cinnamon can reduce blood sugar levels, so individuals with diabetes or hypoglycemia should closely monitor their blood sugar when using cinnamon.

Pregnancy and Breastfeeding: Pregnant or breastfeeding women should consult a healthcare provider before using cinnamon supplements or consuming large quantities of cinnamon.

General Advice: As with any herbal remedy, it is important to start with a lower dose to assess tolerance and gradually increase it as needed. Consulting a healthcare provider before incorporating cinnamon into your routine is advisable, especially if you are taking other medications or have underlying health conditions.

Conclusion: Cinnamon (*Cinnamomum verum*) shows promising benefits for managing PCOS, mainly due to its effects on enhancing insulin sensitivity and lowering blood glucose levels. Its anti-inflammatory and antioxidant properties also contribute to its role in a comprehensive approach to PCOS management. Although generally safe, it's important to use cinnamon in moderation and opt for Ceylon cinnamon to avoid potential issues related to coumarin. Incorporating cinnamon into a balanced diet, with

guidance from a healthcare professional, can improve the management of PCOS symptoms and overall quality of life.

Saw Palmetto (*Serenoa repens*)

Introduction

Saw palmetto, extracted from the fruit of the *Serenoa repens* tree, is well-regarded for its anti-androgenic effects. Traditionally used to address hormonal imbalances in men, its capacity to lower androgen levels makes it a potentially useful herb for managing symptoms of Polycystic Ovary Syndrome (PCOS) in women, such as hirsutism and acne. This section offers a comprehensive examination of the mechanisms, benefits, recommended usage, dosage, and safety considerations associated with using saw palmetto for PCOS management.[4]

Mechanism and Benefits

Mechanism: Saw palmetto primarily works by inhibiting the enzyme 5-alpha-reductase, which converts testosterone into dihydrotestosterone (DHT), a more potent androgen. By decreasing DHT levels, saw palmetto can help lower overall androgen levels in the body, addressing symptoms of hyperandrogenism commonly associated with PCOS.

Benefits:

- **Reduction in Hirsutism:** Lowering androgen levels can significantly decrease excess hair growth on the face and body, a frequent issue for women with PCOS.
- **Improved Skin Health:** Reduced androgen levels can help alleviate acne and enhance overall skin condition.
- **Hormonal Balance:** Saw palmetto's anti-androgenic properties contribute to better hormonal balance, potentially assisting in the regulation of menstrual cycles.

Usage and Dosage

Usage: Saw palmetto is commonly taken as a supplement, available in the form of capsules, tablets, or liquid extracts. It's important to select a high-quality product with standardized extracts to ensure consistent dosing.

Dosage:

Standardized Extract: A typical dosage is 160 mg of standardized extract, taken twice daily. This amount is commonly used in studies and recommended for managing hyperandrogenism symptoms.

Whole Berry Powder: For whole berry powder, the usual dosage is 1 to 2 grams per day.

Safety and Precautions

Safety:

Saw palmetto is generally well-tolerated by most individuals. However, it is important

to monitor for any adverse reactions and consult a healthcare provider if any concerns arise.

Precautions:

- **Gastrointestinal Symptoms**: Some individuals may experience mild gastrointestinal symptoms, such as stomach upset, nausea, or diarrhea. Taking saw palmetto with food can help minimize these effects.
- **Hormonal Interactions**: Since saw palmetto affects hormone levels, it should be used cautiously in conjunction with other hormonal treatments or medications. Women taking oral contraceptives or hormone replacement therapy should consult a healthcare provider before using saw palmetto.
- **Pregnancy and Breastfeeding**: Due to its hormonal effects, saw palmetto is not recommended for use during pregnancy or breastfeeding.

General Advice: Always start with the lowest effective dose and gradually increase as needed, while monitoring for any adverse effects. Consulting a healthcare provider before incorporating saw palmetto into your regimen is advisable, especially if you are taking other medications or have underlying health conditions.

Conclusion: Saw palmetto (*Serenoa repens*) offers promising benefits for women with PCOS, particularly in managing symptoms related to elevated androgen levels, such as hirsutism and acne. By inhibiting the conversion of testosterone to DHT, saw palmetto can help reduce androgen levels and promote hormonal balance. While generally safe, it is important to use saw palmetto under the guidance of a healthcare professional to ensure the best outcomes and avoid potential interactions with other medications. Integrating saw palmetto into a comprehensive treatment plan can significantly improve the quality of life for women managing PCOS.[5]

Licorice Root (*Glycyrrhiza glabra*)

Introduction: Licorice root, derived from the *Glycyrrhiza glabra* plant, has been used in traditional medicine for its anti-inflammatory, anti-viral, and hormone-regulating properties. Its potential benefits for managing Polycystic Ovary Syndrome (PCOS) stem from its ability to influence hormone levels and reduce inflammation. This section explores the mechanisms, benefits, usage, dosage, and safety considerations of licorice root for PCOS management.[6]

Mechanism and Benefits

Mechanism: Licorice root contains glycyrrhizin, a compound that can affect hormone metabolism by inhibiting the enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1). This enzyme is involved in converting inactive cortisone to active cortisol, influencing adrenal and sex hormone levels. By modulating cortisol levels and impacting

androgen metabolism, licorice root can help address symptoms of PCOS related to elevated androgen levels.

Benefits:

- **Reduction in Testosterone Levels**: Licorice root can help lower elevated testosterone levels, thereby reducing symptoms such as hirsutism (excessive hair growth) and acne.
- **Hormonal Balance**: By modulating cortisol levels and influencing hormone metabolism, licorice root can contribute to better overall hormonal balance.
- **Anti-inflammatory Properties**: Licorice root's anti-inflammatory effects can help reduce chronic inflammation, which is often associated with PCOS.
- **Improved Insulin Sensitivity**: Some studies suggest that licorice root may improve insulin sensitivity, which is beneficial for managing PCOS-related insulin resistance.

Usage and Dosage

Usage: Licorice root is available in several forms, including tea, capsules, and tinctures. For PCOS management, the most common forms are capsules or dried root used to make tea.

Dosage:

- **Tea**: Typically, 1 to 2 grams of dried licorice root can be used to prepare tea. Drink 1 to 2 cups of tea daily.
- **Capsules**: The standard dosage is 200 to 400 mg of licorice root extract, taken one to three times daily.

Safety and Precautions

Safety: Licorice root is generally safe when used in moderate amounts for short periods. However, prolonged use or high doses can lead to adverse effects.

Precautions:

- **Hypertension**: Licorice root can cause sodium and water retention, potentially leading to elevated blood pressure. Individuals with hypertension or cardiovascular conditions should use licorice root with caution and monitor blood pressure regularly.
- **Electrolyte Imbalance**: Long-term use of licorice root can lead to hypokalemia (low potassium levels) and other electrolyte imbalances. It is essential to monitor these levels and consult a healthcare provider if symptoms of electrolyte imbalance occur.
- **Pregnancy and Breastfeeding**: Licorice root is not recommended during pregnancy or breastfeeding due to potential hormonal effects and risks of premature labor or developmental issues.

- **Drug Interactions**: Licorice root may interact with medications, including corticosteroids and blood pressure medications. It is crucial to consult a healthcare provider before starting licorice root if you are on other medications.

General Advice: To minimize risks, use licorice root in recommended dosages and for limited durations. Always consult a healthcare provider before starting licorice root, particularly if you have underlying health conditions or are taking other medications.

Conclusion: Licorice root (*Glycyrrhiza glabra*) offers several potential benefits for managing PCOS, particularly through its effects on testosterone levels, hormonal balance, and inflammation. Its ability to modulate hormone metabolism and improve insulin sensitivity makes it a valuable adjunct in a comprehensive PCOS management plan. However, due to potential side effects and interactions, it is crucial to use licorice root under the guidance of a healthcare professional. With proper use, licorice root can contribute to improved symptom management and overall well-being for women with PCOS.[7]

Tribulus terrestris

Tribulus terrestris, also known as puncture vine, is a herb traditionally used for its potential benefits in boosting libido, fertility, and athletic performance. Its possible advantages for managing Polycystic Ovary Syndrome (PCOS) are linked to its effects on modulating hormone levels and supporting reproductive health. This section explores the mechanisms, benefits, recommended usage, dosage, and safety considerations of using *Tribulus terrestris* for PCOS.[8]

Mechanism and Benefits

Mechanism: *Tribulus terrestris* contains active compounds, such as saponins, which are believed to affect hormone levels by stimulating the production of luteinizing hormone (LH) and potentially influencing testosterone and estrogen levels. This can help promote ovulation and enhance fertility, addressing common concerns for women with PCOS.

Benefits:

- **Stimulates Ovulation:** Tribulus may aid in stimulating ovulation by influencing hormone levels, which can be beneficial for women with PCOS who experience irregular or absent ovulation.
- **Improves Fertility:** By supporting hormonal balance and reproductive health, Tribulus has the potential to enhance fertility in women with PCOS.
- **Enhances Libido:** Traditionally used to boost libido, Tribulus may help women experiencing sexual dysfunction related to hormonal imbalances.
- **Supports Hormonal Balance:** By affecting hormone production and regulation, Tribulus can contribute to achieving better overall hormonal balance.

Usage and Dosage

Usage: *Tribulus terrestris* is typically consumed as a supplement, available in forms such as capsules, tablets, and tinctures. Using standardized extracts is important to ensure consistent dosing and effectiveness.

Dosage:

- **Standardized Extract:** The usual dosage is 500 to 1000 mg per day, often divided into two or three smaller doses throughout the day.

  Powder: For the powdered form, the typical dosage is 1 to 2 grams per day

Safety and Precautions

Safety: *Tribulus terrestris* is generally considered safe for most individuals when used as directed. Nevertheless, it's important to adhere to dosage recommendations and seek personalized advice from a healthcare provider.

Precautions:

- **Hormonal Effects:** Tribulus can influence hormone levels, so it should be used cautiously alongside other hormonal treatments or medications. Consult a healthcare provider if you are using oral contraceptives or hormone replacement therapy.
- **Digestive Issues:** Some people may experience gastrointestinal discomfort, such as nausea, bloating, or diarrhea. Taking Tribulus with food can help reduce these effects.
- **Pregnancy and Breastfeeding:** The safety of Tribulus during pregnancy and breastfeeding is not well established. It is advisable to avoid its use during these times unless specifically recommended by a healthcare provider.
- **Medication Interactions:** Tribulus may interact with certain medications, including those affecting hormone levels or blood sugar. Consult a healthcare provider before starting Tribulus if you are taking other medications or have underlying health conditions.

General Advice: Start with the lowest effective dose of Tribulus and observe how your body responds. Gradually increase the dosage, if necessary, while being attentive to any adverse effects. Always consult a healthcare provider before starting any new supplement, especially if you have existing health conditions or are on other medications.

Conclusion: *Tribulus terrestris* may offer benefits for managing PCOS, particularly through its impact on ovulation, fertility, and hormonal balance. By stimulating hormone production and supporting reproductive health, Tribulus can be a valuable addition to a comprehensive PCOS management plan. However, due to its effects on hormones and potential interactions, it is important to use Tribulus under the

supervision of a healthcare professional. With proper use, *TRIBULUS TERRESTRIS* can help improve symptom management and overall reproductive health for women with PCOS.

Vitex (*Vitex Agnus-Castus*)

Introduction: Vitex, or chasteberry, is obtained from the fruit of the *Vitex Agnus-Castus* plant. Historically used in herbal medicine to support menstrual health and fertility, Vitex is gaining attention for its potential benefits in managing Polycystic Ovary Syndrome (PCOS). This section offers a detailed overview of Vitex, covering its mechanisms, advantages, recommended usage, dosage, and safety considerations.[9]

Mechanism and Benefits

Mechanism: *Vitex Agnus-Castus* is believed to primarily affect the pituitary gland, potentially helping to regulate luteinizing hormone (LH) production and reduce prolactin levels. This can contribute to normalizing the menstrual cycle and supporting overall hormonal balance. Additionally, Vitex may indirectly help lower elevated androgen levels, which are commonly associated with PCOS.

Benefits:

- **Menstrual Regularity:** Vitex aids in regulating menstrual cycles by normalizing hormone levels, which can be particularly helpful for women with irregular periods due to PCOS.
- **Reduction in Androgen Levels:** By balancing hormones, Vitex may help alleviate symptoms associated with high androgen levels, such as hirsutism and acne.
- **Improved Fertility:** Stabilizing menstrual cycles and supporting hormonal balance can enhance fertility, which may be beneficial for women with PCOS who are trying to conceive.
- **Reduction in Premenstrual Symptoms:** Vitex is known to relieve symptoms of

Usage and Dosage

Usage: Vitex is most commonly consumed as a supplement, available in capsules, tablets, or tinctures. Standardized extracts are preferred for their consistent dosing and effectiveness.

Dosage:

- **Standardized Extract:** The usual dosage is 400 to 1000 mg per day, typically taken in the morning.
- **Tincture:** If using a tincture, the recommended dosage is 20 to 40 drops (1-2 ml), taken once or twice daily.

Safety and Precautions

Safety: Vitex is generally well-tolerated when used as directed. However, it's important to monitor for any potential adverse effects and consult a healthcare provider if any concerns arise.

Precautions:

- **Hormonal Effects:** Vitex can impact hormone levels, so it should be used with caution alongside other hormonal treatments or medications. Women using oral contraceptives or hormone replacement therapy should consult a healthcare provider before starting Vitex.
- **Gastrointestinal Symptoms:** Some people may experience mild gastrointestinal issues, such as nausea or stomach upset. Taking Vitex with food can help minimize these effects.
- **Pregnancy and Breastfeeding:** The safety of Vitex during pregnancy and breastfeeding is not well established. It is advisable to avoid using it during these times unless specifically recommended by a healthcare provider.
- **Interactions with Medications:** Vitex may interact with certain medications, including those affecting hormonal balance and dopamine levels. Consult a healthcare provider before beginning Vitex if you are taking other medications.

General Advice: Vitex is usually employed as a long-term solution for managing PCOS symptoms, with noticeable improvements often taking several weeks to months. Begin with the recommended dosage and observe how your body responds. It's advisable to consult a healthcare provider before starting Vitex, especially if you have existing health conditions or are on other medications.

Conclusion: Vitex (*Vitex Agnus-Castus*) can offer several benefits for managing PCOS, such as improving menstrual regularity, alleviating symptoms of elevated androgens, and enhancing fertility. By supporting hormonal balance and regulating the menstrual cycle, Vitex can be a useful part of a comprehensive approach to PCOS management. However, due to its impact on hormone levels and potential interactions with other treatments, it's important to use Vitex under the guidance of a healthcare professional. When used appropriately, Vitex can aid in better symptom management and overall reproductive health for women with PCOS.[10]

Conclusion: Managing Polycystic Ovary Syndrome (PCOS) often requires a comprehensive approach due to its complex symptoms and varied nature. Herbal treatments can be a valuable component of this strategy, providing potential benefits for symptom relief and hormonal balance.

Herbs like spearmint, cinnamon, saw palmetto, licorice root, *Tribulus terrestris*, and vitex offer distinct mechanisms that can address different facets of PCOS:

- **Spearmint Tea**: Reduces elevated androgen levels, helping alleviate symptoms such as hirsutism and acne.
- **Cinnamon**: Enhances insulin sensitivity, which is beneficial for managing insulin resistance and metabolic issues related to PCOS.
- **Saw Palmetto**: Inhibits the enzyme that converts testosterone into a more potent androgen, thereby reducing symptoms associated with high androgen levels.
- **Licorice Root**: Modulates hormone levels and reduces inflammation, potentially improving symptoms related to elevated testosterone and insulin resistance.
- ***Tribulus terrestris***: Supports reproductive health and hormonal balance, potentially improving ovulation and fertility.
- **Vitex**: Regulates menstrual cycles and lowers elevated androgen levels, contributing to better overall hormonal balance and fertility.

Integrating these herbs into a PCOS management plan can complement conventional treatments and lifestyle changes, such as diet and exercise, to improve overall well-being. It is crucial to use these herbal remedies with appropriate guidance, taking into account individual health conditions and potential interactions with other treatments.

A holistic approach that combines herbal treatments, medical care, and lifestyle adjustments offers the best chance for effectively managing PCOS and enhancing the quality of life. Regular consultation with healthcare providers ensures that treatments are safe, effective, and customized to individual needs.

References:

1. Jamilian M, Foroozanfard F, Bahmani F, Talaee R, Monavari M, Jafari Z, et al. Effects of the combination of ginger and cinnamon on insulin resistance, glucose metabolism, and lipid profiles in overweight women with polycystic ovary syndrome: a randomized controlled clinical trial. J Tradit Complement Med. 2019;9(4):236-41.
2. Azimi P, Ramezani M, Namiranian N, Asadi M. Effect of *Vitex Agnus-Castus* on metabolic disturbances and hormone profile in women with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. J Diet Suppl. 2021;18(5):516-29.
3. Asemi Z, Esmaillzadeh A. Effects of fenugreek seeds on glycemic control and insulin resistance in women with polycystic ovary syndrome. J Diabetes Metab Disord. 2015;14(1):5.
4. Kamel HH. Role of phytoestrogens in the treatment of postmenopausal women and women with polycystic ovarian syndrome. Eur J Obstet Gynecol Reprod Biol. 2013;170(1):260-3.

5. Khani B, Mehrabani M, Khalesi E, Feizi A, Mofid V, Shokoohi M. Effects of the traditional Persian medicine remedy, Marzeh khormayi, on insulin resistance and androgen levels in women with polycystic ovary syndrome: a randomized double-blind placebo-controlled clinical trial. Phytother Res. 2018;32(9):1790-7.
6. Taghavi SA, Ghanbari E, Hashemipour S, Shahrokh S, Latifi Z, Zadehmodares F, et al. Effects of green tea supplementation on reproductive outcomes in women with polycystic ovary syndrome. J Obstet Gynaecol Res. 2020;46(9):1759-69.
7. Ibrahim ZS, Ahmed WM, El-Baset MA, Mostafa MM, Ellithy MM. Effects of cinnamon on ovarian reserve and metabolic profile in women with polycystic ovary syndrome: a randomized controlled trial. J Obstet Gynaecol Res. 2021;47(1):188-94.
8. Roozbeh N, Mehrabani M, Feizi A, Mofid V, Shokoohi M. The effect of *Tribulus terrestris* on ovarian reserve and metabolic indices in women with polycystic ovary syndrome: a randomized clinical trial. Phytother Res. 2014;28(9):1302-5.
9. Sadeghi MR, Kamalinejad M, Mahdian R, Eslami Rad Z, Mozaffarian V, Ghadirian L. The use of medicinal plants in the treatment of polycystic ovary syndrome: A systematic review. Int J Reprod Biomed (Yazd). 2018;16(10):665-78.
10. Kazemi M, McBreairty LE, Bird J, Alizadeh S, Zello GA. The effect of Licorice (*Glycyrrhiza glabra*) on reproductive hormones and ovarian cyst formation in a rat model of polycystic ovary syndrome. Phytother Res. 2020;34(1):120-30.

A SURVEY STUDY OF ETHNOMEDICINAL VALUE OF BAMBOOS IN THE NORTH EASTERN REGION OF INDIA

Kalyan Malakar
Department of Physics, Silapathar College, Dhemaji, Assam, India
Corresponding author E-mail: kalyanmalakar349@gmail.com

Abstract:

Bamboos are having a great share of importance on the lifestyle of the people in the Indian subcontinent since time immemorial. Be it house making, fishing, food, medicines etc, bamboos touch every aspect of day-to-day life. There are bamboos of different types and sizes available in this region. The medicinal use of bamboos is very pivotal in traditional medicinal systems. Different tribes or communities residing at different locations of this region have been using bamboos according to their traditional belief system. The aim of this article is to give an account of the use of bamboos according to traditions for treating different ailments without any influence of the modern medicine system. The methods discussed in this article are based on data collected by direct observations, key information interviews, appraisal from rural/tribal participants, group discussions, and household survey using questionnaires. A review of different researches conducted in this region by individual researchers are also presented at the end of this article.

Keywords: Bamboo, Ethnomedicinal Use, Indian Subcontinent, Traditional Use.

Introduction:

Bamboos belong to the subfamily *Bambusiodae*, of the family *Poaceae* with 75 genera and 1250 species in the world [6]. India is one of the countries with huge bamboo cover having 23 genera and 128 species [7].

India has a century-old custom of employing herbal remedies and medicinal plants to treat a wide range of illnesses and conditions while also enhancing happiness and health. In addition to seeking the medicinal benefits of plants, people frequently turn to conventional medical systems in the hopes of finding fountains of youth and optimal health. One of the medical systems that is frequently used by the tribal and indigenous people in our nation to treat illnesses is ethnomedicine. The tribal tracts contain vast amounts of knowledge and information on the various applications of plants. Such customary wisdom is, however, vanishing quickly. Documenting this knowledge is imperative, as failure to do so will result in its permanent loss. Our personnel have a truly amazing level of understanding about the utilization of natural plant products.

After food crops, bamboos are the most valuable plant resources for the ethnic communities who live in the mountainous regions of north-eastern India. This is brought on by both the traditional way of life of the ethnic communities and the area's inadequate transportation system. Tender shoots provide them with vegetables, and both mature and immature culms provide the materials for building houses, household items, agricultural implements, and many other items they use in their daily lives, including performing their traditional rites and rituals. As a result, bamboo resources have occupied a special place in their traditional ethnic life [1]. Bamboos has limited known medical history and scant documentation regarding its qualities. Many Ayurvedic literature speak of bamboos most potential medical uses [8]. There is little information available about traditional/ethnobotanical uses, taxonomic descriptions, diversity and distribution, and traditional bamboo management practices from any region of northeastern India, according to a review of the literature on bamboo and cane resources [1]. Because of its numerous industrial and therapeutic applications, as well as the fact that all of its components are rich in nutrients and antioxidants, bamboo is one of the most valuable plants in the world. All plant parts, including the leaves, shoots, culm sheath, and culms, are utilized for food or medicinal purposes. It has recently been noted that the best source of several antioxidants and bioactive chemicals is leaves, which are typically thrown away. Bamboo salt enhanced with various nutrients found in the culms is made from aged culms [2]. Bamboo is becoming more and more well-known around the world as a wholesome food since it has less residual toxicity, is low in fat, high in digestible fibre, and contains a variety of minerals, including potassium, calcium, manganese, zinc, chromium, copper, iron, and smaller amounts of phosphorus and selenium. Bamboo shoots are a good source of highquality vitamins, including A, B1, B3, B6, and E, as well as carbs, proteins, and minerals. Their accessibility to the general public may also assist address the nutritional deficiencies of the rural poor [3]. A staple of the Indian subcontinent's traditional diet is bamboo shoots. Because they are high in proteins, amino acids, carbs, and other essential minerals and vitamins, bamboo shoots have a great deal of potential for usage as a vital health food. A considerable amount of thiamine, niacin, vitamin A, vitamin B6, and vitamin E can be found in freshly harvested bamboo shoots. Additionally, diets based on bamboo shoots are a popular natural health food since they are low in cholesterol and a rich source of dietary fibre and phytosterols [4]. Numerous studies conducted on bamboo in recent years have shown that the leaf has a variety of biological effects, including the ability to scavenge free radicals and act as an antioxidant. It also possesses antimicrobial, anti-aging, and cardio-protective qualities. Additionally, it has anti-diabetic, anti-obesity, and anti-cancerous properties [3]. The food and pharmaceutical industries are putting a lot of effort into characterizing

naturally occurring bioactive chemicals that can be utilized as functional food additives or as medications. Important bioactive substances like phenols, phytosterols, and dietary fibres have been shown in studies to be beneficial against a number of ailments when found in fruits, vegetables, legumes, and whole grains; yet, these same components are absent from bamboos. A common element in South Asian cooking, bamboo shoots are a vegetable high in bioactive substances. Young bamboo shoots are only occasionally utilized as a culinary item, mostly in East and South-East Asian nations, despite the fact that bamboos are widely employed for industrial uses. Shoot consumption is becoming more and more commonplace because to its high nutritional content and several health advantages [10]. India possesses an abundance of traditional medicinal plants, and *D. strictus* has been shown to be a good source of several bioactive substances, including ash, minerals, crude protein, and crude fiber [11].

Numerous chemical compounds identified in the bamboo leaves have been shown to be pharmacologically significant. These constituents have been claimed to be effective in the treatment of numerous specific ailments, including cancer, inflammation, infectious disorders, cardiopathy, diabetes, hepatotoxicity, memory enhancement, and numerous microbial attacks [12, 13, 14, 15].

Several bamboo species have been shown to contain a wide range of important microelements, including cobalt, copper, cadmium, lead, manganese, nickel, selenium, iron, and zinc. In order to improve food-nutritional security, the viability of include raw or processed bamboo shoots in the modern diet and lifestyle is also investigated. In several areas, fermented bamboo shoot (FBS) is utilized for its anti-aging, anti-free radical, antioxidant, and anti-cancer properties. Bamboo shoots' nutritional value was recently examined. FBS supplementation improved the nuggets' physio-chemical, microbiological, and preservation qualities throughout processing [16,19, 20, 23, 25]. FBS is an excellent source of low-fat content, folic acid, protein, fiber, carbs, and vitamin C. They are essential to the body's metabolism, along with a host of other vital elements like potassium, sodium, zinc, magnesium, copper, manganese, and iron, among others. It is safe and beneficial for human consumption due to its significant antioxidant and a-glucosidase inhibitory actions, as well as its allowable number of cyanogenic glycosides. Therefore, the adoption of these items can help combat hunger in developing nations [17, 18, 21, 22, 24, 26, 27].

Bamboos in the North-Eastern region of India:

The importance and abundance of bamboos in the North-Eastern region of India can be understood from the table below:

Table 1: **Total geographic area and the bamboo bearing areas in North Eastern states of India**

States of North Eastern India	Total land area (Sq. Km)	Bamboo bearing area (Sq. Km)	Percentage of land bearing bamboo
Arunachal Pradesh	83,743	15,739	18.8%
Assam	78,438	10,659	13.6%
Manipur	22,327	8,377	37.5%
Meghalaya	22,720	5,007	22%
Mizoram	21,081	4,563	21.6%
Nagaland	16,579	3,947	23.8%
Tripura	10,486	4,201	40.1%

Source: Indian State of Forest Report (ISFR), 2021

India boasts 11.4 million hectares covered with bamboo. Of those, India is home to 129 species in 18 genera. Only the northeast of India is home to 98 different types of bamboo. One of the most significant benefits of bamboo's various qualities is its ability to treat terminal illnesses. The bamboos are thought to be phlegm-relieving, soothing, cool, and tranquil [9]. A review of the literature on cane and bamboo resources reveals a dearth of knowledge regarding traditional and ethnobotanical uses, taxonomic descriptions, diversity and distribution, and traditional bamboo management practices in any region of NorthEastern India. Some of such works can be found in [28, 29, 30, 31, 32, 33, 35, 35].

The Manipuri people's way of life is closely linked to bamboo. Since the locals rely on it for their living, it has a significant impact on their socioeconomic situation. In this area, bamboo shoots - the young, juvenile culm of the bamboo plants that emerge from the subterranean rhizomes - are a staple food item and are utilized in a variety of traditional dishes.

In this condition, the young shoots, both fresh and fermented, are usually eaten as vegetables or as seasonings. In Manipur, the highest protein, amino acid, carbohydrate and fibre content were reported in *B. nutans, D. hamiltonii, D. giganteus* and *B. tulda* respectively [36].

The Naga people have long been associated with bamboo, and they use it in practically every aspect of everyday life. A piece of bamboo is always there, from the time a baby is born till he goes to sleep. They use bamboo to make houses, use the young shoots as fodder, make weapons and hunting and fishing implements out of bamboo, cook using bamboo fuel, store water in bamboo containers, build fences to

tame animals and feed them bamboo leaves, and so on. The umbilical cord that binds a newborn to its mother is carefully severed after the birth, since it is regarded as a highly significant occasion. A small, sharp blade made of split bamboo is employed for this purpose. In addition, they dispose of the umbilical cord by burying it in the ground after carefully wrapping it in banana leaves. These kinds of blades are said to be quite efficient in healing the wound and reducing infection in both the mother and the child. Additionally, if a person passed away, their body was wrapped with bamboo mats made from split bamboo [37].

Bamboo is widely accepted for a variety of purposes and is a priceless gift from nature to the tribes of Mizoram. Bamboo is a prominent plant in the area because of its abundance, accessibility, and important component of plant wealth. Bamboo has played an important part in the lives of the tribes of Mizoram and has been an integral part in the life no such attempt been made to study the ethnobotanical uses on different species of bamboo so this work is an attempt on different uses by the different tribes [43].

The Garo people of Meghalaya are skilled in crafting a wide range of beautiful traditional handicrafts from bamboo, but it is urgently need to refocus attention from these crafts toward high-value industrial applications such as bamboo furniture, agarbati sticks, chopsticks, sleeping mats, and shoot processing, which are more likely to generate jobs and are heavy on technology [50].

The Arunachal Pradesh tribal population is a true repository of traditional knowledge, as evidenced by the centuries-old skill of creating magic from bamboos, the wide array of striking indigenous handicrafts, medicinal systems of treatment, cultural practices, and other customs. However, only a small number of tribes have been the subject of ethnobotanical research thus far, and the state's vast cultural variety is still little known. Thus, there is a pressing need to record the extensive ethnobotanical knowledge that indigenous people possess [32].

Tripura is known for its flourishing bamboo craft history, which relies on a vast workforce of tribal and non-tribal workers. Backward groups have kept the skill alive. This craft generates job opportunities for artists, providing both direct and indirect economic benefits. Tripura has around 16-18 bamboo species utilized for many purposes, including food, medicine, jewellery, crafts, home construction, and incense sticks [51]. Bamboo resources have long been abundant in Tripura's forests. Aside from that, most rural houses have one or two clumps of bamboo in their garden. In order to fulfil their own requirements and to sell the bamboo for a living, many individual families in tribal groups have a tradition of protecting large areas of uplands with naturally growing bamboo, either on their own property or in forestland. As a result,

Tripura is referred to as the "Home of bamboo," and rural craftspeople, primarily women, have long practised producing bamboo sticks [52].

The literature provides a brief overview of the significance of bamboo in daily life, including its usage in building houses, capturing fish, food, medicine, utensils etc. Numerous ethnic groups inhabit the states of the Northeast India, and they are knowledgeable consumers of bamboo goods with a vast body of knowledge passed down through generations in a lineage system from ancestors. The wisdom so passed down has become less significant over time because it has always been oral in character. Numerous age-old lessons have already vanished into the hidden corners of the natural world, where their tribal forefathers once extracted them. The goal of this research is to preserve this historic body of knowledge for human welfare and sustainable development.

Table 2: **The list of bamboo types, which are mainly accounted in this survey**

Sr. No.	Botanical Name (Scientific Name)	Vernacular Name (Local Name)
1	*Bambusa pallida*	Bijuli
2	*Bambusa balcooa*	Bhaluka
3	*Bambusa tulda*	Jati
4	*Malocanna bacciferra*	Muli
5	*Bambusa vulgaris*	Kolosi
6	*Dendrocalamus hamiltonii*	Koko
7	*Dendrocalamus giganteus*	Mokalm
8	*Bambusa arundinacea*	Katah
9	*Bambusa auriculata*	Makal
10	*Bambusa garuchakua*	Ghora
11	*Bambusa multiplex*	Dang
12	*Bambusa vulgaris*	Sonali

Source: Collected from the survey and secondary sources.

Medicinal use of Bamboo in traditional system:

This section covers specific applications of bamboo in ethnomedicine, based on responses from survey participants. We have made an effort to convey their methodology in a way that is consistent with scientific practices. The treatments for tribesmen have been administered in this manner for a very long time, but they have not been thoroughly investigated or validated by science. We have made every effort to accurately convey what they said while preserving the primary points of the survey.

The image of green bamboo leaves in Fig. 2.1 demonstrates that the young and old leaves are held by the bamboo branches and sub-branches. The bamboo plant's

stem, seen in Fig. 2.2, is tough and dark green in colour, with branches emerging from it. A bamboo shoot is seen in Fig. 2.3 in its natural condition. The shoot is protected by a sheath that is coated with spine-like black hairs. An overall bamboo plantation with several bamboo plants growing closely together is seen in Fig. 2.4. Bamboo roots, as seen in Fig. 2.5, are tightly packed, grey roots that provide a firm hold on the ground to support the bamboo plant. Figure 2.6 illustrates the anatomy of a bamboo branch. The branches grow in a manner akin to an intricate neural network, with green stems bearing leaves.

Fig 2.1 (Top Left) – Bamboo leaves. **Fig 2.2** (Top Right) – Bamboo stem.
Fig 2.3 (Middle Left) – Bamboo shoot. **Fig 2.4** (Middle Right) – Bamboo plantation.
Fig 2.5 (Bottom Left) – Bamboo root. **Fig 2.6** (Bottom Right) – Bamboo branch.

Bamboo Slices:

- A sharp blade-like tool crafted from sliced bamboo is used to cut the umbilical cord that connects a newborn to its mother after delivery. These blades are claimed to be particularly effective in healing wounds and reducing infection in both mothers and children. Bamboo slices are said to have been one of the most practical shavings implements long before iron ones were invented.

Bamboo Stems:

- Bamboo stems are locally used to treat fungal nail infections. One end of a thin, dried pith is torched, and the other end is put into one corner of an infected nail. The mildly hot flame moves inside and heals the infected nail.
- A juice is prepared from the stem of the bamboo plant and used to treat diseases like fever, cough, and the common cold. The juice is also provided to someone with sleeping irregularity, stress, or anxiety as a sleep-inducing agent.
- Bamboo has enormous first-aid potential. If we scrape the green stem of young bamboo and collect it carefully without mixing it with other impurities, the resulting powder can be used on new cuts on human or animal skin. The powder has the ability to quickly block blood flow without causing additional infection.
- In ancient wisdom, bamboo stems are said to have the ability to clean the air and provide a climate suitable for human habitation. For this reason, tribal members make mats out of bamboo for sitting, similar mats to lay under mattresses for sleeping, and bamboo ceilings, as was formerly common among ancient people.

Bamboo Powder:

- Finely ground bamboo powder is used as an exfoliator in skincare products like scrubs and cleansers. Due to its softer and less abrasive nature compared to other powders, bamboo powder is ideal for soothing delicate skin.

Bamboo Roots:

- Bamboo roots are used in traditional medicine to treat various illnesses, such as fever, insomnia, and general restlessness. Historically, they were also used to treat rabies.
- The ashes of burned bamboo roots have many significant traditional uses as medicines. Burned roots are a typical treatment for ringworm and bleeding gums. A combination made with mustard oil and charred roots might be utilized to treat joint discomfort (to be applied externally).
- Bamboo roots are a highly beneficial and efficient treatment for Spermatorrhea or "Dhatu Rog" (a condition where semen is released without sexual stimulation). The roots are sun-dried, pulverized, and taken with warmed milk for the cure.

Bamboo Leaves:

- The leaves of bamboo are green in color, long, and thin in structure. Bamboo leaves have many health and cosmetic benefits. They are soaked in water for 5-10 hours, and the resulting water is used as an eye-wash during insect attacks, mechanical injury (without blood loss), and fever to give cooling effects.
- A paste made from bamboo leaves is used to treat snake bites from moderately toxic snakes. Bamboo leaf paste can also be used to treat scorpion stings.
- Bamboo leaves have traditionally been used to treat piles, and they can be sun-dried and ground into powder. The powder, when mixed with hot water and honey, can be used as a weight-loss or fat-loss treatment.
- Bamboo leaves have significant medicinal uses for animals. For example, cows and goats that graze on bamboo leaves produce more concentrated milk, and the leaves help halt blood flow from leech assaults or other external injuries. The leaves are also useful for treating watery and more frequent bowel movements in animals.
- A combination of bamboo leaf paste and honey is used to treat mouth ulcers, providing favorable effects within a day or two.
- Boiling bamboo leaves in water produces a drink that is effective for controlling blood sugar levels in people with diabetes. The drink also exhibits therapeutic effects for dyspepsia, bloating, and constipation.

Bamboo Culm Sheath:

- The culm sheath of bamboo has multiple medicinal properties. A powder made from bamboo culm sheath hairs is applied to new cuts and wounds, utilizing bamboo's antibacterial and therapeutic properties to treat human injuries.
- Soft culms are effective for skincare and overall health maintenance. Young culms are particularly beneficial in restoring the skin's youthful appearance and maintaining a healthy body mass.

Bamboo Seeds:

- Bamboo seeds are thought to provide reproductive benefits, such as aiding infertility.
- However, they are rare since bamboo flowers only once every 50-60 years.

Bamboo Shoots:

- Bamboo shoots are a highly nutritious edible meal that can treat various disorders. A soup made by boiling young bamboo shoots with a few herbs is useful for treating wet coughs, respiratory tract problems, common colds, and fever. The soup also helps stabilize body temperature during cold months.
- In traditional medicine, bamboo shoots are recommended to help empty the stomach and facilitate regular stool movement, thereby treating constipation.

Bamboo Sub-Branches:

- Young bamboo sub-branches have been used since ancient times to clean teeth, tongues, and the entire mouth cavity. They contain compounds that provide teeth with long-life and strength, and they are used only once to reduce the risk of infection.

Bamboo Inter-Nodal Cavity Liquid:

- A liquid material gathered from the inter-nodal cavity of bamboo is used to treat bedwetting, jaundice, and paralytic issues. The liquid is also used as a fever-reducing medication.

Conclusion:

Indigenous people's knowledge of native medicinal plants must be explored and documented before they are fully assimilated into metropolitan areas and integrated into mainstream life. This study demonstrated that medicinal plants continue to play an important part in indigenous people's basic healthcare. The information we got from various sections may be beneficial to other scholars in different fields of study. This study might provide a paradigm for examining the link between plants and humans in the setting of a traditional system.

Finally, it may be worth noting that this study produced a wide range of information about the usage of medicinal herbs by indigenous tribes. As a result, there is an urgent need to document such rapidly disappearing information, to investigate and authenticate biological resources' different uses for future generations, and to establish intellectual property rights for the claim. This will undoubtedly benefit the rural economy and enrich the nation's cultural heritage.

References:

1. Medhi, P., S. K. Borthakur, and D. K. Hore. "Phytoresources from north Cachar hills of Assam, India-IV: bamboos and rattans." *J Bamboo Ratt* 9, no. 3-4 (2010): 115-25.
2. Scurlock JMO, Dayton DC and Hames B. Bamboo: an overlooked biomass resource? *Biomass and Bioenergy*. 2000; 19(4):229-244.
3. Das, Manika. "Bamboo: Inherent source of nutrition and medicine." *Journal of Pharmacognosy and Phytochemistry* 8, no. 2 (2019): 1338-1344.
4. Bao, J. "The nutrition and bioactive function of bamboo shoots." *Food Nut in China* 4, no. 2 (2006): 1-3.
5. Mohan, V. R. "Ethnomedicinal plants of the Tirunelveli district, Tamil Nadu, India." *Ethnobotanical leaflets* 2008, no. 1 (2008): 10.
6. TR, SODERSTROM. "The position of bamboo genera and allies in a system of grass classification." *Grass systematics and evolution* (1987): 225-238.

7. Seethalakshmi, K. K., MS Muktesh Kumar, K. Sankara Pillai, and N. Sarojam. *Bamboos of India: A compendium*. Vol. 17. Brill, 1998.
8. M. Remesh and Muktesh Kumar, Medicinal Bamboos of Kerala, India, SAMAGRA, Vol:7, 2011.
9. Das, Bijaylaxmi, Pankaj Kumar Singh, and Julius Munna. "Miraculous medicinal properties of bamboo." *J Med Plants* 9, no. 5 (2021): 28-30.
10. Nirmala, Chongtham, Madho Singh Bisht, and Manikanta Laishram. "Bioactive compounds in bamboo shoots: health benefits and prospects for developing functional foods." *International Journal of Food Science & Technology* 49, no. 6 (2014): 14251431.
11. Goyal, Arvind Kumar, Sushil Kumar Middha, and Arnab Sen. "In vitro antioxidative profiling of different fractions of Dendrocalamus strictus (Roxb.) Nees leaf extracts." *Free Radicals and Antioxidants* 1, no. 2 (2011): 42-48.
12. Kumar, Mukesh, Sushil Kumar Upadhyay, Ranjana Negi, Indu Sharma, and Raj Singh. "Phytodiversity, Socioeconomic and Ethnomedicinal Study of Bamboos Dendrocalamus Nees in New Forest of Forest Research Institute (FRI), Dehradun (Uttarakhand), India." *International Journal of Botany Studies* 6, no. 3 (2021): 412416.
13. Lv, Zhao-Lin, Xi Lin, Zhi-Hui Miao, Hong-Xuan Guo, Jun-An-Hong Wang, MeiLing Lei, Yue Pan, and Bo-Lin Zhang. "Antioxidant activity of bamboo-leaf extracts from the species Dendrocalamopsis oldhami." *Sci Res Essays* 7, no. 44 (2012): 37893796.
14. Mulyono, Noryawati, Bibiana W. Lay, Sri Rahayu, and Indri Yaprianti. "Antibacterial activity of petung bamboo (Dendrocalamus asper) leaf extract against pathogenic Escherichia coli and their chemical identification." *International Journal of Pharmaceutical & Biological Archive* 3, no. 4 (2012): 770-778.
15. Singhal, Poonam, Santosh Satya, and P. Sudhakar. "Antioxidant and pharmaceutical potential of bamboo leaves." *Bamboo Sci Cult* 24, no. 1 (2011): 19-28.
16. Singhal, Poonam, Santosh Satya, and P. Sudhakar. "Antioxidant and pharmaceutical potential of bamboo leaves." *Bamboo Sci Cult* 24, no. 1 (2011): 19-28.
17. Choudhury, Debangana, Jatindra K. Sahu, and G. D. Sharma. "Bamboo shoot: microbiology, biochemistry and technology of fermentation-a review." (2012).
18. Kumar, Hemant, Mradul Goswami, Sanjay Yadav, and Ch V. Rao. "Evaluation of InVitro Antioxidant Activity in Ficus religiosa (L.) Leaves." *International Journal of Research in Pharmacy & Science* 1, no. 2 (2011).

19. Nirmala Chongtham, Nirmala Chongtham, M. S. Bisht, and Sheena Haorongbam Sheena Haorongbam. "Nutritional properties of bamboo shoots: potential and prospects for utilization as a health food." (2011): 153-168.
20. Nirmala, Chongtham, Elangbam David, and Manohar L. Sharma. "Changes in nutrient components during ageing of emerging juvenile bamboo shoots." *International Journal of Food Sciences and Nutrition* 58, no. 8 (2007): 612618.
21. Owokotomo, I. A., and G. Owoeye. "Proximate analysis and antimicrobial activities of Bambusa vulgaris L. leaves' beverage." *African Journal of Agricultural Research* 6, no. 21 (2011): 5030-5032.
22. Park, Eun-Jin, and Deok-Young Jhon. "Effects of bamboo shoot consumption on lipid profiles and bowel function in healthy young women." *Nutrition* 25, no. 7-8 (2009): 723-728.
23. Satya, Santosh, Lalit M. Bal, Poonam Singhal, and S. N. Naik. "Bamboo shoot processing: food quality and safety aspect (a review)." *Trends in Food Science & Technology* 21, no. 4 (2010): 181-189.
24. Tamang, Jyoti Prakash, Namrata Tamang, Saroj Thapa *at el.* "Microorganisms and nutritional value of ethnic fermented foods and alcoholic beverages of North East India." (2012).
25. Satya, Santosh, Poonam Singhal, Lalit Mohan Bal, and P. Sudhakar. "Bamboo shoot: a potential source of food security." *Mediterranean Journal of Nutrition and Metabolism* 5, no. 1 (2012): 1-10.
26. Singh, S. Anil, H. Dayanidhi Singh, Robbarts Nongmaithem, T. C. Bora, and N. Rajmuhon Singh. "Comparative study of chemical properties of Soibum-A traditional fermented bamboo shoot product and its biological investigation." *International Journal of Bioscience, Biochemistry and Bioinformatics* 1, no. 2 (2011): 1114-1118.
27. Tamang, Buddhiman, and Jyoti Prakash Tamang. "Traditional knowledge of biopreservation of perishable vegetable and bamboo shoots in Northeast India as food resources." (2009).
28. Bhatt, B. P., L. B. Singha, K. Singh, and M. S. Sachan. "Some commercial edible bamboo species of North East India: production, indigenous uses, cost-benefit and management strategies." *Bamboo Science and Culture* 17, no. 1 (2003): 4-20.
29. Barooah, Chandra, and Sochindra Kumar Borthakur. *Diversity and distribution of bamboos in Assam*. Bishen Singh Mahendra Pal Singh (BSMPS), 2003.
30. Nath, A. J., G. Das, and A. K. Das. "Traditional knowledge base in the management of village bamboos: A case study in Barak Valley, Assam, Northeast India." (2009).

31. Sarkar, J., and R. C. Sundriyal. "Indigenous uses, management and conservation of bamboo resource in Arunachal Pradesh, North East India." *Bamboo journal* (2002): 24-39.
32. Sharma, Tika Prasad, and S. K. Borthakur. "Ethnobotanical observations on Bamboos among Adi tribes in Arunachal Pradesh." (2008).
33. Singh, K. A. "Boon of bamboo resources in North East India." *Resource management perspective of Arunachal agriculture* (2002): 69-112.
34. Sundriyal, R. C., T. C. Upreti, and R. Varuni. "Bamboo and cane resource utilization and conservation in the Apatani plateau, Arunachal Pradesh, India: implications for management." *Journal of Bamboo and Rattan* 1, no. 3 (2002): 205-246.
35. Sas Biswas, Sas Biswas. "Studies on bamboo distribution in north-eastern region of India." (1988): 514-531.
36. Premlata[1], Thounaojam, Natasha Saini[1], C. Nirmala[1], and M. S. Bisht. "Nutrient components in young shoots of edible bamboos of Manipur, India." (2015).
37. L. N. Kithan, Socio-economic Importance of Bamboo among the Nagas of Nagaland, *J Hum Ecol*, 48(3): 393-397 (2014), DOI: 10.31901/24566608.2014/48.03.06
38. Naithani, H. B. "Diversity of Indian bamboos with special reference to North-east India." (2008): 765-788.
39. Thokchom, A., and P. S. Yadava. "Bamboo and its role in climate change." *Current Science* (2015): 762-763.
40. Tripathi S.K. and Singh K.P. (2008) Role of Active components in carbon and nutrient cycling of bamboo ecosystems in Indian dry tropical region, J. Bamboo and Rattan 7(1-2): 141 – 150.
41. Tripathi, S. K., K. P. Singh, and P. K. Singh. "Temporal changes in spatial pattern of fine-root mass and nutrient concentrations in Indian bamboo savanna." *Applied Vegetation Science* 2, no. 2 (1999): 229-238.

NEEM (*AZADIRACHTA INDICA* A. JUSS.) MICROBIOTA: ENDOPHYTIC BACTERIA AND RHIZOSPHERE MICROORGANISMS

Girish K

Department of Microbiology,

Government College for Women, Kolar – 563 101, India.

Corresponding author E-mail: girishk77@yahoo.com

Abstract:

Azadirachta indica A. Juss., (neem), which is native to India, is globally well known for its medicinal properties. Neem products are known to have antibacterial, antifungal, insecticidal and other versatile biological activities. Since times immemorial, neem plant has been used for the treatment of several human ailments. Plants provide an important biological niche for the growth of a wide variety of microorganisms and neem plant is no exception to this. Many endophytes and rhizosphere microorganisms with numerous biological properties and functions have been reported associated with neem. Endophytic microbes are the storehouse of new bioactive metabolites, possessing a wide variety of biological activities as antibiotic, antiviral, anticancer, anti-inflammatory, antioxidant, etc. The rhizosphere, the narrow zone surrounding and influenced by plant roots, is a hot spot for numerous microorganisms with multifarious bioactivities.

Keywords: Neem, *Azadirachta indica*, Microbiota, Endophytes, Bioprospecting, Rhizosphere Microorganisms

Introduction:

Azadirachta indica A. Juss., commonly known as *neem*, is a tree that grows in tropical regions such as India. *Neem* has been extensively used in Ayurveda, Unani and Homoeopathic medicine. All parts of the neem tree- leaves, flowers, seeds, fruits, roots and bark have been used traditionally for the treatment of inflammation, infections, fever, skin diseases and dental disorders. Its role as health-promoting component is attributed to it rich source of phytochemicals (Girish and Shankara Bhat, 2008).

Endophytes constitute plant-colonizing microorganisms in a mutualistic symbiosis relationship. They live in apparently healthy internal plant tissues, without causing disease (Schulz and Boyle, 2006). Endophytes are found in plants of most ecosystems and are of importance since they help to improve yields, by stimulating plants growth and immune response, excluding plant pathogens by niche competition, as well as actively participating in antioxidant activities (Pandey *et al.,* 2018).

The root and its surrounding region or rhizosphere is a novel ecosystem which is the harbor of wide variety of microorganisms including bacteria, fungi, algae etc. (Prashar *et al.,* 2014). The rhizospheric microbial population has immense role in agriculture and crop improvement. Many studies have reported that these rhizosphere microorganisms can have profound effects on seed germination, seedling vigor, plant growth and development, nutrition, diseases, and productivity (Rodrigo Mendes *et al.,* 2013)

Endophytic bacteria from *Azadirachta indica*

Among plant microbiota, endophytic bacteria can be found in most plant species and be recovered from roots, leaves, stems, and a few from flowers, fruits, and seeds (Lodewyckx *et al.,* 2002). Many studies have emphasized endophytic bacteria from neem plant and their application in different areas

Verma *et al*. (2009) isolated 55 different isolates of endophytic actinomycetes from neem plant. They reported *Streptomyces* to be the dominant species followed by *Streptosporangium, Microbispora, Streptoverticillium, Saccharomonospora* sp., and *Nocardia*. Actinomycetes were recovered more from roots (54.5%), followed by stems (23.6%), and leaves (21.8%). A *Streptomyces* strain was isolated from the neem. The isolate was closely related to the type strain of *Streptomyces plicatus* sharing a 16S rRNA gene sequence similarity of 96% and this new strain was named as *Streptomyces* sp. mrinalini7 (Singh and Padmavathy, 2014). Seven novel endophytic bacterial species *viz*., *Bacillus amyloliquefaciens* (JNU-001), *Burkholderia denitrificans* (JNU-002), *Pseudomonas aeruginosa* (JNU-003), *Xanthomonas campestris* (JNU-004), *Azotobacter tropicalis* (JNU-005), *Acetobacter xylinum* (JNU-006) and *Azospirillum lipoferum* (JNU-007) were recovered from native neem varieties of Rajasthan state, India. Among these endophytic bacterial isolates obtained, *Bacillus amyloliquefaciens* (JNU-001) was dominant (Tiwari and Thakur, 2014).

An actinomycetes strain was isolated from neem leaves and named as NEK5 (Vijayan *et al.,* 2014). Endophytic actinomycetes were isolated from healthy leaves, stem and root samples of *A. indica*, with highest species richness. *Streptomyces* species was the predominant actinomycetes isolated, while other actinomycetes isolated were *Kocuria, Microbispora, Micrococcus, Micromonospora,* and *Timonella* (Gohain *et al.,* 2015). The endophytic *Streptomyces coelicolor* strain AZRA 37 was isolated from the surface sterilized root of neem plant (Kumar *et al.,* 2016). An actinomycete closely related to *Micromonospora costi* and *Micromonospora avicenniae* (98.75% similarity in 16S rRNA gene sequences) was isolated from the roots of neem and was named

Micromonospora azadirachtae sp. nov. (Kuncharoen *et al.,* 2019). Seven endophytic bacterial strains (6 Gram positive and 1 Gram-negative bacteria) were isolated from 12 neem samples collected in Phan Thiet and Lagi (Binh Thuan province), Vung Tau (Ba Ria - Vung Tau province), Vietnam (Linh *et al.,* 2020).

Bioprospecting of Neem Endophytic Bacteria

Endophytes are a source of large number of bioactive secondary metabolites with unique structures including alkaloids, benzopyranones, flavonoids, phenolic acids, quinines, steroids, terpenoids, tetralones, xanthones and others (Tan and Zou, 2001). Such bioactive metabolites find wide range of applications such as agrochemicals, antibiotics, immunosuppressants, antiparasitic, antioxidant and anticancer agents (Gunatilika, 2006). The bioactive compounds found in the host plant tissues might be due to the associated endophytes. A few reports in the recent years show that the endophytic bacteria from *A. indica* produce bioactive compounds (Verma *et al.,* 2011b; Arun Kumar *et al*. 2015).

1. Antimicrobial Activity

Actinomycetes isolated from neem plant were screened for their antibacterial and antifungal acti*vities. Streptomyces* had acute activity against *Pseudomonas fluorescens* and *Escherichia coli*, while an isolate of *Nocardia* sp., from leaves showed antagonism against *Bacillus sub*tilis. A few isolates of *Streptomyces, Nocardia* sp., and *Streptosporangium* sp., also showed significant antagonistic activity against root pathogens, including *Pythium* sp., and *Phytophthora* sp. (Verma *et al.,* 2009). Endophytic actinomycetes species isolated from neem effectively inhibited the growth of the *Alternaria alternata* causing early blight disease in tomato (Verma *et al.,* 2011).

Actinomycetes strain NEK5 isolated from neem leaves showed good antifungal activity. The ethyl acetate extract of culture filtrate of NEK5 isolate inhibited the growth of *Fusarium* sp., *Pythium* sp., *Curvularia* sp. and *Cercospora* sp. (Vijayan *et al.,* 2014). Methanol and ethanol extracts of endophytic bacterial (*Bacillus cereus* NRL2) cells isolated from neem were screened for antibacterial activity by agar well diffusion assay. Methanol and ethanol extracts showed significant antibacterial activity against *S. aureus* with IZ of 33 mm and 29 mm, respectively. Four major compounds having antimicrobial activity were obtained from this bacteria such as Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-, Methyl-2-O-methylál-arabinopyranoside, Propionylfilicinic acid and Benzene carboxylic acid (Arun Kumar *et al*. 2015).

The crude extract from *Streptomyces* species isolated from neem, grown in Casein-starch peptone-yeast (CSPY) broth, showed significant inhibition of bacteria

Pseudomonas syringae (MTCC 673) and *S. aureus* (MTCC 96), and fungi *C. albicans* (MTCC 3017), and *Rhizoctonia solani* (MTCC 4634) (Gohain *et al.,* 2015). The *Streptomyces coelicolor* was treated with different concentrations of 5-azacytidine and evaluated for its antibacterial potential against five human pathogenic bacteria (*Aeromonas hydrophila* IMS/GN11, *Enterococcus faecalis* IMS/GN7, *Salmonella typhi* MTCC 3216, *Shigella flexneri* ATCC 12022 and *S. aureus* ATCC 25923. The crude extract obtained from cultures treated with 25 µM concentration of 5-azacytidine, was found effective against all the five pathogenic bacteria tested (Kumar *et al.,* 2016).

A total of 25 endophytic bacterial isolates were obtained from neem fresh and healthy leaves. Maximum isolates were Gram-positive and rod shaped. In disk diffusion assay using culture broth of endophytic bacteria, significant antibacterial activity was observed against *Bacillus cereus, E. coli, Klebsiella pneumoniae, S. aureus, Salmonella typhimurium,* and *Streptococcus pyogenes.* Out of 25 isolates 2 isolates were effective against *B. cereus,* 23 against *E. coli,* 22 against *K. pneumoniae,* 20 against *S. aureus,* 22 against *S. typhimurium,* and 21 against *S. pyogenes* (Singh *et al.,* 2017). A total of 80 bacterial endophytes were isolated from various parts of neem plant such as leaf, flower, seed, bark, cortex and root using novel neem-based media. Out of them, only three bacterial isolates showed antifungal potential by inhibiting the growth of *Magnaporthe oryzae* causing blast of rice (Agasimundin *et al.,* 2019).

Antifungal and antibacterial activities of endophytic bacteria from *A. indica* were studied by well diffusion agar method. KT2 strain inhibited *Salmonella typhi* and *Staphylococcus aureus*, KT3 strain showed inhibitory activity against three human pathogenic fungi such as *Candida albicans*, *Trichophyton mentagrophytes*, and *Trichophyton rubrum*, while KT1 and KT2 strains showed antifungal activity against *C. albicans* and *T. rubrum*. By biochemical tests, KT2 strain was identified as the *Bacillus subtilis* (Linh *et al.,* 2020).

2. Other Activities

Endophytic actinomycetes species isolated from *A. indica,* significantly improved the growth of tomato plant by producing the phytohormone indole acetic acid (IAA) and siderophores. *Streptomyces* strain AzR-051 produced the highest amount of IAA at 13.73 µmol / ml, compared to strains AzR-049 and AzR-010 that produced 9.22 µmol / ml and 10.43 µmol / ml respectively. The *Streptomyces* strain also produced siderophores (Verma *et al.,* 2011). The *Streptomyces* sp. mrinalini7 isolate when inoculated into model tomato plants significantly enhanced the biomass production of the plant and seed germination (Singh and Padmavathy, 2014).

Rhizosphere Microorganisms of Neem

The rhizosphere is a narrow zone adjacent to and influenced by living plant roots (Kennedy, 1999). It is a site of high microbial activity in and around roots in soil (Sorenson, 1997). It harbors a great diversity of microorganisms affecting plant growth and health (Campbell and Greaves, 1990; Boehm *et al.,* 1993). The diversity and composition of microbial taxa in the rhizosphere can be affected by several factors including plant species (Miller *et al.,* 1989). The composition of microbial community in the rhizosphere is important for the performances of the plant, as microbial species can have beneficial, neutral or harmful relationships with the roots (Buchenauer, 1998; Atkinson and Watson, 2000; Sylvia and Chellemi, 2001). Microorganisms in the rhizosphere are found to be more in population and are having high metabolic rate than in non rhizosphere soil (Tamilarasi *et al.,* 2008). There are many reports of neem rhizosphere microorganisms and their associated bioactivities.

Field investigation was carried out to determine the arbuscular mycorrhizal fungi (AM) population and their diversity in neem-based agroforestry fields. *Glomus*, *Gigaspora* and *Sclerocystis* were the genera of AM present in the neem-based agroforestry system. Among the three genera, *Glomus* occurred most frequently with 15 species, three species were of *Gigaspora* and two were of *Sclerocystis*. *Glomus fasciculatum* was the predominant AM fungus infecting neem (Pande and Tarafdar, 2004). Arbuscular mycorrhizal (AM) fungi are recognized as an essential component of sustainable agricultural ecosystems (Jefferies, 1987; Barea, 1991).

Rhizosphere microflora of medicinal plants including *A. indica* was estimated. The total number of heterotrophic bacteria in the neem rhizosphere was 41 x 10^4 cfu / g, actinomycetes population was 17 x 10^2 cfu / g and fungal population was 18 x 10^2 cfu / g. The predominant bacterial genus was *Bacillus* followed by *Pseudomonas, Enterobacter, Corynebacterium, Micrococcus* and *Serratia*. Among the fungus the most dominant genus was *Rhizopus* followed by *Aspergillus, Penicillium, Mucor* and *Fusarium*. Among the actinomycetes, isolates of *Streptomyces* was found to be maximum followed by *Frankia* sp. (Tamilarasi *et al.,* 2008).

Both Vesicular-arbuscular mycorrhizal fungi (VAM) and soil fungal diversity and frequency were studied in the neem rhizosphere from five ecogeographically different regions. Mycofloral diversity included *Aspergillus niger, A. flavus*, *A. nidulans, A. versicolor, A. fumigatus, Alternaria tenuis, A. alternata, Cladosporium* sp., *Cephalospora* sp., *C. albicans, Fusarium oxysporum, Pestalotia monorhinca, Paecilomyces, Monilia sitophila, Nigrospora oryzae* and *Rhizopus nigricans*. Saline-arid-parched soil exhibited

three unique fungal species namely, *Monilia sitophila, Aspergillus versicolo*r and *Paecilomyces fusisporus*, whereas, the delta-wet region exhibited *Rhizopus nigricans* as its unique species. Overall, in the five regions studied, three VAM genera with nine species were observed, with *Glomus* being the predominant genus *viz., Glomus mosseae, Glomus microcarpum, Glomus macrocarpum, Glomus constrictum, Glomus fasciculatum, Glomus multisubstance, Glomus deserticola, Gigaspora albida, Gigaspora margarita* and *Acaulospora* sp. (Chary, 2011).

Phosphate solubilizing bacteria (PSB) isolates namely N-B (col-1) and N-C (col-2), were isolated from neem rhizosphere (Shankarrao, 2012). Neem rhizosphere soil can be a rich source for the isolation of phosphate solubilizing microorganisms, due to high phosphate requirements of neem tree and other medicinal plants (Phavaphutanon *et al.,* 1996), or due to long term association and interaction between neem root and microorganisms found in the rhizosphere environments (Lucas Garcia *et al.,* 2001). The bacterial phosphate solubilization activity is due to secretion of organic acids such as oxalic, citric, formic, acetic, propionic, lactic, succinic and gluconic acid which chelate the cation bound to phosphate and convert it to soluble forms through their hydroxyl and carboxyl groups and production of acid /alkaline phosphatase enzyme (Chen *et al.,* 2006).

Rhizospheric bacteria were isolated from neem and identified as *E. coli, Lactobacillus fermentum, Micrococcus luteus, Neisseria sicca, Sporosarcina* sp.*, Streptococcus* sp. and *Streptococcus faecalis* (Pandey and Singh, 2013). Phosphate-solubilizing rhizosphere fungus, *Talaromyces funiculosus* SLS8, was isolated from neem (*A. indica*) on saline soil. The fungus was tolerant to environmental stressors, salinity and agricultural systemic fungicides (Kanse *et al.,* 2015). The rhizosphere soil of *neem* showed maximum population density of AM fungi, PSB, *Azotobacter* sp. and *Azospirillum* sp. (Maohan and Saranya Devi, 2015). A total of 27 bacterial cultures were isolated from the neem rhizosphere samples. Then three cultures were characterized and found to be *Micrococcus luteus, Sporosarcina* sp.*,* and *Staphylococcus epidermidis* (Mhatre and Nanoty, 2015). Bacterial strains named RHSAN-1 to 6 were isolated from neem rhizosphere of North 24 Parganas district of West Bengal (Biswas *et al.,* 2016).

A total of six morphologically different fungal isolates (NS1, NS2, NS3, NS4, NS5, NS6) were isolated from the rhizospheric soil of neem and were identified as *Aspergillus* sp. (NS1, NS3, NS4, and NS6), *Fusarium* sp. (NS2) and *Penicillium* sp. (NS5) based on colony characterization (colony color, colony growth) and sporulating structures. One

isolate was subjected for molecular characterization and found to be *Aspergillus niger* (Nisha Rani *et al.,* 2017). Arbuscular mycorrhizal fungi (AMF) species belonging to five genera (*Glomus, Acaulospora, Gigaspora, Sclerocystis* and *Scutellospora*) were isolated from the rhizosphere soil of *A. indica*. *Glomus* species was dominant followed by *Gigaspora* and *Acaulopsora* (Anusha Duvva *et al.,* 2018). Eleven bacterial isolates that produced indole acetic acid (IAA) were obtained from the rhizosphere of *A. indica* (Damle and Kulkarni, 2018). N_2-fixing bacteria *Azotobacter paspali*, *Azotobacter vinelandii* and *Actinomycetes* sp., were isolated from the rhizosphere of neem (Hala and Ali, 2019).

Microorganisms are also intentionally introduced into the rhizosphere environments to enhance certain agriculturally beneficial activities mainly aiming at plant growth promotion (Tamilarasi *et al.,* 2008). Inoculation of neem rhizosphere with AM fungi (Habte *et al.,* 1993; Phavaphutanon *et al.,* 1996) reduced fertilizer requirement in plant production. The effect of inoculation of neem with VA-mycorrhizal fungi (*Glomus fasciculatum*) and PSB was examined under nursery conditions to understand the compatibility between phosphate solubilizing and phosphate mobilizing organisms in the neem rhizosphere. The results clearly indicated that combined inoculation markedly increased the plant growth of the neem seedlings when compared to individual inoculants or uninoculated control, showing the synergistic effect (Kalavathi *et al.,* 2000).

Neem seedlings were inoculated with arbuscular mycorrhizal (AM) fungi, *Glomus intraradices*, *Azospirillum brasilense* and PSB. Microbial inoculation resulted in greater plant height, increased mycorrhizal colonization, leaf area and number, root collar diameter, biomass, phosphorus, nitrogen and potassium content, and seedling quality. Microbial inoculation effects were greatest when seedlings were inoculated with a combination of microbes rather than individually. This clearly indicated that these microorganisms act synergistically (Muthukumar *et al.,* 2001).

Bioprospecting of Neem Rhizosphere Microorganisms

The isolates of bacteria, actinomycetes and fungi isolated from neem rhizosphere were evaluated for IAA production. Among them 62.5% of fungal isolates produced IAA followed by 52.17% of actinomycetes and 23.7% of bacterial isolates (Tamilarasi *et al.,* 2008). The microbial diversity of VAM and soil fungi observed in neem rhizosphere could be correlated with the azadirachtin-A content of the neem trees (Chary, 2011).

PSB isolates from neem rhizosphere N-B (col-1), N-C (col-2) showed potent antifungal activity against *Helminthosporium gramineum* and *Rhizopus oryzae*. Isolate N-B (col-1) also showed good antifungal activity against *Aspergillus niger* and *Ustilago maydis*. Comparitively, *R. oryzae*, *H. gramineum, A. niger* and *U. maydis* showed more sensitiveness to tested isolates than *Alternaria brassicicola*, *A. solani* and *Sclerotium rolfsii*. Both the isolates exhibited maximum antibacterial activity against *S. aureus*, followed by *Ps. aeruginosa* and *S. typhimurium* (Shankarrao, 2012). N-B (col-1) showed more than one PGPR trait such as phosphate solubilization, antifungal and antibacterial activity and phytohormone production. This isolate might promote plant growth directly, indirectly or synergistically in the soil environment (Shankarrao, 2012).

Rhizospheric bacteria isolated from neem were subjected for extraction of intracellular secondary metabolites using methanol and extracellular secondary metabolites using chloroform. These extracts exhibited significant antibacterial activity against *E. coli, Ps. aeruginosa* and *S. aureus. Sporosarcina* sp., *M. luteus* and *N. sicca* inhibited all the tree bacteria *viz., E. coli* (IZ 17.5, 12.5 and 13 mm), *Ps. aeruginosa* (21, 17.5 and 28.5 mm) and *S. aureus* (11.5, 13 and 27.5 mm) respectively. *Streptococcus* sp., and *S. faecalis* inhibited *E. coli* (36.5 and 17 mm) and *S. aureus* (11 and 10 mm) respectively. *Lactobacillus fermentum* inhibited only *E. coli* (13.5 mm) and rhizosphere *E. coli* inhibited only *Ps. aeruginosa* (12.5 mm) (Pandey and Singh, 2013).

Phosphate solubilization under different nutritional conditions was investigated by culturing *T. funiculosus* SLS8 in Pikovskaya liquid medium. The highest concentration of solubilised phosphate (187 mg / l) was achieved after 5 days of incubation in the medium with glucose and ammonium sulphate (Kanse *et al.,* 2015). The soil fungi have been reported to solubilize insoluble phosphates by secreting weak organic acids (Maliha *et al.,* 2004). Three bacterial cultures *M. luteus, Sporosarcina* sp.*,* and *S. epidermis* obtained from neem rhizosphere were screened for antibacterial activity using culture broth extract by agar well diffusion method. All the bacteria tested showed significant antibacterial activity. *M. luteus* exhibited IZ of 14, 13 and 13 mm, *Sporosarcina* sp. showed IZ of 16, 16.5 and 16 mm, and *S. epidermis* exhibited IZ of 14, 15 and 14 mm against *E. coli, Ps. aeruginosa* and *S. aureus,* respectively (Mhatre and Nanoty, 2015).

The culture filtrates of three fungal strains NS1, NS4 (*Aspergillus* sp.) and NS5 (*Penicillium* sp.) isolated from neem rhizosphere were screened for antibacterial activity by agar well diffusion assay. All the three isolates showed significant antibacterial activity against *E. coli* MTCC 40 with IZ of 20.33, 14.66 and 17.0 mm,

respectively. NS1 that showed the best activity was identified as *A. niger* and studied for optimization of fermentation conditions to maximize antibiotic production. Process using glucose as carbon source, ammonium nitrate as nitrogen source, at pH 5 and 25^{o}C resulted in maximum yield of antibiotic (Nisha Rani *et al.,* 2017). Good amount of IAA was produced by bacterial isolates obtained from neem rhizosphere. The range of IAA of the isolates ranged from 10.425 to 43.404 µg/ ml. The highest amount of IAA was produced by the isolate 6 (Damle and Kulkarni, 2018).

Conclusion and Recommendations:

Endophytic bacteria of neem are an under-investigated group of microorganisms that represent an abundant and renewable source of bioactive and chemically new compounds with potential for exploitation in a wide variety of fields including medicine, agriculture, and industry. The microbial population of the rhizosphere of this plant also has not been studied so far in detail. Further studies are required in this direction to explore and evaluate the enormous microbial population in these ecosystems. Endophytic bacteria with their peculiar potential compounds might provide a range of bioactive compounds catering to the need of novel drugs. The rhizosphere isolates of neem plant might have sufficient bioprospective potentiality like biofertilizer formulations for better crop production as well as therapeutic drugs for human diseases.

References:

1. Agasimundin, V.B., Rangiah, K., Sheetal, A. and Gowda, M. 2019. Neem microbiome. In: The Neem Genome, Compendium of Plant Genomes (Eds. Gowda M, Sheetal A, Kole C). Springer, Cham. pp 111-123. ISBN 978-3-030-16122-4
2. Anusha Duvva, Dayakar Govindu, Sharat Bellamkonda and Srinivas Podeti. 2018. Diversity of arbuscular mycorrhizal fungi in the rhizosphere soils of four agroforestry tree species of North Telangana. *Asian J. Microbiol. Biotechnol. Environ. Sci.,* 20(2): 277-283.
3. Arun Kumar G., Robert Antony, A. and Rajesh Kannan, V. 2015. Exploration of endophytic microorganisms from selected medicinal plants and their control potential to multi drug resistant pathogens. *J. Med. Plants Studies* 3(2): 49-57.
4. Atkinson, D. and Watson, C.A. 2000. The beneficial rhizosphere: A dynamic entity. *Appl. Soil Ecol.,* 15: 99-104.
5. Barea, J.M. 1991. Vesicular arbuscular mycorrhizae as modifiers of soil fertility. *Adv. Soil Sci.,* 15: 1–40.

6. Biswas, K., Basu, J., Ghosh, A. and Giri, P. 2016. Study of rhizospheric bacterial population of *Azadirachta indica* (neem) of North 24 Parganas district of West Bengal for bioprospective consideration. *Int. J. Exp. Res. Rev.,* 6: 62-66.
7. Boehm, M., Madden, V and, Hoitink, H.A.J. 1993. Effect of organic matter decomposition level on bacterial species diversity and composition in relation to *Pythium* damping off severity. *Appl. Environ. Microbiol*., 59: 4171-4179.
8. Buchenauer, H. 1998. Biological control of soil borne diseases by rhizobacteria. *J. Plant Dis. Protect.*, 105: 329-348.
9. Campbell, R. and Greaves, M.P. 1990. Anatomy and community structure of the rhizosphere. *In: The Rhizosphere* (Ed. Lynch JM). Wiley, New York. pp. 11-34.
10. Chary, P. 2011. A comprehensive study on characterization of elite neem chemotypes through mycofloral, tissue-cultural, ecomorphological and molecular analyses using azadirachtin-A as a biomarker. *Physiol. Mol. Biol. Plants* 17(1): 49–64.
11. Chen, Y.P., Rekha, P.D., Arun, A.B., Shen, F.T., Lai, W.A. and Young, C.C. 2006. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. *Appl. Soil. Ecol*., 34: 33–41.
12. Damle, N. and Kulkarni, S. 2018. *In vitro* screening of indole acetic acid (IAA) producing rhizobacteria from some medicinal plants. *DAV Int. J. Sci.,* 7(1): 1-5. http://davijs.com/2018/DAVIJS-Vol-7-1-4.pdf
13. Girish, K. and Shankara Bhat, S. 2008a. Neem – A green treasure. *EJBio*., 4(3): 102-111.
14. Gohain, A., Gogoi, A., Debnath, R., Yadav, A., Singh, B.P., Gupta, V.K., Sharma, R. and Saikia, R. 2015. Antimicrobial biosynthetic potential and genetic diversity of endophytic actinomycetes associated with medicinal plants. *FEMS Microbiol. Lett*., 362(19): https://doi.org/10.1093/femsle/fnv158
15. Gunatilaka, A.A.L. 2006. Natural products from plant-associated microorganisms: Distribution, structural diversity, bioactivity and implication of their occurrence. *J. Nat. Prod.*, 69: 509-526.
16. Habte, M., Muruleedhara, B.N. and Ikawa, H. 1993. Response of neem (*Azadirachta indica*) to soil P concentration and mycorrhizal colonization. *Arid Land Res. Manage*., 7: 327–333.
17. Hala, Y. and Ali, A. Isolation and characterization of *Azotobacter* from neem rhizosphere. *J. Phys.: Conf. Ser.* 1244 012019. doi:10.1088/1742-6596/1244/1/012019

18. Jeffries, P. 1987. Use of mycorrhizae in agriculture. *Crit. Rev. Biotechnol*., 5: 319–357.
19. Kalavathi, B.P., Santhanakrishnan, P. and Divya, M.P. 2000. Effect of VA-mycorrhizal fungus and phosphorus solubilising bacterium in neem. *Indian For.,* 126(1): 67-70.
20. Kanse, O.S., Whitelaw-Wecker, M., Kadam, T.A. and Bhosale, H.J. 2015. Phosphate solubilization by stress-tolerant soil fungus *Talaromyces funiculosus* SLS8 isolated from the neem rhizosphere. *Ann. Microbiol*., 65(1): 85–93.
21. Kennedy, A.C. 1999. The rhizosphere and spermosphere. *In: Principles and Applications of Soil Microbiology* (Eds. Sylvia DM, Fuhrmann JJ, Hartel PG, Zuberer DA). Prentice Hall, New Jersey.
22. Kumar, J., Sharma, V.K., Singh, D.K., Mishra, A., Gond, S.K., Verma, S.K., Kumar, A. and Kharwar, R.N. 2016. Epigenetic activation of antibacterial property of an endophytic *Streptomyces coelicolor* strain AZRA 37 and identification of the induced protein using MALDI TOF MS/MS. *PLoS ONE* 11(2): e0147876. doi:10.1371/journal.pone.0147876
23. Kuncharoen, N., Kudo, T., Ohkuma, M. and Tanasupawat, S. 2019. *Micromonospora azadirachtae* sp. nov., isolated from roots of *Azadirachta indica* A. Juss. *var. siamensis* Valeton. *Antonie Van Leeuwenhoek* 112: 253–262.
24. Linh, D.N., Thien, B.V., A Ni, T.T., Duy, N.T., Minh, N.V. and Dong, T.C. 2020. Studies on antimicrobial activities of endophytic bacteria isolated from neem tree (*Azadirachta indica*). *Ho Chi Minh City Open Univ. J. Sci.,* 10(1): 72-80.
25. Lodewyckx, C., Vangronsveld, J., Porteous, F., Moore, E.R.B., Taghavi, S., Mezgeay, M., et al. (2002). Endophytic bacteria and their potential applications. *Crit. Rev. Plant Sci.,* 21: 583–606. doi: 10.1080/0735-260291044377
26. Lucas Garcia, J.A., Probanza, A., Ramos, B. and Gutierrez Manero, F.J. 2001. Genetic variability of rhizobacteria from wild populations of four Lupinus species based on PCR-RAPDs. *J. Plant Nutr. Soil Sci*., 164: 1–7.
27. Maliha, R., Samina, K., Najma, A., Sadia, A. and Farooq, L.. 2004. Organic acid production and phosphate solubilization by phosphate solubilizing microorganisms under *in vitro* conditions. *Pak. J. Biol. Sci*., 7: 187–196.
28. Mhatre, S.R. and Nanoty, V.D. 2015. Antimicrobial activity of rhizospheric bacteria of *Azadirachta indica* producing metabolites against human bacterial pathogens. *Int. J. of Life Sci.,* Special issue A3: 21-25.

29. Miller, H.J., Henken, G. and Van Veen, J.A. 1989. Variation and composition of bacterial populations in the rhizosphere of maize, wheat and grass cultivars. *Can. J. Microbiol*., 35(6): 656-660.
30. Mohan, V. and Saranya Devi, K. 2015. Study on the status of beneficial microbes from afforested textile and urban waste water polluted sites in Tirupur district, Tamil Nadu, South India. *ENVIS Centre Newsletter* 13(1). Available from: www.envismadrasuniv.org
31. Muthukumar, T., Udaiyan, K. and Rajeshkannan, V. 2001. Response of neem (*Azadirachta indica* A. Juss) to indigenous arbuscular mycorrhizal fungi, phosphate-solubilizing and asymbiotic nitrogen-fixing bacteria under tropical nursery conditions. *Biol. Fertil. Soils* 34: 417–426.
32. Nisha Rani, Pranay Jain and Geetanjali. 2017. Isolation of antimicrobial compound producing fungi from the rhizospheric soil of the medicinal plant *Azadirachta indica. J. Chem. Pharma. Res.,* 9(9): 265-270.
33. Pande, M. and Tarafdar, J.C. 2004. Arbuscular mycorrhizal fungal diversity in neem-based agroforestry systems in Rajasthan. *Appl. Soil Ecol*., 26: 233–241.
34. Pandey, A. and Singh, A. 2013. A comparative study on secondary metabolites producing microbes isolated from rhizospheric and non-rhizospheric region of *Azadirachta indica* and *Oscimum tenuiflorum*. *Int. J. Pharm. Res. Allied. Sci*., 2: 36-48.
35. Pandey, P.K., Singh, S., Singh, M.C., Singh, A.K., Yadav, S.K., Pandey AK., et al. (2018). Diversity, ecology, and conservation of fungal and bacterial endophytes. *In: Microbial Resource Conservation* (Eds. Sharma SK, Varma A.). Springer International Publishing, New York. Pp. 393–430.
36. Phavaphutanon. L., Davies, F.T.Jr. and Duray, S.A. 1996. Growth, root alteration, and nutrient uptake of neem tree (*Azadirachta indica* A. Juss) seedlings in response to vesicular-arbuscular mycorrhizal fungi and phosphorus nutrition*. Int. Tree Crops J*., 9: 59-67.
37. Prashar, P., Kapoor, N. and Sachdeva, S. 2014. Rhizosphere: its structure, bacterial diversity and significance. *Rev. Environ. Sci. Biotechnol.,* 13: 63-77. https://doi.org/10.1007/ s11157-013-9317-z
38. Rodrigo Mendes, Paolina Garbeva and Jos M. Raaijmakers. 2013. The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS Microbiol. Rev.,* 37(5): 634–663. https://doi.org/10.1111/1574-6976.12028

39. Schulz, B. and Boyle, C. 2006. What are endophytes? *Soil Biol.,* 9: 1-14. doi: 10.1007/3-540-33526-9_1
40. Shankarrao, O. 2012. Isolation and characterization of phosphate solubilizing bacteria from rhizospheric soil samples. *Online Int. Interdiscip. Res, J.*, 2(4): 28-39.
41. Singh, A.K., Sharma, R.K., Sharma, V., Singh, T., Kumar, R and Kumari, D. 2017. Isolation, morphological identification and *in vitro* antibacterial activity of endophytic bacteria isolated from *Azadirachta indica* (neem) leaves. *Veterinary World* 10(5): 510-516.
42. Singh, M.J. and Padmavathy, S. 2014. Isolation, screening and characterization of endophytic PGPR actinomycetes present commonly in neem and tulsi leaves - *in vitro* study (tomato). *Int. J. Recent. Sci. Res.,* 5(3): 574-579.
43. Sorensen, J. 1997. The rhizosphere as a habitat for soil microorganisms. *In: Modern soil Microbiology* (Eds. Van Elas JD, Trevors JT, Wellington EMH). Marcel Dekker, New York. pp. 21-45.
44. Sylvia, D.M. and Chellemi, D.O. 2001. Interactions among root-inhabiting fungi and their implications for biological control of root pathogens. *Adv. Agron*., 73: 1-33.
45. Tamilarasi, S., Nanthakumar, K., Karthikeyan, K. and Lakshmanaperumalsamy. 2008. Diversity of root associated microorganisms of selected medicinal plants and influence of rhizomicroorganisms on the antimicrobial property of *Coriandrum sativum. J. Environ. Biol.*, 29(1): 127-134.
46. Tan, R.X. and Zou, W.X. 2001. Endophytes: A rich source of functional metabolites. *Nat. Prod. Rep.,* 18: 448-459.
47. Tiwari, K. and Thakur, H.K. 2014. Diversity and molecular characterization of dominant *Bacillus amyloliquefaciens* (JNU-001) endophytic bacterial strains isolated from native neem varieties of Sanganer region of Rajasthan. *J. Biodivers. Biopros. Dev*., 1: 115. doi:10.4172/2376-0214.1000115
48. Verma, V.C., Gond, S.K., Kumar, A., Mishra, A., Kharwar, R.N. and Gange, A.C. 2009. Endophytic actinomycetes from *Azadirachta indica* A. Juss.: Isolation, diversity, and anti-microbial activity. *Microb. Ecol*., 57: 749–756.
49. Verma, V.C., Singh, S.K. and Prakash, S. 2011. Bio-control and plant growth promotion potential of siderophore producing endophytic *Streptomyces* from *Azadirachta indica* A. Juss. *J. Basic Microbiol*., 51: 550–556.
50. Vijayan, V.M., Radhakrishnan, M. and Balagurunathan, R. 2014. Bioprospecting of endophytic actinomycetes for antiphytofungal activity. *Int. J. Chem. Tech. Res*., 6(5): 2689-2694.

URBAN HORTICULTURE: INNOVATIONS AND CHALLENGES

Mahendra Kumar Yadav* and Gayathri P. M
School of Agriculture, RNB Global University, Bikaner, Rajasthan
*Corresponding author E-mail: mahendra.yadav@rnbglobal.edu.in

Introduction:

Plant production in highly populated urban contexts is the focus of the rapidly expanding field known as urban horticulture. The necessity of incorporating horticulture into urban design has become more and more obvious as cities grow and green places disappear. Urban horticulture is a broad field that includes anything from large-scale rooftop farms to small-scale community gardens. It is essential to improving environmental quality, food security, and urban sustainability. Horticulture in urban environments has traditionally only been practiced in public parks and private gardens. However, a rebirth in urban horticulture has been brought about by recent technological advancements and an increased consciousness of environmental concerns. New techniques and systems are being created to optimize the productivity of constrained urban environments. This chapter examines the major developments that have revolutionized urban horticulture, the problems now facing it, and possible future paths for this important area of study. Urban horticulture functions at the nexus of landscape architecture, urban planning, and agriculture, converting underused or unusable urban spaces into fruitful green spaces. It makes use of cutting-edge technology and innovative solutions to solve the particular problems presented by urban surroundings, such as restricted space, contaminated soil, and scarce water supplies. Urban horticulture is vital to enhancing the quality of life in cities, supporting ecological balance, and creating a sense of community among urban dwellers. Examples of this include growing vegetables on a balcony, tending to a community garden, and installing green roofs on high-rise buildings.

Definition

- Urban horticulture refers to the practice of growing horticultural plants within city environments, encompassing a wide range of activities that involve the cultivation, management, and use of horticultural plants in urban spaces. This includes not only traditional gardening in private yards but also innovative methods such as vertical gardening, rooftop gardens, hydroponics, and community gardens.

- Urban horticulture refers to the practice of growing various horticulture crops including fruits, vegetables, leafy greens, ornamentals, medicinal and aromatic plants in the cities i.e.; urban areas in a limited space but in an effective manner.

Urban Horticulture

Objectives of Urban Horticulture-

- Urban horticulture aims to enhance food security by promoting local food production and reducing food miles, which helps cities become more self-sufficient and less reliant on external food sources.
- Additionally, it is essential for advancing environmental sustainability since it supports urban biodiversity conservation and the construction of green infrastructure, such vertical gardens and green roofs.
- Urban horticulture improves the aesthetics of cities and enhances the quality of life for residents by transforming neglected areas into beautiful, functional green spaces that offer recreational opportunities**.**
- Urban horticulture encourages the creation of community gardens where residents can come together, share knowledge, and build relationships.
- Urban horticulture supports economic development by creating jobs in urban farming, landscaping, and related industries, and by fostering entrepreneurship in areas such as farmers' markets and green businesses.
- Urban horticulture contributes to climate change mitigation by helping to sequester carbon dioxide and improving the energy efficiency of buildings through the use of green roofs and walls.
- It also emphasizes resource efficiency by incorporating water conservation practices like drip irrigation and rainwater harvesting, and by promoting waste reduction through composting.

- Urban horticulture adapts to the challenges of urban environments by developing innovative solutions for space constraints, such as vertical gardening and hydroponics, and addressing issues related to soil quality and water availability through alternative growing methods.

Innovations in Urban Horticulture

1. Vertical Gardening

Vertical gardening is one of the most significant innovations in urban horticulture. This method involves growing plants on vertically inclined surfaces, allowing for the cultivation of a large number of plants in a relatively small area. Vertical gardens can be installed on the exteriors or interiors of buildings, making them a versatile solution for space-constrained urban environments.

Techniques and Systems: There are several ways to build vertical gardens, such as using trellises, stacked containers, and hydroponic systems. Using modular panels to create a green facade that can be fastened to walls is one common technique. These panels may hold soil or other growth media and are frequently constructed from strong, lightweight materials.

2. Rooftop and Balcony Gardens

In metropolitan areas, where space is limited, rooftop and balcony gardens are growing in popularity. These rooftop and balcony gardens repurpose usually unutilized areas to cultivate a range of plants, from food crops to decorative flowers.

Design and Implementation: Rooftop gardens range in complexity from a few potted plants to an intricately designed green roof system. In addition to providing insulation, lowering storm water runoff, and enhancing air quality, green roofs are made to encourage plant growth. Usually, they are made up of many layers, such as a growth medium, drainage system, waterproof membrane, and plants.

Benefits: Rooftop gardens offer numerous benefits, including the reduction of the urban heat island effect, increased biodiversity, and the provision of local food sources. They also serve as recreational spaces for residents and can improve the aesthetic appeal of buildings.

Challenges: Despite their benefits, rooftop gardens face several challenges, including structural limitations, water management, and the need for regular maintenance. It is essential to consider the load-bearing capacity of a building before installing a rooftop garden, as the added weight of soil, water, and plants can be significant.

3. Hydroponics and Aquaponics in Urban Settings

Due to their efficiency and capacity to be used in small places, soilless growing techniques like hydroponics and aquaponics have become more and more popular in urban horticulture.

i. Hydroponics: In hydroponics, plants are grown without the need of soil in a nutrient-rich water solution. With this technique, the growing environment can be precisely controlled, which promotes quicker plant development and larger harvests. Urban rooftops, balconies, and even interior areas may be effortlessly incorporated with hydroponic systems.

ii. Aquaponics: Hydroponics and aquaculture—the raising of fish or other aquatic animals—are combined in aquaponics. In an aquaponic system, the plants aid in filtering and purifying the water while the aquatic creatures' waste supplies nutrients for the plants. Because of this symbiotic relationship, aquaponics is a productive and sustainable way to grow food, especially in cities with limited resources and space.

Applications in Urban Agriculture: The fact that hydroponics and aquaponics both require little area and can be used inside makes them ideal for urban agriculture. From modest residential setups to big commercial farms, these systems may be adjusted to meet a variety of areas. To illustrate how these methods might support urban food security, the Plantagon International Association in Sweden has created a vertical greenhouse that employs hydroponics to produce vegetables within a high-rise structure.

4. Community Gardens and Urban Farms

Urban horticulture cannot exist without community gardens and urban farms, which offer chances for education, social engagement, and local food production. These areas, which are run by locals, groups, or governments, are frequently built on empty lots, roofs, or inside public parks.

i. The Rise of Community Gardening: Community gardens have seen a resurgence in recent years, driven by a growing interest in local food systems and sustainable living. Residents may learn about horticulture, cultivate their own food, and strengthen their feeling of community by using these gardens. By improving access to wholesome, fresh produce in cities, especially those located in food deserts, they also help ensure food security.

ii. Social and Environmental Benefits: Community gardens have many positive social and environmental effects in addition to producing food. They act as urban green spaces, lowering temperatures, enhancing biodiversity, and enhancing air quality. They also offer chances for social engagement and community development, which encourages participants' sense of pride and ownership.

Challenges: Community gardens face several challenges, including securing land tenure, managing resources, and ensuring long-term sustainability. Since many community gardens are situated on short-term or borrowed property, they are susceptible to changes in the form of redevelopment. The success of community gardens

also depends on efficient administration and planning, as these initiatives need for constant upkeep and cooperation from all involved parties.

5. Smart Technology in Urban Horticulture

The way we cultivate and maintain plants in cities is changing as a result of the incorporation of smart technologies into urban gardening. Urban horticulture methods are being optimized by technology, which includes smartphone applications and Internet of Things devices, to make them more accessible and efficient.

i. Internet of Things and Sensors: Urban horticulture is using the Internet of Things (IoT) and sensor technologies more and more to monitor and control environmental factors including temperature, humidity, soil moisture, and light levels. Real-time data gathering and analysis made possible by these technology improves plant productivity and health by giving growers exact control over growth conditions.

ii. Mobile Applications: Urban horticulture is benefiting greatly from the usage of mobile applications, which provide consumers access to information on pest control, plant maintenance, and garden design. Through arranging activities, exchanging materials, and bringing gardeners together, these applications may help promote community involvement.

iii. Examples of Smart Technology in Use: Numerous intelligent gardening devices have been created specifically for urban settings. For instance, the Edyn Garden Sensor is a solar-powered gadget that tracks soil conditions and gives gardeners immediate feedback via a smartphone app. Comparably, the Parrot Flower Power is a wireless sensor that monitors plant health and notifies customers when their plants want fertilizer or water.

Challenges in Urban Horticulture

Urban horticulture has many advantages, but in order to reach its full potential, it must overcome a number of obstacles.

1. Space Constraints

The lack of available space is one of the biggest obstacles in urban gardening. Land for horticulture is sometimes expensive and difficult to get in urban areas due to their crowded population.

i. Maximizing Space: Urban horticulturists are using creative methods like container gardening, rooftop gardens, and vertical growing to get around space limits. Due to the effective use of available space, plants may now be grown in locations that would not be suited for traditional gardening.

ii. Creative Solutions: To optimize space in urban contexts, new designs and technologies are being created in addition to conventional ways. For instance, you may grow more plants in a less space by using stacked or rotating garden systems. In order

to maintain horticulture's place in the urban environment, urban planners are now introducing green areas within recently constructed buildings.

2. Water Management

Horticulturalists depend heavily on water, and managing water in urban settings may be difficult, especially in areas with scarce water supplies.

i. Water Scarcity: Water shortage is a common issue in urban areas because of the high demand and limited supply. Climate change has the potential to make this scarcity worse by causing more frequent and severe droughts.

ii. Efficient Irrigation Systems: Urban horticulturists are implementing effective irrigation techniques like drip irrigation, which minimizes waste by delivering water straight to the plant roots, in response to the scarcity of water. By only using water when necessary, automatic irrigation systems and moisture sensors may also assist optimize water consumption.

iii. Greywater and Rainwater Harvesting: In urban gardening, rainwater collecting systems and greywater—recycled water from sinks, showers, and washing machines—are growing in popularity. By collecting and reusing water that would otherwise be wasted, these systems help to lower the demand for fresh water and offer urban gardens a reliable supply of water.

3. Soil Quality and Contamination

Urban soils frequently have problems with pollution and low quality, which can be harmful to plants and their growth.

i. Soil Contamination: Urban soils can be contaminated with heavy metals, chemicals, and other pollutants from industrial activities, vehicle emissions, and construction. These toxins can build up in plants, rendering them unfit for human consumption and degrading the garden's general health.

ii. Solutions for Contaminated Soil: In urban horticulture, there are several approaches that may be used to manage soil pollution. Using clean, imported soil or growth media, raised beds and container gardening are good strategies to prevent contaminated soil.

References:

1. Manoharan, S. And Kamath, S.R. (2023). Urban horticulture: a way for self-reliance and nutritional, Just agriculture, August.
2. Jill L. Edmondson. (2024). Urban horticulture: Building the evidence base to support integration into cities and towns. Plants people planet, 6:777–779.
3. https://www.freepik.com/free-photos-vectors/urban-farming.

BIOSYNTHESIS OF ZINC OXIDE (ZnO) AND MAGNESIUM OXIDE (MgO) NANOPARTICLES USING FRUIT WASTES AND ITS APPLICATION IN AGRICULTURAL SECTOR AS A BIOPLASTIC MULCH

S. S. Patil[1], B. S. Pal[1], S. K. Naik[1], S. R. Wadekar[1], M. S. Hate[1] and R. S. Chaughule[1,2]

[1]Department of chemistry, Ramnarain Ruia Autonomous College, L.N. Road, Matunga (east), Mumbai-400019.

[2]Tata Institute of Fundamental Research, Mumbai, India

Corresponding author E-mail: satwikpatil9@gmail.com, bhavanaapal@gmail.com, sandhya03012003@gmail.com, shravaniwadekar@ruiacollege.edu, manishhate@ruiacollege.edu, rsctifr@yahoo.com

Abstract:

The ample quantity of food leftovers from variety of sources is an environmental hazard if not disposed of appropriately. Thus, introduction of a cheap and easy approach for food waste utilization is an ideal option to seek at present. (e.g., formation of valuable items while reducing the quantity of waste). The adoption of such a process is expected to reduce the production cost of biodegradable plastics as well as simultaneously reduce the dependence on nonbiodegradable and single use plastics. This article focuses on the production of bioplastics from banana peels. These were proposed to replace the use of polyethylene as an agricultural mulch. In this respect, detailed attention was paid to the development of ZnO and MgO nanoparticles using ultra sonication and fusing these with the bioplastic films. The efficiency of these bioplastic mulches was studied on the growth of plants for a period of three months while considering other factors viz health of the plants, biodegradability and tensile strength of the mulches. In this research, zinc oxide nanoparticles (ZnO NPs) were synthesized using an eco-friendly approach, by deploying *Citrus limetta* fruit peel methanolic extract as a stabilizer as well as bio reducing agent and zinc nitrate hexahydrate as a zinc precursor and Magnesium oxide (MgO NPs) using *Punica granatum* fruit peel methanolic extract as a reducing agent and magnesium nitrate hexahydrate as a Magnesium precursor. The synthesized NPs were confirmed by UV, FTIR, and XRD

Keywords: Bioplastics, Mulch, ZnO NPs, MgO NPs, Biopolymer, Plant Extract, Bio-Nanocomposite Films.

Introduction:

Nanotechnology is a growing area of research not just limited to materials but also include biomedical and engineering applications, hence Nano biotechnology, a

novel multidisciplinary approach which serves as a technique in production of the nanoparticles by green nature friendly methods for biomedical and agricultural applications. Nanoparticles have an ample of applications in various fields such as energy science, catalysis, single electron transistors, mechanics, optics, electronics, optoelectronics, nonlinear optical devices, space industries, chemical industries, biomedical sciences, drug delivery, tissue engineering, food, health care agricultural field and cosmetics, because the present world utilizes plastics for daily purposes, about 266 million tones of plastics generated worldwide in the year 2010, of which 57 million tones were generated in just Europe [1]. Low and high-density polyethylene, polyvinyl chloride are some of the most commonly available plastics used for packing of items. Unfortunately, these cannot be degraded by present natural microorganisms upon their disposal in the environment [2]. The early interest in bioplastics started in the early 20th century when scientist Henry Ford took corn and soybean oils to manufacture the automotive parts [3]. Large structures from natural polymers and smaller molecules such as sugar, disaccharides and fatty acids have been studied as major raw materials for the production of bioplastics. Starch is the most studied sources for the development of bioplastic.

Many previous studies have been performed with starch as a natural polymerizing agent. Starch consists of a straight, branched and long chains of two beta d-glucose units connected together by glyosidic linkage. The branched polymerized amylopectin and the straight chain amylose, which gives its granular structure. Due to its easy availability, cheap cost renewable nature and biodegradability, starch is mostly used in the production of bioplastics. Starch can behave like a thermoplastic with a plasticizer, in presence of application of heat and mechanical treatment [4]. As earlier starch-based films are prone to high water affinity and brittleness, other natural biopolymers are mixed with starch as fillers to improve the tensile strength of the films' and other properties [5]. Banana peel is the most common form of starch and a large bio waste around the world especially in tropical countries of Asia like India, Indonesia, etc. The disposal of these large amounts of wet organic waste can ultimately harm the environment and lead to health problems such as respiratory disorders due to production of gases like methane. [6]. Banana peels have high percentage of starch, about 18.5% [7]. As banana peels ripe, the glucose in them increases. But, if the peels are too ripe, starch will be converted into glucose while fully, unripe peels are very firm although are high in starch. [8]. *Punica granatum (P. granatum F.)* or pomegranate is described as a power fruit, known for its superior pharmacological properties and health prospects to consumers worldwide [9]. P. granatum F. peel makes around one third of the whole fruit and thus becomes a byproduct which is thrown after

consumption. The actual percentage of edible portion of the pomegranate to the inedible portion (peels) is very low.

The utilization of these agriculture-wastes from fruits has gained tremendous attention in recent years due to their easy availability and cost-effectiveness [10]. *P. granatum F.* peel has been found to contain extraordinarily high concentration of phenolic compounds as natural antioxidants which include punicalagin, gallic acid, ellagic acid, chlorogenic acid, caffeic acid, punicalin, apigenin, quercetin, and anthocyanins like pelargonidin, cyanidin, granatin A and granatin B. [11]. These are mostly concentrated in the peels of P. granatum and are solely responsible for the reduction of metal precursors to aid in the green synthesis of a whole variety of different NPs [12]-[14]. The usage of biological methods is environmentally safe and friendly, as tiny microorganisms or plants bring about the formation NPs with an mean size of up to 100-110 nanometers [15]. Plants and microorganisms are used for bio reduction and synthesis of stable NPs thanks to coating agents, with the possibility of the extra/intracellular synthesis of NPs [16]. In addition, plant synthesis methods are the best biological options for getting NPs at a low cost, easily, and in huge quantities, with better control over their size, shape, and their ability to interact positively with the biological surfaces like membranes , which allows them to be incorporated in the medical treatment of deadly illnesses like cancer, drug delivery, and as a antibacterial, insecticidal and antifungal agents, as well as enabling their successful use in insecticides and in agricultural sector [17].

A Mulch is used to hold moisture in the soil, it also decreases the growth of weed or blocks weeds, keeps the soil and roots cool from the sunlight, prevents freeze drying of plants in winter, and makes the garden and landscape look more attractive if applied aesthetically. In general words a mulch is any material, which is used to cover soil's top or upper surface. If they are infused with essential micro nutrients they can also add nutrients to the soil. There are two types of mulches: Organic- These are natural substances that are devoid of anything synthetic and is the good option for adding beneficial nutrients to soil. Inorganic- This contains a synthetic chemical. It can be a best option for entirely blocking weeds and retaining water and moisture in the soil and also for trapping heat in the soil. Inorganic mulch lasts longer than organic mulch but doesn't add nutrients to the soil but ultimately pollute the soil [18]. Plastic mulch films were first used in the late 1950s in research and have been used commercially for vegetable production from the early 1960s onwards [19]. The increase in production and lack of biodegradability of commercial polymers, particularly commodity plastics used in agriculture and packaging, focused public attention on a potentially huge environmental accumulation and plastic pollution problem that could persist for over

centuries Removal of the plastic is expensive and is time-consuming and despite use of machines still requires hand labor as small bits of plastic cannot be cleaned by machines. The residual film particles if left in the field may interfere with root development of the subsequent crop.

Materials and Methods:

Citrus fruits peels and pomegranate peels were obtained from local vendors of Dadar Market for the bio reduction of Zn and Mg salts respectively. AR grade Zinc Nitrate and Magnesium nitrate Heptahydrate were used. Analytical Grade NaOH of Loba Chemials was used. For mulch synthesis, banana peels, lab grade chitosan and Agar were used. Analytical grade Hydrochloric Acid and glycerol were used. Instruments used were Sonicator, Magnetic Stirring Apparatus, Hot air oven, crucibles and Crucible furnace. For testing the effectiveness of the synthesized bioplastic mulch model plant selected was *Trigonella Foenum-Graecum (f*enugreek) plant.

A) Preparation of methanolic extract of *Citrus limetta:* The fruit peels were washed with distilled water and kept drying for 1 day at 60°C and then was crushed into fine powder. Methanol was added to it and it was kept in Ultrasonicator for efficient extraction. After completion of extraction the extract was filtered using Whatmann filter paper No 41.

B) Preparation of methanolic extract of *Punica granatum*: The fruit peels were washed with distilled water and kept drying for 1 day at 80°C and then was crushed into fine powder. Methanol was added to it and it was kept in Ultrasonicator for efficient extraction. After completion of extraction the extract was filtered using Whatmann filter paper No 41.

C) Preparation of ZnO NPs: Appropriate quantity of the methanolic extract was taken in a beaker and placed in the sonicator and Zinc nitrate hexahydrate was added. Further NaOH was added drop wise till precipitate forms and the pH of the solution becomes basic. Then it was kept into sonicator for more 2 hours. After completion of precipitation the extract was filtered and the precipitate was washed with deionized water and kept for drying. Then it was heated in a crucible furnace for 2 hours at 450°C for annealing and thus ZnO NPs were formed which were further characterized.

D) Preparation of MgO NPs: Appropriate quantity of the methanolic extract was taken in a beaker and placed in the sonicator and Magnesium nitrate hexahydrate was added.

Further NaOH was added dropwise till precipitate forms and the pH of the solution becomes basic. Then it was kept into sonicator for more 2 hours. After completion of precipitation the extract was filtered and the precipitate was washed with

deionized water and kept for drying. Then it was heated in a crucible furnace for 2 hours at 450°C for annealing and finally MgO NPs were formed which were further characterized.

E) Preparation of ZnO and MgO mixed bioplastic film: Banana peels obtained were washed with dH_20 and then cleaned properly. They were then dipped into acetic acid for some time and then added into water to boil for half an hour. HCl was added and Glycerol was mixed in increasing concentration of Chitosan and Agar was added as a co – Biopolymer to achieve an ideal biofilm and then it was continued stirring for 30 minutes. Then NaOH was added and stirred and the an appropriate quantity of finely divided ZnO and MgO NPs were mixed in and stirred. Then the mixture was poured into a mold and spread into a thin layer with a wooden rod. The molds were then placed in a 130^0C oven and baked for 30 minutes.

F) Tensile Strength: A small piece of bioplastic film was hooked onto the spring balance and weight was added to check the tensile strength. The weight was added until film broke. This was done 4-5 times. and tensile strength was calculated by the following formula

Tensile Strength = Weight Load (N) /Area of cross section of biofilm (M2).

The biofilms were then used as a mulch to check the efficiency of growth in fenugreek plants, the antibacterial and insecticidal activity as well as the biodegradability of the mulch. The plants were monitored daily for a period of three months. Regular watering of plants was done according to the need.

Results and Discussions:

1) UV Analysis:

For MgO NPs: The absorption peak at 280 nm proved that the NPs has unique properties related to their size, shape, and composition. The lambda max in the range of 260-280 matched with the literature values

For ZnO NPs: The lambda max (wavelength at which maximum absorption takes place) from the spectrum is observed at 372 nm which matches with that of ZnO NPs as given in the literature.

2) FTIR Analysis:

For ZnO NPs: Different bands are observed in the 1600–800 cm^{-1} regions which corresponds to the organic content from plant extract. A sharp peak at 530 cm^{-1}, corresponds to the characteristic bending bond vibration of Zn–O, which confirms or proves that the ZnO NPs are synthesized.

For MgO NPs: The FTIR analysis of the nanoparticles as well peel extract shows the presence of four peaks at wavenumbers 3450, 2050, 1630, 1050 cm^{-1}. The

peak at 3450 cm^{-1} is that of alcohol group which shifts to lower value of 3370cm^{-1}after the preparation of MgO NPs. Similarly all the different peaks are either shifted to higher or lower values of wavenumbers The unique peak obtained at wavenumber value of 525 cm^{-1} relates to metal oxygen bond i.e Mg-O bond in MgO NPs.

3) XRD Analysis:

For ZnO NPs. XRD analysis is used to determine the crystalline size and phase identification of the nanoparticles. The XRD pattern of synthesized ZnO NPs was matching with standard JCPDS File. The XRD pattern indicates that the ZnO NPs have hexagonal structure. The diffraction peaks of ZnO nanoparticles are at 2θ = 35.45, 44.72 and 67.30 in planes of 101, 102 and 202,respectively. For MgO NPs: Five peaks, 109.12, 199.12, 220.13, 310.44, and 220.13, were observed at 2-theta degrees of 38.8◦ , 44.7◦ , 61.3◦ , 73.6◦ , and 77.7, respectively. in accordance to the JCPDS file 75-0447.

SEM Results:

For ZnO NPs: SEM images of ZnO nanoparticles can show that they are irregular or spherical in shape.

For MgO NPs: SEM images of MgO nanoparticles have shown that they are spherical in shape and have an average size of less than 20 nanometers.

TEM Results:

For ZnO NPs: TEM images of ZnO nanoparticles can show that they are about 20 nanometers in size.

For MgO NPs: TEM analysis has confirmed that MgO nanoparticles have a cubic structure.

Conclusion:

The ZnO and MgO NPs were successfully synthesized by bio reduction from waste peels of *Citrus limetta* and *Punica granatum* respectively. By using variable concentrations of chitosan and agar the NPs induced bioplastic mulch showed great tensile strengths as well as rapid growth with big leaf sizes. The bioplastic mulch also showed biodegradability as it completely biodegraded within one month of use.

The synthesis of ZnO NPs and MgO NPs from fruit wastes and their incorporation into bioplastic mulch films represents a novel and sustainable approach to enhancing agricultural practices. This study successfully demonstrates that fruit waste can be utilized as an eco-friendly and cost-effective source for the green synthesis of nanoparticles, which are characterized by desirable properties such as antimicrobial activity and nutrient delivery. The application of these nanoparticles in bioplastic mulch films offers a promising alternative to conventional plastic mulches, addressing key

environmental issues such as plastic pollution and soil degradation. The biodegradable nature of the mulches, combined with the functional benefits of ZnO and MgO nanoparticles, leads to improved crop protection, enhanced soil health, and increased agricultural productivity. This project paves the way for a circular economy in agriculture, where waste products are repurposed into valuable inputs, contributing to more sustainable and resilient farming systems. The results suggest that further optimization and field trials are needed to fine-tune the nanoparticle concentrations and evaluate long-term impacts on soil and plant health. Overall, this innovative approach holds significant potential for transforming agricultural practices, reducing environmental footprints, and promoting sustainable resource use.

Future Scopes:

1) Nanoparticles can be coated with plant growth regulators and can be engineered to release them slowly when required.
2) NPs of ZnO and AgO contains excellent antimicrobial activity against soil borne pathogens and can be used to reduce the dependency on the use of chemical pesticides.
3) Arsenic nanoparticles could be used incorporated in the mulches to repel of insect species like grasshoppers
4) Nanoparticles could enhance the physical properties of bioplastic mulches, such as their ability to improve soil aeration and reduce compaction.
5) As technologies advance and become more cost-effective and the knowledge of these bio mulches reaches the common crowd, the use of nanoparticle-enhanced bioplastic mulches could become economically viable for broader applications, making them accessible to a wider range of agricultural operations.
6) Development and integration of certain attractant chemicals (to attract potential pollinators for plants) with the bio mulches could also be made to increase the overall pollination of the plant species.
7) The synthesized nanoparticles could be explored for other applications, such as in water purification, food packaging, or as antimicrobial agents in other sectors, thereby expanding their commercial value and impact.
8) The success of this approach could inspire more research into the green synthesis of nanoparticles using other types of agricultural and industrial waste, broadening the scope of eco-friendly nanotechnology

References:

1. Sprajcar, M., Horvat, P., & Kržan, A. (2012). Biopolymers and bioplastics: Plastics aligned with nature. National Institute of Chemistry.

2. Qin, Y., Yang, J., & Xue, J. (2015). Characterization of antimicrobial poly (lactic acid)/poly (trimethylene carbonate) films with cinnamaldehyde. *Journal of Materials Science*, 50, 1150-1158.
3. Bioplastics Industry Overview Guide. (2012). R.
4. Agustin, M. B., Ahmmad, B., Alonzo, S. M. M., & Patriana, F. M. (2014). Bioplastic based on starch and cellulose nanocrystals from rice straw. *Journal of Reinforced Plastics and Composites*, 33, 2205-2213.
5. Bof, M. J., Bordagaray, V. C., Locaso, D. E., & García, M. J. (2015). Chitosan molecular weight effect on starch-composite film properties. *Food Hydrocolloids*, 51, 281-294.
6. Nasution, Z., Lim, R. Y., & WZS, W. H. (2012). Banana peel flour: An alternative ingredient for wholemeal bread. Unpublished PhD thesis, University Malaysia Terengganu.
7. Astuiti, P., & Erprihana, A. A. (2014). Antimicrobial edible film from banana peels as food packaging. *American Journal of Oil & Chemical Technologies*, 2(2), 65-70.
8. Soltani, M., Alimardani, R., & Omid, M. (2010). Prediction of banana quality during ripening stage using capacitance sensing system. *Australian Journal of Crop Science*, 4(6), 443-447.
9. Chaudhary, A., & Rahul, S. N. (2017). Antibacterial activity of *Punica granatum* (pomegranate) fruit peel extract against pathogenic and drug resistance bacterial strains. *International Journal of Current Microbiology and Applied Sciences*, 6(12), 3802-3807.
10. Adelere, I. A., & Lateef, A. (2016). A novel approach to the green synthesis of metallic nanoparticles: The use of agro-wastes, enzymes, and pigments. *Nanotechnology Reviews*, 5(6), 567-587.
11. Singh, B., et al. (2018). Phenolic compounds as beneficial phytochemicals in pomegranate (*Punica granatum* L.) peel: A review. *Food Chemistry*, 261, 75-86.
12. Fuku, X., Diallo, A., & Maaza, M. (2016). Nanoscaled electrocatalytic optically modulated ZnO nanoparticles through green process of *Punica granatum* L. and their antibacterial activities. *International Journal of Electrochemistry*, 1-10.
13. Nasiriboroumand, M., Montazer, M., & Barani, H. (2018). Preparation and characterization of biocompatible silver nanoparticles using pomegranate peel extract. *Journal of Photochemistry and Photobiology B: Biology*, 179, 98-104.
14. Phongtongpasuk, S., & Poadang, S. (2015). Green synthesis of silver nanoparticles using pomegranate peel extract. *Advanced Materials Research*, 1131, 227-230.

15. Vijayaram, S., Razafindralambo, H., Sun, Y. Z., Vasantharaj, S., Ghafarifarsani, H., Hoseinifar, S. H., & Raeeszadeh, M. (2023). Applications of green synthesized metal nanoparticles—A review. *Biological Trace Element Research*, 1-27.
16. Kulkarni, D., Sherkar, R., Shirsathe, C., Sonwane, R., Varpe, N., Shelke, S., More, M. P., Pardeshi, S. R., Dhaneshwar, G., Junnuthula, V., et al. (2023). Biofabrication of nanoparticles: Sources, synthesis, and biomedical applications. *Frontiers in Bioengineering and Biotechnology*, 11, 1159193.
17. Ismael, A. F., Ahmed, N. M., Ibrahim, K. H., & Al-Kubaisi, A. A. (2023). Tea plant leaves for green synthesis of metallic nanoparticles. *Macromolecular Symposia*, 407, 2100377.
18. Iqbal, R. (2020). Potential agricultural and environmental benefits of mulches—a review. *Environmental Science and Pollution Research*, 44, Article 75.
19. Kasirajan, S., & Ngouajio, M. (2012). Polyethylene and biodegradable mulches for agricultural applications: A review. *Horticultural Science*, 32, 501-529.

EARTHWISE PADS: ANTIMICROBIAL AND BIODEGRADABLE SANITARY PADS WITH NANOMATERIALS FUSED POLYMERS

D. K. Rokade[1], D. R. Gawade[1], S. P. Disale[1], S. S. Kamble[1],
A. M. Purandare[1], S. R. Wadekar[1], M. S. Hate[1] and R. S. Chaughule[1,2]
[1]Department of Chemistry,
Ramnarain Ruia Autonomous College, L. N. Road, Matunga (East), Mumbai – 400019
[2]Tata Institute of Fundamental Research, Mumbai, India
Corresponding author E-mail: rokadediksha141@gmail.com,
deeshagawade03@gmail.com, shrutidisale@gmail.com, sakshikamble889@gmail.com,
athangpurandare414@gmail.com, shravaniwadekar@ruiacollege.edu,
manishhate@ruiacollege.edu, rsctifr@yahoo.com

Abstract:

Menstrual hygiene and awareness have been the need of the hour for women's physical and mental health. Menstruation is an integral part of women's life and so are sanitary napkins. While on her menstruation periods, a woman surely experiences discomfort, irritation, and menstrual pain. Use of ashes, rages, leaves, and other unsafe material have been a practice before the evolution of napkins. Advent of napkins has been from early 19th century and the study still thrives for betterment. Modern era has led women's from using commercially available sanitary napkins made of synthetic and non-biodegradable materials, not having any cotton materials and there are dangerous chemicals which may stay in body for 20yrs after the exposure. In similar ways the fragrance and deodorants used in napkins nowadays can enter the blood stream and affect locally in the vagina which causes several discomfort and diseases viz. cervical cancer, Urinary Tract Infection, Polycystic Ovary Syndrome (PCOS), rashes, allergic conditions, etc. Herbal sanitary pads represent an innovative approach to menstrual hygiene management by integrating natural products with conventional pad technology. Sanitary pads made from natural fibers and infused with herbal extract. The inclusion of natural fibers and herbal extracts aim to enhance comfort, reduce menstrual discomfort, and promote skin health. Moreover, Nanomaterials are significantly enhancing the performance of herbal sanitary pads. Nanomaterials enhance absorption, lock in moisture provides comfort and reduces the risk of leaks. Nanoparticles like silver provide antimicrobial benefits, helping to reduce odors and prevent infections by inhibiting the growth of bacteria and fungi. They also create better air permeability, allowing for improved ventilation and reducing the chances of irritation and rashes. Nanotechnology allows for the efficient delivery of herbal extracts and active ingredients, ensuring they are effectively incorporated into the pad's structure and

provide sustained benefits such as soothing and anti-inflammatory effects. Nano-sized materials can create a softer, gentler surface, which can be less irritating to sensitive skin compared to traditional materials. This paper reviews the composition and efficacy of herbal sanitary pads infused with nanomaterials, evaluating their performance compared to traditional and organic alternatives. Additionally, it examines the potential benefits for women with sensitive skin or specific health concerns, as well as the environmental implications of using biodegradable and natural materials. The findings suggest that herbal sanitary pads offer a promising solution for improving menstrual hygiene while supporting eco-friendly practices.

Keywords: ZnO NPs, AgO NPS, Herbal Extract, Biodegradable Materials, Urinary Tract Infection, PCOD.

Introduction:

Nanotechnology represents a revolutionary path for technological development that concerns the management of material at the nanometer scale (one billion times smaller than a meter). It has the potential to change our perspectives and expectations and provide us with the capability to resolve global issues. The discovery and use of carbon nanomaterials has allowed the introduction of many new areas of technology in nanomedicine, biosensors, and bioelectronics. [1-3] Nanotechnology holds the promise of revolutionizing many fields by providing new solutions to complex problems. Its continued development and integration into various industries could lead to significant advancements in medicine, technology, and environmental sustainability. In summary, nanotechnology leverages the unique properties of materials at the nanoscale to create innovative solutions across diverse sectors, offering the potential for ground-breaking improvements in both everyday applications and advanced technologies. [4],[5] The essence and promise of nanoscience and nanotechnology are illustrated in the fact that the properties of materials (i.e., chemical, physical, and biological) at the nanoscale may be quite different from those within a bulk material. When the dimensions of a material are reduced below 100 nm dramatic alterations may occur in their properties. [6] Materials therefore might be nanostructured in order to provide a specific performance or to provide new properties to a material in addition to changes linked specifically to size and structure. Such macromolecules and particles made of a small number of molecules, at sizes of 1–50 nm, possess distinct physicochemical properties. Compared with bulk materials, nanoparticles (NPs) possess properties of increased performance when they are used for similar applications [7].

Menstruation is (often called as periods) a biological and natural process occurring in a woman's body monthly. This process of shedding of the endometrium lining lasts for 3-7 days. The span of menstrual cycle is of 28 days. [8]-[10] Over centuries, women used fabrics, leaves, etc as an absorbing material to absorb the blood

discharge. Such materials used to cause health issues in women. So, menstrual hygiene products are essential for women's health and well-being. Evolution of sanitary napkins from 19th century has slowly replaced the use of old fabrics, leaves etc., during menstruation. Most of the commercial sanitary napkins have SAP (superabsorbent polymer) and synthetic materials.[11] These materials are non-biodegradable. These conventional sanitary pads often raise concern due to their synthetic materials, potential skin irritants, and environmental impact. They contribute significantly in global waste and posing long term environmental challenges. The sap ingredient used in commercial pads are non-biodegradable and cause irritation/discomfort, eventually affecting the menstrual health. Considering these challenges, there is a growing demand for more natural, sustainable alternatives that cater to the needs of modern consumers. Herbal sanitary pads present a novel solution by combining the benefits of natural fibers with the antibacterial and antifungal properties of herbal extracts. Lemon extract is also used for fragrance. [12]-[14] For these natural fibers are used as an absorbing material. Herbal pads function similarly to regular menstrual pads in terms of absorbency and comfort. The key difference is the addition of herbal components. Herbal pads typically have a core layer designed to absorb menstrual flow, just like conventional pads. The herbal ingredients are usually integrated into the pad's top layer or sometimes in a separate compartment. [15]-[17] This layer is in contact with the skin, allowing the herbs to interact with the body. Some individuals may be sensitive or allergic to certain herbs. It's important to review the ingredient list and conduct a patch test if you have sensitive skin. The scientific evidence supporting the specific benefits of herbal pads is limited. While anecdotal evidence suggests benefits, more research is needed. Moreover, users should consider their personal comfort and needs. Some may prefer herbal pads for their added benefits, while others might opt for traditional pads based on effectiveness and simplicity.[18]

Composition of Herbal Pads -

1. Absorbent Core Material: The core of herbal pads is made from absorbent materials like coconut fiber, cotton. This core design captures and holds menstrual blood, ensuring dryness and comfort.

2. Herbal Infusion: The top layer of the pad may be infused with various herbal extracts. This layer is in direct contact with the skin. Herbs can be incorporated in several ways:

a. Herbs are embedded into the pad's surface or core.

b. Herbal materials might be added as a separate layer between the absorbent core and the outer lining.

3. Outer Layers:

Top Sheet: It is made up of soft, breathable materials like cotton or a cotton blend. It may be treated to keep the herbal extracts in place.

Back Sheet: It is usually a waterproof material which prevent leaks and protect clothing.

Methodology:

Step 1: synthesis of ZnO and AgO Nps :

1) Synthesis of ZnO nps – 20 ml of the plant extract was heated at 50 °C for 10 min and 50 ml of 91 mM of zinc acetate solution (1 g of zinc acetate was dissolved in 50 ml of distilled water) was added drop wise to it under stirring. The reaction mixture became yellowish and cream-colored precipitate of zinc hydroxide was formed. The reaction mixture was left for 30 min for complete reduction to zinc hydroxide. Then the precipitate was collected by centrifugation at 16 000 rpm for 10 min at 4 °C. The precipitate was vacuum dried at 30 °C and the sample (PZN30) was stored for further studies. [19]-[23]
2) Synthesis of AgO Nps – 1 mM silver nitrate solution in double distilled water was the source of silver. Silver nitrate and seed extract were mixed in a ratio of 1:9. The reaction mixture was heated below the boiling point and continuously stirred at 800 rpm using magnetic stirrer. The mixture turned reddish brown in color within 1 h. The whole reaction was carried out in the dark. The obtained suspension was centrifuged at 15,000 rpm for 45 min. The pellet containing silver nanoparticles was washed 3–4 times with deionized water to remove silver ions and seed extract residue.[24],[25]

   The lemons were bought from the local vendors of Matunga. The lemon peels were then dried in the oven and a fine powder of them was prepared. Neem powder was bought from local market.

Step 2: Preparation of extract:

a. Neem extract made using 1:1 methanol
b. Lemon peel extract using 1:1 methanol

Post filtration, extract was ready.

Step 3: Absorbent sheet making:

a. PVA + water + aloe vera gel + coconut coir this mixture was used as a gel and then spread on a cotton layer

b. Kept in oven for 2-3 hrs.

This sheet works as an absorbent layer, effectively absorbs menstrual blood.

Step 4: Pad making process:

a. Lower layer of thick non-woven cotton sheet

b. absorption layer of cotton and coconut coir (coconut fiber)

c. antibacterial layer of extract
d. top layer of thin non-woven absorbent sheet.

Results and Discussions:

The research project on the development of herbal pads has yielded promising results. Through extensive experimentation and testing, we have successfully created a prototype that combines the natural benefits of herbal extracts with the convenience of disposable pads. The herbal pads have shown to overcome menstrual problems such as irritation, foul smell of blood, discomfort, eventually leading to a healthy menstrual health for the women. Additionally, the natural materials used in our pads have made them biodegradable unlike the commercial pads, and hence their way of disposable was environment friendly. These findings suggest that our herbal pads are not only an effective solution for menstrual comfort but also a sustainable alternative for environmentally conscious consumers. Further research and development are underway to refine our product and bring it to market.

Herbal sanitary pads are an innovative product designed to blend traditional herbal remedies with modern menstrual hygiene solutions. Here is a detailed discussion on their various aspects:

1. Composition and Herbal Ingredients

Herbal sanitary pads incorporate natural plant extracts and essential oils aimed at providing additional benefits beyond basic menstrual protection. Common herbs used include:

- **Aloe Vera:** Known for its soothing and moisturizing properties, it helps reduce irritation and discomfort.
- **Chamomile:** Has anti-inflammatory and calming effects, potentially alleviating menstrual cramps, and discomfort.
- **Lavender:** Offers antibacterial properties and a calming scent, which can help with relaxation.
- **Witch Hazel:** Often used for its astringent properties to reduce swelling and inflammation.

2. Benefits

- **Reduced Irritation:** The natural herbal ingredients are typically gentler on the skin compared to synthetic materials, which can be beneficial for people with sensitive skin or allergies.
- **Antibacterial and Antifungal Properties:** Many herbal extracts used in these pads have natural antimicrobial properties, helping to reduce the risk of infections and odor.

- **Comfort and Absorption:** Advances in nanotechnology enhance the absorption and breathability of herbal pads, making them more effective and comfortable to wear.

3. Environmental Impact

- **Biodegradability:** Many herbal pads are designed to be more eco-friendly compared to conventional pads. They often use biodegradable materials, which helps reduce the environmental footprint.
- **Natural Materials:** The use of natural fibers and herbal extracts aligns with a growing trend towards sustainable and environmentally conscious products.

4. Challenges

- **Efficacy and Consistency:** The effectiveness of herbal pads can vary depending on the quality and concentration of the herbal ingredients used. There is also a need for rigorous testing to ensure that these pads meet hygiene standards.
- **Cost:** Herbal and eco-friendly products can be more expensive due to the cost of natural materials and manufacturing processes.
- **Consumer Awareness:** While herbal pads offer numerous benefits, not all consumers may be aware of these advantages or may be skeptical about their effectiveness compared to traditional options.

5. Market Trends

- **Growing Demand:** There is an increasing interest in natural and organic products, including menstrual hygiene items. Consumers are becoming more conscious of the materials they use and their impact on health and the environment.
- **Innovation:** The integration of advanced materials like nanotechnology into herbal pads is driving innovation, leading to improvements in performance, comfort, and efficacy.

6. Regulatory and Safety Considerations

- **Regulation:** Herbal sanitary pads must comply with regulations and standards to ensure safety and efficacy. This includes testing for potential allergens and ensuring that the herbal ingredients do not cause adverse reactions.
- **Transparency:** Brands need to provide clear information about the ingredients used and their benefits, allowing consumers to make informed choices.

Nanomaterials of silver and zinc oxide are known for antimicrobial activities but the antimicrobial activity is lost due to oxidation and nanomaterials pose toxicity on human tissues. In the present study, nanomaterials were coated with agarose to prevent oxidation and retain antimicrobial activity for a long period without toxicity to humans.

Challenges:

a) Material Constraints: Creating a pad that is both biodegradable and highly absorbent can be challenging. Also, biodegradable materials may not always match the absorbency levels of conventional pads.

Solution are as follows:

Material Innovation: Research and develop new biodegradable materials that offer high absorbency and comfort. Innovations in plant-based fibres and advanced manufacturing techniques can help address this issue.

Testing and Quality Assurance: Conduct thorough testing to ensure that biodegradable pads perform effectively throughout their intended use.

b) Degradation of Herbs: Herbal ingredients may degrade or lose their potency over time, especially when combined with biodegradable materials that break down faster.

Solution: Use packaging that protects the herbal ingredients from environmental factors like moisture and air, which can affect their stability.

c) Market Availability: Limited availability in some regions can make it difficult for consumers to find and purchase biodegradable herbal pads.

Solution: Increase distribution channels to make these products more widely available.

Key risks associated with nanoparticles:

Uncertainty and Unknowns

Limited Data: The long-term effects of exposure to nanoparticles are not well understood due to the relatively recent development of nanotechnology. There is a need for more research to assess potential risks thoroughly.

Varied Properties: Different nanoparticles have distinct properties (size, shape, composition) that can influence their behavior and toxicity, making it challenging to generalize risks across all types of nanoparticles.

Limitations:

Herbal pads infused with nanoparticles are a promising innovation in the field of personal care, offering potential benefits like enhanced antimicrobial properties, better absorption, and improved skin health. However, they also come with several limitations:

Safety Concerns: The long-term safety of nanoparticles, especially when used in intimate areas, is still under scrutiny.

Regulatory Challenges: Regulatory bodies are still developing guidelines for the use of nanoparticles in consumer products.

Cost: The incorporation of nanoparticles into herbal pads can increase production costs, making these products more expensive than conventional options.

Effectiveness: The actual effectiveness of nanoparticles in enhancing the properties of herbal pads may vary.

Consumer Acceptance: Some consumers might be hesitant to use products with nanoparticles due to concerns about safety, the environment, or simply a preference for more natural or traditional products.

Future Scope:

The future scope for herbal sanitary pads looks promising due to increasing awareness of environmental and health concerns. Herbal sanitary pads, which often use natural and biodegradable materials, can tap into this growing market. There is room for innovation in terms of the herbs and natural materials used. Enhanced formulations that provide additional benefits, such as antimicrobial properties or improved comfort, could attract more users. Research into the specific health benefits of the herbs used can provide a competitive edge. If certain herbs are found to have therapeutic properties, such as reducing menstrual discomfort or maintaining pH balance, these benefits can be highlighted. Making herbal sanitary pads more affordable and widely available, especially in underserved regions, could help broaden their adoption. This might involve scaling up production or finding cost-effective materials.

Conclusion:

This study explored the effectiveness and acceptability of herbal sanitary pads as an alternative to traditional products. Our findings suggest that herbal sanitary pads made from natural ingredients such as cotton, coconut coir, and herbal extracts (neem and lemon peels) offer several benefits, including:

a) Improved menstrual health and hygiene
b) Reduced incidence of vaginal infections and irritation
c) Enhanced comfort and breathability
d) Increased eco-friendliness and sustainability
e) Addition of fragrance (foul smell of blood masked)

These results indicate that herbal sanitary pads are a viable option for women seeking a more natural, comfortable, and environmentally conscious solution for their menstrual needs. Further research can build upon these findings to optimize product design, ingredients, and accessibility. By promoting herbal sanitary pads, we can empower women to make informed choices about their reproductive health while supporting a more sustainable future.

Nanotechnology improved sanitary pads:

a) Enhanced Absorption and Leak Protection
b) Improved Breathability
c) Antimicrobial Properties
d) Skin Sensitivity and Comfort
e) Odor Control

References:

1. Kaur, R., K. Kaur and R. Kaur, (2018). Menstrual hygiene, management, and waste disposal: Practices and challenges faced by girls/women of developing countries. J. Environ. Public Health, Vol. (2018). 10.1155/(2018)/1730964.

2. Mahajan, T., (2019). Imperfect information in menstrual health and the role of informed choice. Indian J. Gender Stud., 26: 59-78.

3. Said, N.B. and V.C.L. Chiang, (2020). The knowledge, skill competencies, and psychological preparedness of nurses for disasters: A systematic review. Int. Emerg. Nurs., Vol. 48. 10.1016/j.ienj.(2019).100806.

4. Dasgupta, A. and M. Sarkar, 2008. Menstrual hygiene: How hygienic is the adolescent girl? Indian J. Community Med., 33: 77-80.

5. Mudey, A.B., N. Kesharwani, G.A. Mudey and R. Goyal, (2010). A cross-sectional study on awareness regarding safe and hygienic practices amongst school going adolescent girls in rural area of Wardha District, India. Global J. Health Sci., 2: 225-231.

6. Khanna, A., R.S. Goyal and R. Bhawsar, (2005). Menstrual practices and reproductive problems: A study of adolescent girls in Rajasthan. J. Health Manage.,

7: 91-107. 7. Offit, P.A., (2018). Bad Advice: Or Why Celebrities, Politicians, And Activists Aren't Your Best Source of Health Information. Columbia University Press, Columbia, Pages: 251.

8. Tudu, P.N., (2020). Saathi sanitary pads: Eco-friendly pads which will make you go bananas! J. Philanthropy Mark., Vol. 25. 10.1002/nvsm.1667.

9. Sivakami, M., A.M. van Eijk, H. Thakur, N. Kakade and C. Patil *et al.,* (2019). Effect of menstruation on girls and their schooling, and facilitators of menstrual hygiene management in schools: Surveys in government schools in three states in India, 2015. J. Global Health, Vol. 9. 10.7189/jogh.09.010408.

10. Kim, K.M. and J.S. Choi, (2020). Female university students' menstrual-hygiene management and factors associated with genitourinary-tract infections in Korea. Women Health, 60: 559-569.

11. Benjamin, S., E. Masai, N. Kamimura, K. Takahashi, R.C. Anderson and P. Abdul Faisal, (2017). Phthalates impact human health: Epidemiological evidences and plausible mechanism of action. J. Hazard. Mater., 340: 360-383.

12. Bolden, A.L., J.R. Rochester, K. Schultz and C.F. Kwiatkowski, (2017). Polycyclic aromatic hydrocarbons and female reproductive health: A scoping review. Reprod. Toxicol., 73: 61-74.

13. Rathi, B.S., P.S. Kumar and D.V.N. Vo, (2021). Critical review on hazardous pollutants in water environment: Occurrence, monitoring, fate, removal technologies and risk assessment. Sci. Total Environ., Vol. 797. 10.1016/j.scitotenv.(2021).149134.

14. Loo, S.L., L. Vásquez, A. Athanassiou and D. Fragouli, (2021). Polymeric hydrogels-A promising platform in enhancing water security for a sustainable future. Adv. Mater. Interfaces, Vol. 8. 10.1002/admi.(2021)00580.

15. White, H.L., T. Mwapasa, M. Mphasa, P.K. Kalonde and N. Feasey *et al.,* (2023). Open defaecation by proxy: Tackling the increase of disposable diapers in waste piles in informal settlements. Int. J. Hyg. Environ. Health, Vol. 250. 10.1016/j.ijheh.(2023).114171.

16. Sommer, M., S. Chandraratna, S. Cavill, T. Mahon and P. Phillips-Howard, (2016). Managing menstruation in the workplace: An overlooked issue in low-and middle-income countries. Int. J. Equity Health, Vol. 15. 10.1186/s12939-016-0379-8.

17. Tan, W., Z. Ma, L. Zhang, M. Zhang, T. Yu, Y. Zhang and T. Zhao, (2022). Study of polyvinyl alcohol/polymalic acid hydrogel dressing pads incorporated with curcumin as chronic wound dressing. Polym. Bull., 10.1007/s00289-022-04494-7.

18. Peter, A. and K. Abhitha, (2021). Menstrual cup: A replacement to sanitary pads for a plastic free periods. Mater. Today: Proceed., 47: 5199-5202.

19. Ajmeri, J.R. and C.J. Ajmeri, (2016). Developments in the Use of Nonwovens for Disposable Hygiene Products. In: Advances in Technical Nonwovens: A Volume in Woodhead Publishing Series in Textiles, Kellie, G. (Ed.), Woodhead Publishing, Sawston, Cambridge, ISBN: 9780081005750, pp: 473-496.

20. Kombe, A.J.K., B. Li, A. Zahid, H.M. Mengist, G.A. Bounda, Y. Zhou and T. Jin, (2020). Epidemiology and burden of human papillomavirus and related diseases, molecular pathogenesis, and vaccine evaluation. Front. Public Health, Vol. 8. 10.3389/fpubh.(2020).552028.

21. Lomonaco, T., E. Manco, A. Corti, J. La Nasa and S. Ghimenti *et al.,* (2020). Release of harmful volatile organic compounds (VOCs) from photo-degraded plastic debris: A neglected source of environmental pollution. J. Hazard. Mater., Vol. 394. 10.1016/j.jhazmat.(2020).122596.

22. DeVito, M.J. and A. Schecter, (2002). Exposure assessment to dioxins from the use of tampons and diapers. Environ. Health Perspect., 110: 23-28.

23. Bhandari, V., S. Jose, P. Badanayak, A. Sankaran and V. Anandan, (2022). Antimicrobial finishing of metals, metal oxides, and metal composites on textiles: A systematic review. Ind. Eng. Chem. Res., 61: 86-101.

24. Rtimi, S., D.D. Dionysiou, S.C. Pillai and J. Kiwi, (2019). Advances in catalytic/photocatalytic bacterial inactivation by nano Ag and Cu coated surfaces and medical devices. Appl. Catal. B : Environ., 240: 291-318.

25. Suvarna, V., A. Nair, R. Mallya, T. Khan and A. Omri, (2022). Antimicrobial nanomaterials for food packaging. Antibiotics, Vol. 11. 10.3390/antibiotics11060729.

BIOSYNTHESIS OF ZnO NANOPARTICLES AND ITS APPLICATION IN HERBAL PRODUCTS

A. D'lima[1], N. N. Jagdale[1], S. R. Pawaskar[1], Y. M. Mayekar[1], S. R. Wadekar[1], M. S. Hate[1] and R. S. Chaughule[1,2]

[1]Department of Chemistry,

Ramnarain Ruia Autonomous College, L.N. Road, Matunga (East), Mumbai-400019

[1,2]Tata Institute of Fundamental Research, Mumbai, India

Corresponding author E-mail: avrildlima4@gmail.com jagdaleneha.004@gmail.com, sharvaripawaskar29@gmail.com, yashmayekar2508@gmail.com shravaniwadekar@ruiacollege.edu, manishhate@ruiacollege.edu, rsctifr@yahoo.com

Abstract:

With the advent of advanced technology in every sphere of science, traditional practices have lost their importance. When it comes to hair care products, the massive use of harmful chemicals presently, like sulphates, parabens etc. practically lead to buildup of residues, blocking of hair follicles and stripping the hair off its natural oils. Hence our aim behind formulating a herbal shampoo is to provide a healthier way of nourishing the scalp and hair which is also eco-friendly and sustainable. We have chosen ingredients like Lemon grass, Scutch grass, grapefruit seeds, Shikakai, Neem which are traditionally used and are known for their exceptional antibiotic, antifungal and moisturizing properties which rejuvenates the scalp. Along with effective contribution of the above-mentioned ingredients, we have inculcated the use of nano technology by introducing ZnO nano particles. ZnO nano particles promote the growth of strong and resilient hair strands. By the virtue of their size, they can penetrate better into the hair follicles and scalp which most shampoos today fail to do naturally.

Keywords: ZnO Nanoparticles, Bio-Nanocomposites, Herbal Extract, Eco-Friendly, Sustainable.

Introduction:

Hair is a characteristic feature of mammals which serves several functions, namely thermal insulation, protection, perspiration facilitation and pheromone evaporation, sensory activity and, in some cases. Hair in mammals is expressed in different shapes, sizes, colors, and growth patterns [1]-[3].

Though not a necessary organ for survival but provides thermoregulation, hair has a major impact on cosmetic appearance, and it is an indicator of health, youth, and vitality. Besides, the diversity of its properties such as curvature, thickness and color can constitute a particular signature of each ethnicity around the globe [4]-[6].

The most widely used cosmetic products for cleansing hairs and scalp in our daily life are shampoos, which are basically a solution of a detergent containing suitable additives for other benefits such as hair-conditioning enhancement, lubrication, medication etc. Synthetic surfactants are added to shampoo primarily for the foaming and cleansing action but their regular use leads to dryness of hairs, hair loss, irritation to scalp and eyes. Herbal formulations are considered as alternative to synthetic shampoo [7]-[10]

Lemon grass introduced in a solvent mixture like water & alcohol was sonicated for 2hrs to obtain the extract of lemon grass. In 50 ml of extract, 5g of zinc nitrate was added and heated gently simultaneously to obtain precipitate. The acidity was balanced by Sodium hydroxide. The precipitate was later filtered and dried by placing in hot air oven. After 24 hours of drying, it was placed in a crucible in the mantle at 300 C, for 2 hours. A fine power was obtained and sampled to be given for UV [11]-[13].

The other ingredients used in the shampoo are as follows:

Pictures	**Common Name**	**Scientific Name**	**Effective ingredient**	**Role in shampoo**
	Lemon grass	*Cymbopogon citratus*	Citral, Geraniol, vitamin C	Antimicrobial & Antifungal
	Scutch Grass (Durva)	*Cynodon dactylon*	Flavonoids, tannins, saponins	Cleanse, tighten & tone the scalp
	Shikakai	*Acacia concinna*	Antioxidants, Vitamin D & Vitamin E	Protects hair from oxidative stress
	Aloe Vera	*Aloe barbadensis*	Vitamin B12 and Salicylic acid	Moisturizer

	Neem	*Azadirachta indica*	Triglyceride, limonoids, antioxidants	Prevents premature greying and dandruff
	Rose water	*Rosa rubiginosa*	PH balancing properties	Balances pH of shampoo
	Grape seed	*Vitis vinifera*	Vitamin E, Antioxidant, Folic acid	Anti-microbial agent

Methodolody:

1. Preparation of ethanolic mixture of *Cymbopogon citratus* (lemon grass)

30g of *Cymbopogon citratus* (lemon grass) was weighed and then was finely cut into pieces and was immersed in aqueous alcohol mixture (100 ml ethanol + 100 ml distilled water). The mixture was sonicated for 2 hours [14],[15].

2. Preparation of ZnO Nanoparticles:

Appropriate amount of sonicated mixture was filtered using Whatmann Filter paper No. 41. We took 50 ml of the filtered mixture and added 5g of Zinc nitrate. The resulting mixture was again sonicated for 2 hours post which 0.1 N NaOH was added dropwise (during sonication itself). A precipitate was formed, and the pH of the solution becomes basic which was filtered and kept for UV drying. Then it was heated in a crucible furnace for 2 hours at 450°C for annealing and thus ZnO NPs were formed which were further characterized.

3. Testing foaming capacity of Shikakai (*Acacia concinna)* and *Cyclea peltata:*

I. Shikakai (*Acacia concinna):*

Grind dried Shikakai (*Acacia concinna)* till it is a fine powder. Weigh 1g of the powder into a clean beaker and add 50 ml of Distilled water, shake it vigorously. Keep it undisturbed for 5 minutes and then and then measure the foam formed.

II. Shikakai (*Acacia concinna)* and *Cyclea peltata* in combination:

Repeat the above steps with 2.5g of Shikakai and 2.5g of *Cyclea peltata* together. Whichever procedure forms better foam, it is considered as the foaming agent for the herbal shampoo.

4. Preparation of Anti-microbial extract:

Keep scutch grass (durva), grape seeds and neem leaves in the oven till they are dry. Then by extraction process using Soxhlet Extractor, individually we obtained the extract of scutch grass (durva), neem and grape seed using distilled water in combination with ethanol as a solvent.

5. Preparation of herbal shampoo:

On addition of ZnO nanoparticles with prepared Foaming Agent and Antimicrobial extract we will be getting our herbal shampoo of appropriate texture, color, and odor.

Result and Discussion:

Appearance, Color, pH, Foam Volume (ml), Cleaning action are to be tested,

Parameters	**Ideal results**
Appearance	Clear
Color	Brown black
pH	4.33
Foam Volume	166 ml
Cleaning action	Good

Appearance: The formulated shampoos were evaluated for physical characteristics such as color, odor, and transparency. A previously prepared shampoo was transparent and had good odor. The same is expected in our formulated shampoo.

Color: Black Brown, Dandruff Cleansing Shampoo - 50 ml.

pH: pH range expected for the shampoo is between 4.33 and 4.73. This pH is acid balanced which is near to the skin pH. The pH of shampoo is important for improving and enhancing the qualities of hair, minimizing irritation to the eyes and stabilizing the ecological balance of the scalp. Mild acidity prevents swelling and promotes tightening of the scales, there by inducing shine.

Foaming Stability: The stability of the foam will be determined using cylinder shake method. About 50 ml of formulated shampoo (1%) solution will be taken in a graduated cylinder of 250 ml capacity and shaken for 10 times vigorously. Foam stability will be measured by recording the foam volume of shake test after 1 min and 4 min, respectively. From the consumer point of view, foam stability is one of the important needs of a shampoo. It is an important parameter that was considered in the shampoo

evaluation. The foam volume produced by the formulated shampoo is supposed to be above 50 ml. The prepared shampoo should generate uniform, small sized, compact, denser, and stable foam. The foam volume remains same throughout the period of about 4 min showing that the generated foam by the shampoo has good stability.

Cleaning action: About 1 g of grease is spread on non-adsorbent cotton and kept in conical flask containing 1% shampoo solution. The conical flask is shaken for 1 hour in mechanical shaker. Cotton is collected, dried, and weighed.

The amount of grease removed is determined by using the equation given below:

$DP = 100 (1-T/C)$

Where;

C - Weight of grease in control sample.

T - Weight of grease in test sample.

DP - Percentage of detergency power.

The created cleanser should be clear and good appealing. It should demonstrate good froth stability, good cleansing and should have good conditioning properties.

Conclusion:

The main purpose behind this was to develop a stable and functionally effective shampoo. The study was carried out with the aim of preparing the herbal shampoo that provides smooth and holistically fulfilling effects for hair, safer than the chemical conditioning agents. Herbal shampoo was formulated with an alcoholic extract of Lemon grass and extracts of plants that are commonly used for cleansing and smoothening hair traditionally. To provide the effective conditioning effects, the present study involves the use of Grape seeds, Aloe vera, Scutch grass, Shikakai and Neem instead of synthetic cationic conditioners. Factors like UV radiations, use of harsh chemical products have direct and indirect action on the hair and scalp. The present study focuses on the potential of herbal extracts from cosmetic purposes. Hence with this plan we hope to conclude that the formulation of this herbal shampoo is effective in providing smoothening, shiny and better conditioning effect.

Limitations:

1. Herbal shampoos have a shorter shelf life.
2. Some herbal ingredients may evoke allergies in certain amount of composition.
3. Herbal shampoos produce comparatively less lather as they lack the common foaming agent sulfate, which some might find unsatisfactory.
4. Naturally occurring herbs possess a limited potential in removing heavy dirt, oil or buildup, compared to harsh chemicals.

Challenges:

1. Having a consistent supply of high-quality ingredients is difficult as based on factors like seasonality, geography, and cultivation methods, their quality varies.
2. Herbal ingredients can be unpredictable in how they affect the pH of the final product.
3. Herbal ingredients can vary in potency, leading to inconsistent results for users.
4. Scaling up production while maintaining quality can be difficult, especially when dealing with delicate herbal ingredients that may not behave the same way in larger batches.

Future Prospects:

1. Herbal shampoos, being free from harsh chemicals, are likely to see increased demand as consumers become conscious of haircare products they use and their preference of organic and healthier alternatives.
2. As sustainability becomes a priority, herbal shampoos that use eco-friendly packaging and sustainable sourcing practices will gain popularity.
3. Due to various healing properties of herbs, personalized formulations that cater to individual hair types, needs, and preferences could become more common.
4. As more awareness spreads about the traditional, indigenous herbal practices and their benefits, shampoos that incorporate ancient remedies and lesser-known herbs could gain attraction.

References:

1. Aniket Pawar, Rakesh Pomnar, Mahesh Sawant, Rohit Aher, Komal Suryawanshi, (2022), The Formulation and Evaluation of Herbal Shampoo Vol. 10, page no. 16-19
2. Manali V. Gharat, Rutuja V. Dalavi, Amit A. Dound, Aniket R. Chikane, Prof. Kajal A. Walunj, (2022), A Research Article on Formulation and Evaluation of Herbal Shampoo, Vol. 2, page no. 1-4
3. Prerana V. Poojary, Swohinee Sarkar, Adithi Ananda Poojary, Pooja Mallya, Raja Selvaraj, Ananthamurthy Koteshwara, Jesil Mathew Aranjani, Shaila Lewis, (2015), Novel anti-dandruff shampoo incorporated with ketoconazole-coated zinc oxide nanoparticles using green tea extract, Vol. 23
4. Suyog Sunil Bhagwat, Dr. N. J. Paulbudhe, (2022)0, Formation and Evaluation of Herbal Shampoo, Vol. 8, page no. 7-9
5. Gore SS, (2022), Chemical Route Synthesis and Sonication of ZnO Nanoparticles, Vol. 10, page no. 2

6. M. Sankara Bhavani, Shaik Mehamuda Jan, Kota Sandhya Rani, M. Srirekha, (2023), Formulation, Evaluation and Comparison of the Herbal shampoo with commercial shampoos
7. Pawan Maurya, Shashikant Maury, Piyush Yadav, Manoj Kumar Yadav, Suraj Maurya, Satyam Jaysawal, (2021), A Review Article on: Herbal Shampoo, Vol. 8, page no. 3
8. Sakshi More and Sonal Kumbhar, (2024), Formulation And Evaluation Of Herbal Shampoo, Vol. 2
9. X. Fuku, A. Diallo, M. Maaza,(2016), Nano-scaled electrocatalytic optically modulated ZnO nanoparticles through green process of *Punica granatum*l and their antibacterial activities
10. Shreshta Roy Goswami and Mukesh Singh, 2018, Microwave-mediated synthesis of zinc oxide nanoparticles: a therapeutic approach against Malassezia species
11. P Vignesh, K Sudhakar, (2022), Nature inspired nano additives for biofuel applications
12. Xian-Quin Zhou, Zakir Hayat, Dong-Dhong Zhang, Meng Yao Li, Si Hu, Qiong Wu, Yu-Fei Cao and Ying Yuan, (2023), Zinc Oxide Nanoparticles: Synthesis, Characterization, Modification, and Applications in Food and Agriculture
13. Prerna V Poojari, Swohinee Sarkar, Adithi Ananda Poojary, Pooja Mallya, Novel anti-dandruff shampoo incorporated with ketoconazole-coated zinc oxide nanoparticles using green tea extract
14. Rajni Bala, Reecha Madaan, Sandeep Arora, Vibhu, (2017), Green Synthesis and Characterization of silver nanoparticles using Kinnow mandarian peels extract and its application in Shampoo Formulation
15. Neeta Rai, A. Jain, John Abraham, (2013), Formulation and Evaluation of Herbal Antidandruff Shampoo Containing Garlic Loaded Solid Lipid Nanoparticles.

USE OF CHEWING STICKS IN THE ERA OF TOOTHBRUSH: A CASE STUDY FROM JHARIGAON BLOCK OF NABARANGPUR DISTRICT, ODISHA

Maninee Sahu[1], Sunil Kumar Sahu[2] and Alok Ranjan Sahu*[3]

[1]Odisha Adarsha Vidyalaya, Jharigaon, Nabarangpur 764076

[2]Odisha Adarsha Vidyalaya, Chandahandi, Nabarangpur 764077

[3]Department of Botany, Vikash Degree College, Bargarh 768040

*Corresponding author E-mail: alok.btgene@gmail.com

Abstract:

Keeping in mind more dental facilities, more dental problems, to find out the fact behind the statement the present study was carried out in tribal dominated areas of Odisha. In this present research work we have documented a total of 31 plant species having a place with 30 genera and 20 families have been recorded to use as toothbrushes by the native of Jharigaon Block of Nabarangpur district. A maximum numbers of five species are contributed from family Fabaceae, followed by three species from Malvaceae, two species each from five families (Anacardiaceae, Euphorbiaceae, Moraceae, Rutaceae and Sapotaceae), rest 13 families contributed one species each. The distribution of plant species as habit, among 31 plant species, 22 numbers of tree (71%), Seven numbers of shrub (23%), while one species is contributed from both herb (3%) and climber (3%). Documentation of these plant species used by the natives to maintain oral hygiene will also be helpful for pharmaceutical agencies to develop dental care products in the near future. It will also be helpful for the economic development of tribal communities of the region.

Keywords: Documentation, Tooth Brush, Jharigaon Block, Nabarangpur

Introduction:

As per the WHO report, more than 80% of the world population depend on traditional medicine for their primary health care purpose (Sahu *et al.,* 2017). India has one of the world's most primitive indigenous medical cultures, with a continuous practice coming down across more than four millennia. Though this medical tradition is quite a few centuries old, even at present people in the rural and remote areas depend upon it for their health care requirements. Exploration of conventional medicine is an unaccountably gorgeous yet scientifically essential and economically crucial assignment of ethnobotanists. In the last few decades significant work in identification, documentation and discovery of conventional medicine has been made in India. Ethnobotanical enumerations are often major in enlightening locally vital plant species

mainly for the discovery of crude drugs (Jain, 1981). Right from its start, the documentation of conventional knowledge, mainly medicinal uses of plants, has given several important drugs of the modern day (Pushpagadan and Kumar, 2005). Exploration of conventional medicine is very essential for the wellbeing of rural and tribal communities for treatment of conventional illness. This is due to the health care facilities in rural areas being insufficient and expensive too. Moreover, conventional medicine based on plants provides utmost rural or tribal healthcare, because 80% of the raw materials used in the preparation of drugs obtained from medicinal plants (Sahu *et al.,* 2010; Sahu *et al.,* 2013; Sahu and Sahu, 2017; Sahu *et al.,* 2020).

Oral hygiene is an essential part of a person. Oral health when ignored, results in different types of oral ailments similar to dental caries and periodontal diseases. Oral disorders can considerably influence the general well-being of a person by causing huge pain and anxiety, thus affecting their quality of life. Teeth are very hard but sensitive organs which are fixed in the jaw bones. They not only help in the biting and grinding of food but also aid speech. Dental caries and periodontal diseases are the two common threats to oral health and are important public health problems because of their prevalence, their impact on individuals and society, and the expense of their treatment. Oral diseases are caused due to bacterial infections, food habits and lifestyle. Any disease of the gums or faulty teeth disturbs the method of digestion. Lack of oral hygiene, surplus content of fleshy food and sweets harm our teeth by instigating toothache, pyorrhoea, bleeding gums and dental caries (Dhilon, 1994). The preparation of herbal plants to treat dental problems has been reported by many researchers, viz. the use of *Acacia nilotica* L., *Azadirachta indica* A. Juss., and *Vitex negundo* L. in dental health care has been reported by several authors (Sahu *et al.,* 2010; Sahu *et al.,* 2013; Sahu and Sahu, 2017; Sahu *et al.,* 2020). Most of these plants are alkaline with high antibacterial activity. Hence these plants help to maintain acid alkaline equilibrium of the saliva, decrease plaque/calculus development and are less disposed to periodontal infections. It is also noticed that the microorganisms originating in inflamed gums are resistant to antibiotics but not to antibacterial plant extracts like neem. One of the common conventional practices followed is the use of herbal 'chewing sticks' instead of plastic brushes to maintain oral health and hygiene. The best known examples of conventional chewing sticks and tongue cleaner are *Acacia nilotica* L., *Azadirachta indica* A. Juss., *Lawsonia alba* Lamk., *Madhuca indica* Gmel., *Millettia pinnata* (L.) Panigrahi, *Mimusops elengi* L. *Shorea robusta* Roth, *Sida acuta* Burm. f., *Terminalia arjuna* Retz., and *Vitex negundo* L (Sahu *et al.,* 2010; Sahu *et al.,* 2013; Sahu and Sahu, 2017; Sahu *et al.,* 2020). Tooth powder is the first evidence and was made up of elements like powdered charcoal, powdered bark and some flavouring agents, applied

to teeth using a simple stick. The rural people of Jharigaon Block of Nabarangpur district depend on plant resources for their primary health care needs. This present work aims to document the toothbrush plants and also integrate traditional knowledge with modern dental care practices to formulate their sustainable utilization. No such studies related to toothbrush plants have been conducted and documented from the region. That's why an attempt has been made to explore and document the traditional uses of some of the plants used by the native people for maintaining their oral health.

Materials and Methods:

Study Area

Odisha with its widened topography, variable climatic conditions, huge forest districts and immense familial people give a fascinating area to ethnobotanical considers. Nabarangpur district lies between 19° 23' North latitude and 82° 55' East longitudes and has 5,294 Sq Km of geographical area, most of the area is covered with dense forest. Nabarangpur district is a tribal dominated district having a population of 12,20,946 (Census of India, 2011). The population density of this district is 230 inhabitants per square K.M. It has a sex ratio of 1018 females for every 1000 males and the literacy rate is 48.2%. The Scheduled Castes and Scheduled Tribes are 14.53% and 55.79% of the population respectively. This district is well known for its tribal culture. Some of the largest tribes are the Bhottadas and Gonds, they have their language, customs and folk dances. The river Indravati forms the boundary between Nabarangpur and Koraput districts.

Collection of Data and Analysis

Before starting fieldwork, a literature survey of ethnomedicinal work in Odisha was carried out (Sahu *et al.,* 2010, Sahu *et al.,* 2013, Dhal *et al.,* 2015, Sahu *et al.,* 2021). The study area was frequently visited and close interaction was made with the tribal peoples of Jharigaon Block. The plant species were collected and identified by using local flora books (Haines, 1925; Saxena and Brahman, 1994-96). The local names were cross checked by using earlier published literature of different districts of Odisha (Sahu *et al.,* 2010, Sahu *et al.,* 2013, Dhal *et al.,* 2015, Sahu *et al.,* 2021, Sahu and Sahu 2022; 2023; 2024). The authority plant names were affirmed at http://worldfloraonline.com.

Enumeration

During the field survey, ethnobotanical data of 31 species of plants belonging to 20 families were gathered from various habitats of the study areas and each was documented with regard to its local name, part used, collection, mode of administration and habitats of the species. The prevalent oral health problems in the surveyed villages are dental caries and periodental diseases like gingivitis, pyorrhea, etc. In the following enumeration, the species are arranged alphabetically in botanical name, followed by

local name and family name in tabular format. The histogram for number of species vs family and pie-chart for number of species vs habits were drawn by using MS-EXCEL 2007.

Results and Discussion:

An aggregate of 31 plant species having a place with 30 genera and 20 families have been recorded to use as toothbrush (Table 1). Greatest numbers of five species are from family Fabaceae, three species from Malvaceae, two species each from five families (Anacardiaceae, Euphorbiaceae, Moraceae, Rutaceae and Sapotaceae), rest 13 families contributed one species each (Table 1, Figure 1). The distribution of plant species as habit, among 31 plant species, 22 numbers of tree (71%), Seven numbers of shrub (23%), while one species is contributed from both herb (3%) and climber (3%) as shown in Figure 2.

At present, world markets are overwhelmed with a variety of tooth glues or pastes, tooth brushes, gel mouthwash fluids and crores of rupees are spent on their notice, circulation, advancement advertisement. But, as distant as the fetched figure is concerned, few individuals in India can bear it when the population underneath the destitution line in India and Odisha are 29.9 and 44.7 separately (Sahu and Sahu, 2017). The financial situation of other creating nations is no better than India. Besides, a few corrective and medication companies in Egypt, India, Pakistan, Switzerland and U.K. have too been connected to this information for manufacturing tooth pastes. It is logically demonstrated to decrease tartar and plaque, battles germs and microbes to keep gums solid, makes a difference, anticipates tooth rot, disposes of awful breath and guarantees solid teeth. The greatest and limited sources of India request the alternative way of medicinal and herbal treatment technology can be visualized and for implementation (Sahu and Sahu, 2017; 2020). In this context, phytotherapy resources for herbal health care show up important because it requires no extraordinary property, sophistication or skill in generation, planning and utilization. So it has ended up a need to gather records and pharmacologically assess the valuable secondary metabolites like alkaloids, tannins, gums or any other useful plant item accessible from the neighborhood vegetation for superior herbal and dental care in Odisha. The herbal plants which make the spine of ancient traditional medicine in the final few decades have been the subject for exceptionally solid pharmacological studies; this has been brought around by the acknowledgement of the worth of therapeutic plants as expected sources of unused compounds of therapeutic value. Traditional pharmaceutical may be an ability practiced by few elderly individuals whose test information is acknowledged by everybody within the town.

Table 1: List of plants used as toothbrush by the tribal peoples of Jharigaon block of Nabarangpur District, Odisha, India.

Botanical Name	Local Name	Family
Abutilon indicum (L.) Sweet	Kuthelchitra	Malvaceae
Acacia catechu (L. f.) Willd	Khayar	Fabaceae
Acacia nilotica (L.) Willd. Ex Delile	Bamur	Fabaceae
Achyranthes aspera L.	Kukurdanti	Amaranthaceae
Aegle marmelos L.	Bael	Rutaceae
Alstonia scholaris L. R. Br.	Chatiana	Apocynaceae
Annona squamosa L.	Sitaphal	Annonaceae
Azadirachta indica A. Juss.	Neem	Meliaceae
Bambusa arundinacea (Retz.) Roxb.	Baunsa	Poaceae
Buchaania lanzan Spreng.	Char	Anacardiaceae
Butea monosperma (L.) Taub.	Palsa	Fabaceae
Cajanus cajan (L.) Millsp.	Kandul	Fabaceae
Emblica officinalis Gaertn	Anla	Euphorbiaceae
Ficus racemosa L.	Dumer	Moraceae
Hibiscus rosa-sinensis L.	Mandar	Malvaceae
Jatropha curcas L.	Ramjada	Euphorbiaceae
Lawsonia alba L.	Benjati	Lythraceae
Madhuca longifolia (Koenig.) Macbride	Mahul	Sapotaceae
Mangiferara indica L	Amba	Anacardiaceae
Millettia pinnata (L.) Panigrahi	Karanj	Fabaceae
Mimusops elengi L.	Baul	Sapotaceae
Morus indica L.	Tut	Moraceae
Murraya paniculata (L.) Jacq.	Kamini	Rutaceae
Phoenix sylvestris Roxb.	Khajur	Areaceae
Psidium guajava L.	Jam	Myteraceae
Shorea robusta Roth	Sargi	Dipterocarpaceae
Sida acuta Burm. f.	Bajarmuli	Malvaceae
Smilax zeylanica L.	Muturi	Smilacaceae
Tamirindus indica L.	Tentel	Caesalpiniaceae
Terminalia arjuna Retz.	Kau	Combretaceae
Vitex negundo L.	Nirgundi	Verbenaceae

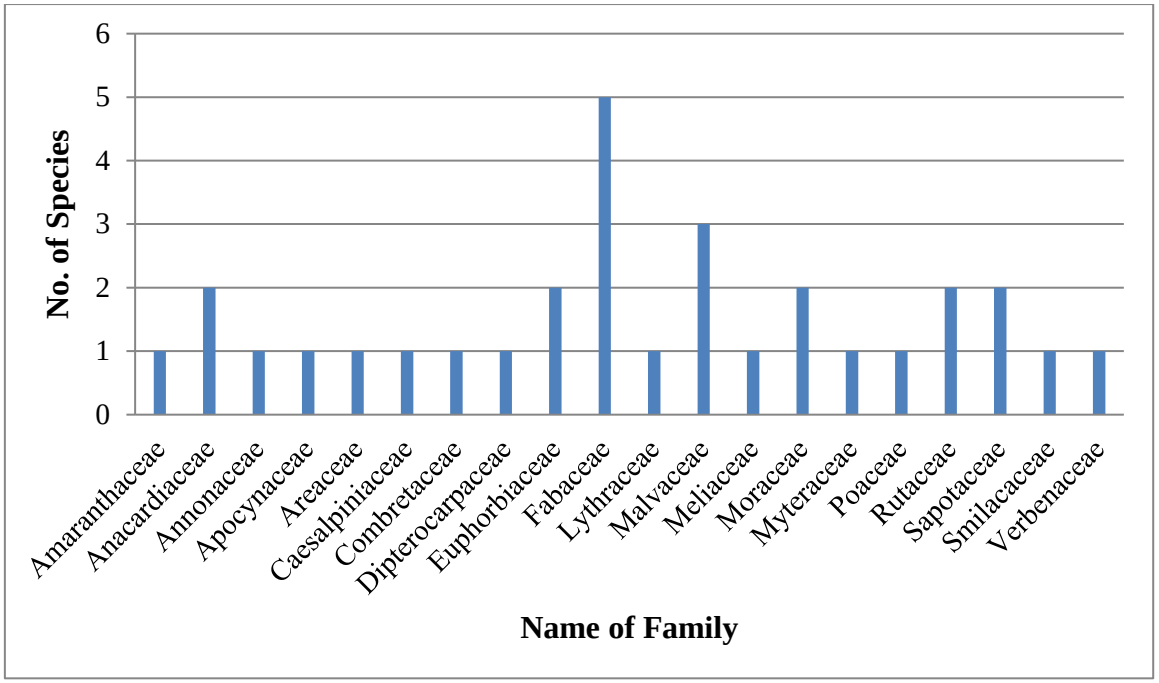


Figure 1: Family-wise distribution of plants Used as toothbrush by the tribal peoples of Jharigaon block of Nabarangpur District, Odisha, India

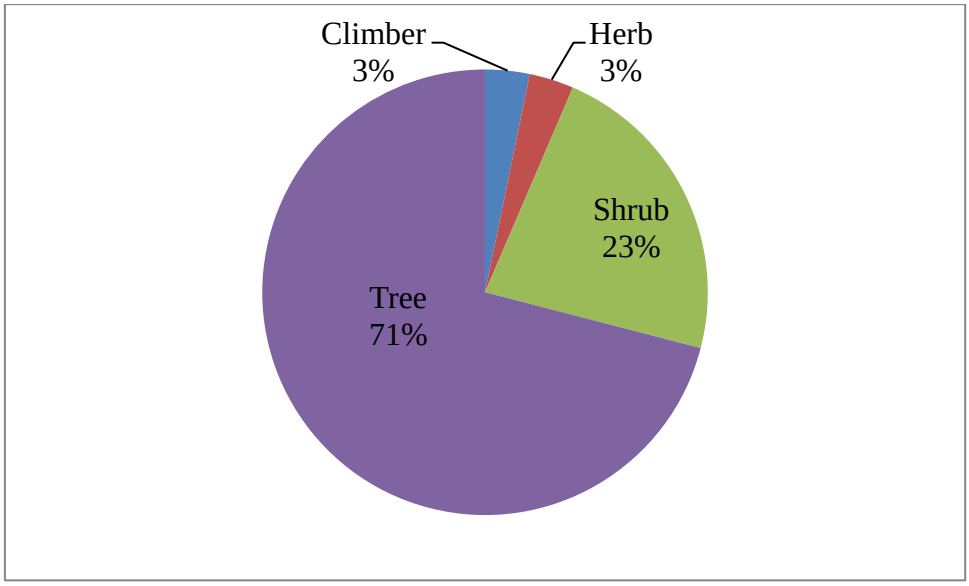


Figure 2: Diversity of used plant species by habit

Plant-based traditional knowledge has gotten to be a standard instrument in hunting for modern sources of drugs, it is evident that these home grown solutions can offer a stage for advance inquiry in dentistry. During the survey time, it was observed that elderly individuals have more information and knowledge about these conventional home grown drugs. Recent research revealed that therapeutic plants proceed to play a key part in dental care needs of local people groups of Jharigaon of

Nabarangpur district. Thus there's a pressing need to secure biodiversity as well as the conventional information by rectifying documentation and to assist inquire about dentistry.

Acknowledgement:

The author (ARS) is especially thankful to the Chairman, the Principal and the Staff of Vikash Degree College, Bargarh for providing necessary facilities, moral support, and encouragement. The authors are especially thankful to the tribal and local health healers of Nabarangpur district for their cooperation and voluntarily shared a wealth of their accumulated knowledge regarding the ethnomedicinal practices of their communities and for their hospitality.

References:

1. Dhal NK, Panda SS, Muduli (2015): Traditional uses of medicinal plants by native people in Nawarangpur district, Odisha, India. Asian J. Plant Sci. Res., 5: 27-33.
2. Dhilon MS (1994): Oral health for a healthy life. Swasth Hind., 38(3-4):75-79.
3. Haines HH (1921-1925): The Botany of Bihar and Orissa, Adlard and Sons and West Newman, London, I-VI.
4. Jain SK (1981): Glimpses of Indian Ethnobotany. Oxford and IBH Publication, New Delhi.
5. Pushpagadan P, Kumar (2005): Ethnobotany B. CBD, WTO and the Biodiversity Act of India. Ethnobotany, 17:2-12.
6. Sahu AR, Behera N, Mishra SP (2010): Use of Ethnomedicinal Plants by Natives of Bargarh District of Orissa, India, Ethnobotanical Leaflets, 14: 889-910.
7. Sahu AR, Nayak AK, Panigrahi SK (2013): Survey of some important ethno-medicinal plants of Sohela Block, Western Odisha, India. Life Sciences Leaflets, 11:1-9.
8. Sahu AR, Sahu M (2020): A preliminary report on the ethnobotanical plants used for dental care by the tribal of Bargarh District, Western Odisha. World Journal of Pharmacy and Pharmaceutical Sciences, 9(2):1020-1028. (DOI: 10.20959/wjpps20202-15463).
9. Sahu AR, Sahu M (2022): Green leafy vegetables used by the Tribal Peoples of Jharigaon Block of Nabarangpur District, Odisha, India. In Ecology Research, Jachak *et al.,* Bhumi Publishing, Nigave Khalasa, Kolhapur 416207, Maharashtra, INDIA. Volume V, Chapter 7:52-59.
10. Sahu AR, Sahu M (2023): A preliminary Report on Ethnomedicinal Study of Plants Used to Treat Asthma by the Gond Tribes of Nabarangpur District, Odisha, India. In **Frontiers in Life Science Volume X**; Parimala B, Mishra P, Yadav KK, and Sahu

AR (Ed.). Bhumi Publishing, Nigave Khalasa, Kolhapur 416207, Maharashtra, India. Chapter 1, Pp. 1- 10.

11. Sahu AR, Sahu M, Raal A (2021): An Ethnobotanical Study on Native Plants of Bargarh of Western Odisha, India in relieving Urogenital ailments, Ethnobotany Research & Applications, 21:29 (http://dx.doi.org/10.32859/era.21.29.1-11).
12. Sahu M, Sahu AR (2017): A Preliminary Report on the Traditional Practice for Dental and Oral Health Care in Bargarh District of Western Odisha, India. Journal of Medicinal Plants Studies, 5(5):120-125.
13. Sahu M, Sahu AR (2024): A Report on Ethno-Colour Concept among the Gond Tribal peoples of Jharigaon block, Nabarangpur District, Odisha, India. In From Cells to Ecosystems: Exploring Life Science Research; Kadam SS, Sahu AR, Sarvajana S, and Verma S (Ed.). Bhumi Publishing, Nigave Khalasa, Kolhapur 416207, Maharashtra, INDIA. (ISBN: 978-93-95847-22-3), Volume II, Pp. 108-116.
14. Saxena HO, Brahman M (1994-96): The flora of Orissa, Regional Research Laboratory, Orissa and Orissa Forest Development Corporation Ltd., I-IV.

Plant Science: From Fundamentals to Advanced Research Volume II

(ISBN: 978-93-95847-68-1)

About Editors

Dr. Prem Kumar Gautam M.Sc. in Botany [1999], Ph.D. in Botany [2000-2003], with nearly 13+ years of teaching experience. About 2 and 1/2 years of research experience as a Plant Breeder in various Seeds companies. Presently, Dr. Gautam serves as Associate Professor and Head in P.G. & Research Department of Botany, NTVS'S G.T. Patil College, Nandurbar (M.S.). A total of 15 research papers were published in reputed journals, along with 30 Workshops/Seminars/webinars/international/national conferences attended. Five chapters are published in different books and Twelve books are published. As a recognised supervisor/Guide to Ph.D. Students of KBC North Maharashtra University Jalgaon, (One student was awarded Ph. D. Degree and five are working.)

Dr. Niharika Dewangan is an Assistant Professor and Head of the Department of Life Science at Shri Shankaracharya Professional University, Junwani, Bhilai, C.G. She holds a B.Sc. in Biology, an M.Sc. in Botany with merit distinction, an M.Phil in Botany, and a Ph.D. in Botany, with her research focusing on the "Impact of Urea on Plant Growth Promoting Rhizobacteria with Reference to Rice Crop." Dr. Dewangan has 18 years of teaching experience and 8 years of research experience. Her work has resulted in 13 research papers, 4 book chapters, and 1 patent on "Novel Bioengineered Microorganisms for Efficient Biofuel Production from Agricultural Waste." Her research interests include Plant Growth Promoting Rhizobacteria, Phytochemistry, Plant Ecology, and Medicinal Botany. Currently, she is supervising four Ph.D. scholars. Dr. Dewangan also qualified for the CG SET in 2017.

Dr. Pallavi Purushottamrao Ulhe (M.Sc. Ph.D) is working as an Associate Professor and Head in Department of Botany, Narayanrao Kale Smruti Model College (Art, Commerce and Science) Karanja (gh.) Dist- Wardha with teaching experience 14 years at UG level. She was completed her M.Sc. (Botany) with specialization "Cytogenetics". She awarded a Ph.D. in 2008 on topic "Seed Morphological And Micropropagation Studies Of Some Medicinal Plants". She was completed one minor research project entitled "Evaluation Of Biochemical And Phytochemical Analysis Of Some Apiaceae (Umbelliferae) Seeds" funded by UGC. She has published 28 research papers in reputed National and International journals. She has attended more than 43 National and International conferences/workshops/symposia/seminars and presented a paper. She was an organizing secretary of One Day Virtual National Conference On "New Trends in Biodiversity" (NCNTB-2021). Her area of interest Cytology, Seed Biology, Biotechnology.

Dr. Pravin Dinkar Patil, M. Sc. Botany (Cytogenetics and Plant Breeding), Ph. D. (Molecular Systematics)., working as Assistant Professor in Department of Botany at Shankarlal Agrawal Science College, Salekasa, District Gondia. He has teaching experience of 10 years at undergraduate level. His area of research interest is molecular phylogeny, DNA barcoding, plant breeding and microscopy. He published more than 20 research papers in national and international journals.

Bhumi Publishing

ISBN 978-93-95847-68-1
9 789395 847681