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PROCEEDINGS OF IC-SABS 2025:

EMERGING FRONTIERS IN BIOTECHNOLOGY AND SCIENTIFIC INNOVATION

IS-EDESM 2025

under the

INTERNATIONAL SUMMIT

on

Empowering Development through Engineering, Science & Management towards Sustainable Future

KUNWAR SATYAVIRA

COLLEGE OF ENGINEERING AND MANAGEMENT

&

RAJKIYA ENGINEERING COLLEGE, BIJNOR

In Association with

LINCOLN UNIVERSITY COLLEGE, MALAYSIA

Summit Date & Venue

7th November 2025 at Kunwar Satyavira College of Engineering & Management, Bijnor, (U.P.), India

8th November 2025 at Rajkiya Engineering College, Bijnor, (U.P.), India

Hybrid Mode : Online & Offline

Chief Patron

Prof. (Dr.) J.P. Pandey
Vice Chancellor, Dr. APJ Abdul Kalam
Technical University, Lucknow

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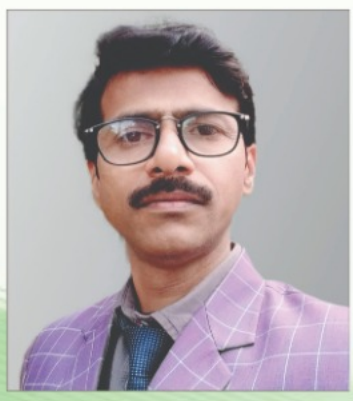
Prof. (Dr.) Amit Kumar Bansal
Director, KSVCEM

Organizing Secretary

Prof. (Dr.) Lokesh Kumar Agrawal
Dean, Student Welfare, KSVCEM

Convener



Prof. Manoj Kumar Kushwaha
Dean Academic, KSVCEM

Dr. Ishan Bhardwaj
HoD IT, REC, Bijnor

Proceedings of IC-SABS 2025: Emerging Frontiers in Biotechnology and Scientific Innovation

Authors:

Dr. Amit Kumar Bansal

Manoj Kumar Kushwaha

Sangeeta Sharma

Ujjwal Kumar

कर्म विद्याहि कौशलम्
KUNWAR SATYAVIRA
College of Engineering and Management
Estd. 1999

Kunwar Satyavira College of Engineering & Management

Regd. Office : Meerut Road Opp. BSNL Office, Bijnor-246701 [U.P.]
Phone No : 01342-263514 Mob: 7055522100
email: director@ksvira.edu.in
Website: www.ksvira.edu.in/publications
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Published in 2026.

ISBN: 978-81-963190-1-4

First Edition: 2026

About the Conference

The **Conference** is a prestigious academic event that brings together a global community of researchers, educators, industry professionals and policy-makers dedicated to exploring **cutting-edge developments in Sciences and Biotechnology**.

In a world facing unprecedented challenges such as emerging diseases, climate change, population growth and diminishing natural resources, the role of **Science and Biotechnology** is more critical than ever.

Theme of the Conference

"Innovating Life: Interdisciplinary Approaches in Sciences and Biotechnology"

The modern era of science is witnessing a paradigm shift, where **interdisciplinary research** is no longer an option but a necessity. The convergence of Biotechnology & Science with fields such as materials science, computational modeling, data science, nanotechnology and environmental engineering is redefining how we understand life processes and solve complex global challenges.

This year's theme, *"Innovating Life: Interdisciplinary Approaches in Sciences and Biotechnology,"* highlights the importance of **collaborative, cross-disciplinary innovations** in transforming the fields of medicine, agriculture, industrial processes and ecological sustainability.

The theme emphasizes:

- **Integration** of diverse scientific disciplines to create holistic solutions.
- **Innovation** through collaborative research and application-driven discoveries.
- **Impact** on real-world problems such as disease diagnostics, food security, clean energy and environmental health.

It encourages scientists, technologists and thought leaders to break disciplinary boundaries, explore shared methodologies and translate research into meaningful societal benefits.

Preface

It gives us immense pleasure to present the proceedings of **IC-SABS 2025: Emerging Frontiers in Biotechnology & Scientific Innovation**, organized under the prestigious **INTERNATIONAL SUMMIT on Empowering Development through Engineering, Science & Management towards Sustainable Future**.

The conference was envisioned as a multidisciplinary platform to bring together researchers, academicians, industry experts, policymakers, and students to exchange innovative ideas and discuss recent developments in biotechnology, applied sciences, sustainability, and emerging technologies.

This volume contains selected research papers presented during the conference, highlighting recent advancements, practical applications, and future directions in biotechnology and scientific innovation. The contributions reflect the dedication of researchers working toward sustainable development and global progress.

We hope that this proceeding will serve as a valuable reference for researchers, educators, and professionals, inspiring further scientific exploration and collaborative innovation.

Acknowledgement

The Organizing Committee of **IC-SABS-2025** expresses sincere gratitude to all individuals and institutions whose support made this conference a grand success.

We extend our heartfelt thanks to the keynote speakers, invited guests, session chairs, reviewers, and contributors for sharing their expertise and valuable insights.

We also acknowledge the efforts of the scientific committee, technical teams, faculty members, volunteers, and administrative staff for their dedication and commitment in ensuring the successful conduct of the conference.

Special appreciation is extended to all participating researchers, scholars, and students whose enthusiastic involvement enriched the academic spirit of the event.

We gratefully recognize the support of partner institutions, sponsors, and collaborators for encouraging research, innovation, and sustainable development.

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Vice Chancellor

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उत्तर प्रदेश, लखनऊ
Dr. A.P.J. ABDUL KALAM TECHNICAL UNIVERSITY
Uttar Pradesh, Lucknow

Dated: 03.06.2026

Message

It gives me immense pleasure to extend my warm greetings to the organizers, distinguished speakers, researchers, industry experts and participants of the **International Summit on Empowering Development through Engineering, Science & Management towards a Sustainable Future (IS-EDESM 2025)** and the **International Conference on Sustainable Advances in Biotechnology & Sciences (IC-SABS-2025)**.

The theme of the summit, *"Bridging Innovation and Sustainability through Engineering, Sciences & Management,"* is both timely and visionary. In an era marked by rapid technological transformation, environmental challenges, and evolving socio-economic landscapes, the integration of interdisciplinary knowledge has become imperative. Engineering, science and management are no longer independent domains; rather, they are interconnected pillars that collectively shape sustainable progress.

The parallel conference, IC-SABS-2025, focusing on *"Innovating Life: Interdisciplinary Approaches in Sciences and Biotechnology,"* addresses some of the most pressing global challenges emerging diseases, food security, environmental degradation, and sustainable industrial practices. These collaborative approaches are essential for achieving breakthroughs in healthcare, agriculture, clean energy and ecological sustainability.

As Vice Chancellor, I strongly believe that higher education institutions must act as catalysts for transformative change. Conferences of this magnitude not only disseminate knowledge but also inspire young minds to pursue research that is ethical, impactful and socially responsible.
I commend the organizing committee for their vision, dedication and commitment in hosting this prestigious international summit. I am confident that the deliberations and outcomes of IS-EDESM 2025 and IC-SABS-2025 will contribute meaningfully to the global discourse on sustainable development.

I extend my best wishes for the grand success of the summit and hope that it fosters enduring collaborations, innovative research pathways and transformative advancements for a sustainable future.

(Prof. Jai Prakash Pandey)
Vice Chancellor

Sector-11, Jankipuram Extension, Lucknow - 226031 (U.P.) India, Website : www.aktu.ac.in
(Office) : +91 522 2772189, 2772194 E-mail : vc@aktu.ac.in

College Code : 017

Estd.1999

KUNWAR SATYAVIRA

College of Engineering and Management

(Approved by AICTE, New Delhi & Affiliated to Dr. APJ AKTU, Lucknow)

(Formerly VIRA College of Engineering)

Website: www.ksvira.edu.in Email: director@ksvira.edu.in

Message

It is a matter of great pride and privilege to welcome distinguished scholars, researchers, industry leaders, academicians and students to the **International Summit on Empowering Development through Engineering, Science & Management towards a Sustainable Future (IS-EDESM 2025)** and the **International Conference on Sustainable Advances in Biotechnology & Sciences (IC-SABS-2025).**

At Kunwar Satyavira College of Engineering and Management, we firmly believe that education must transcend classroom boundaries and actively contribute to societal transformation. The vision behind IS-EDESM 2025 reflects this commitment—creating a multidisciplinary platform where innovation meets responsibility, and knowledge translates into sustainable development.

The summit's theme, *"Bridging Innovation and Sustainability through Engineering, Sciences & Management,"* emphasizes the integration of technical expertise with strategic leadership and ethical responsibility. Today's global challenges—climate change, rapid urbanization, digital transformation and resource scarcity—demand collaborative solutions that are technologically sound, economically viable and socially inclusive. Through keynote sessions, technical presentations, panel discussions and interactive workshops, this summit aims to inspire thought leadership and practical innovation.

The conference IC-SABS-2025, under the theme *"Innovating Life: Interdisciplinary Approaches in Sciences and Biotechnology,"* further strengthens our commitment to research that directly impacts human life and environmental well-being. Biotechnology and scientific research are pivotal in addressing global issues such as disease diagnostics, sustainable agriculture, renewable energy and ecological preservation. By encouraging interdisciplinary collaboration, we aim to nurture discoveries that are application-oriented and beneficial to society at large.

I extend my heartfelt appreciation to the organizing committee, advisory board members, collaborators and sponsors whose collective efforts have made this prestigious event possible. I am confident that the deliberations and research outcomes presented during IS-EDESM 2025 and IC-SABS-2025 will pave the way for innovative, sustainable and impactful solutions.

I wish the summit tremendous success and hope it continues to serve as a beacon of knowledge, collaboration and sustainable progress for years to come.

Mr. Amitaba Vira
Chairman, Kunwar Satyavira College of Engineering & Management (KSVCEM)
Patron IS-EDESM 2025

Campus: Bijnor City, Bijnor-246701 [U.P.] T: 01342-263514, 263833 Mob: 7830824300 / 7055522100

College Code : 017

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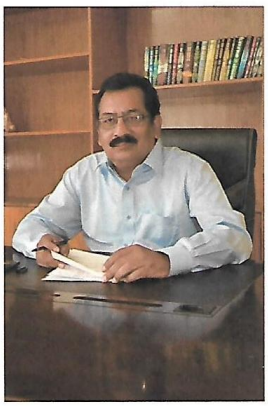

Message

It gives me immense pleasure to extend my warm greetings to all delegates, academicians, researchers, industry experts, and students participating in the *International Summit on Empowering Development through Engineering, Science & Management towards a Sustainable Future (IS-EDESM 2025)*, along with the *International Conference on Sustainable Advances in Biotechnology & Sciences (IC-SABS-2025)*.

The theme of this summit, *"Bridging Innovation and Sustainability through Engineering, Sciences & Management,"* is both timely and transformative. In an era defined by rapid technological evolution and pressing global challenges—ranging from climate change and resource depletion to emerging health concerns and population growth—the responsibility of academic and research institutions has expanded beyond knowledge dissemination to active societal transformation.

At **Kunwar Satyavira College of Engineering & Management (KSVCEM)**, we believe that sustainable development can only be achieved through interdisciplinary collaboration and forward-thinking innovation. IS-EDESM 2025 has been envisioned as a global platform where diverse domains converge to create actionable solutions. By integrating engineering excellence, scientific inquiry, and strategic management practices, the summit aspires to foster innovations that are scalable, inclusive, and environmentally responsible.

The parallel conference, IC-SABS-2025, with its compelling theme *"Innovating Life: Interdisciplinary Approaches in Sciences and Biotechnology,"* reflects the growing need for collaborative research across biotechnology, data science, nanotechnology, environmental engineering, and materials science. The future of healthcare, agriculture, clean energy, and ecological sustainability depends on our ability to transcend disciplinary boundaries and convert research into impactful real-world applications.

This summit not only provides a platform for presenting research papers and engaging in technical discussions but also promotes networking, industry-academia partnerships, and the exchange of global perspectives. I am confident that the deliberations, keynote sessions, and panel discussions will inspire innovative thinking and lay the groundwork for meaningful collaborations.

I congratulate the organizing committee for their dedication and meticulous planning in bringing together such an esteemed gathering of thought leaders and innovators. I extend my best wishes to all participants for fruitful discussions and enriching experiences.

Kunwar Udayan Vira
Secretary, Kunwar Satyavira College of Engineering & Management (KSVCEM)
Patron IS-EDESM 2025

Campus: Bijnor City, Bijnor-246701 [U.P.] T: 01342-263514, 263833 Mob: 7830824300 / 7055522100

राजकीय इंजीनियरिंग कॉलेज,बिजनौर
Rajkiya Engineering College, Bijnor
An Institute of Government of Uttar Pradesh
(AICTE APPROVED)
Jaleelpur Road, Near Eidgah,
Chandpur, Distt- Bijnor,
Uttar Pradesh, India -246725,
Website : www.recb.ac.in
Ph: 01345-221301
Fax 01345-221302

Message

It is a matter of great pride and honor to convey my best wishes for the successful organization of the *International Summit on Empowering Development through Engineering, Science & Management towards a Sustainable Future (IS-EDESM 2025)* and the *International Conference on Sustainable Advances in Biotechnology & Sciences (IC-SABS-2025).*

The vision behind IS-EDESM 2025 resonates deeply with the contemporary needs of global academia and industry. The theme, *"Bridging Innovation and Sustainability through Engineering, Sciences & Management,"* underscores the essential synergy between technological advancement and responsible development. Engineering and scientific research must now be guided not only by innovation but also by sustainability, ethics, and societal impact.

As Director of **Rajkiya Engineering College, Bijnor**, I firmly believe that interdisciplinary platforms such as this summit play a crucial role in nurturing research culture, fostering innovation, and encouraging young minds to think beyond conventional frameworks. The integration of engineering principles with scientific advancements and managerial strategies is pivotal in addressing global concerns like energy sustainability, environmental preservation, and technological inclusivity.

The focus of IC-SABS-2025 on *"Innovating Life: Interdisciplinary Approaches in Sciences and Biotechnology"* is particularly commendable. The convergence of biotechnology with computational modeling, nanotechnology, materials science, and environmental engineering is redefining problem-solving approaches in medicine, agriculture, industrial biotechnology, and ecological management. By promoting cross-disciplinary collaboration, this conference encourages researchers to translate theoretical discoveries into practical solutions that benefit society at large.

Such academic congregations also provide invaluable exposure to students and early-career researchers, enabling them to interact with global experts, present their findings, and gain insights into emerging research trends. The exchange of ideas and collaborative discussions during this summit will undoubtedly contribute to strengthening research networks and advancing sustainable technologies.

I congratulate the organizers for conceptualizing and executing this prestigious academic event. I am confident that IS-EDESM 2025 and IC-SABS-2025 will inspire meaningful dialogue, innovative research partnerships, and impactful outcomes aligned with global sustainability goals.

I extend my heartfelt wishes for the grand success of the summit and look forward to the transformative ideas and collaborations it will foster.

Badal

Prof. (Dr.) Neelendra Badal
Director, Rajkiya Engineering College (REC), Bijnor
Patron IS-EDESM 2025

Dr. Amiya Bhaumik
Founder & President
Lincoln University College, Malaysia
Co-Patron, IS-EDESM 2025

Message

It gives me immense pleasure to extend my heartfelt greetings to all participants of the International Summit on Empowering Development through Engineering, Science & Management towards a Sustainable Future (IS-EDESM 2025) which will be held 7th and 8th November, 2025. As Co-Patron of this significant academic gathering, I am delighted to witness an inspiring convergence of intellect, innovation, and interdisciplinary collaboration that truly embodies the spirit of sustainable progress.In today's dynamic world, the challenges of sustainability demand a holistic approach - one that integrates the creativity of science, the precision of engineering, and the strategic insight of management. This Summit provides an exceptional platform for scholars, researchers, policymakers, and industry professionals to exchange transformative ideas and devise solutions that contribute to a balanced and sustainable future. Lincoln University College, Malaysia, has always believed in fostering global partnerships that advance education, research, and innovation. Through our association with Kunwar Satyavira College of Engineering & Management and Rajkiya Engineering College, Chandpur, Bijnor, we reaffirm our shared commitment to nurturing knowledge that not only drives technological excellence but also uplifts humanity. I am confident that IS-EDESM 2025 will inspire meaningful dialogues, stimulate groundbreaking research, and strengthen global networks that collectively work towards sustainable development goals (SDGs) by supporting UNSDGs. I commend the organizers for their vision and dedication in orchestrating this international endeavour, and I extend my best wishes for the success of this Summit.

Ruchi Vira
Member of Parliament
(Lok Sabha)
Moradabad (U.P.)

302, Yamuna Appartments
Bishamber Das Marg
New Delhi - 110 001
Phone : 011-23350302

Message

It gives me immense pleasure to extend my warm greetings and best wishes to all distinguished delegates, researchers, academicians, industry experts and students participating in the International Summit on Empowering Development through Engineering, Science & Management towards a Sustainable Future (IS-EDESM 2025) and the **International Conference on Sustainable Advances in Biotechnology & Sciences (IC-SABS-2025).**

The theme of the summit, *"Bridging Innovation and Sustainability through Engineering, Sciences & Management,"* reflects a powerful vision for our times. In an era defined by rapid technological transformation and complex global challenges, it is imperative that innovation is guided by responsibility and sustainability. Engineering excellence, scientific exploration and strategic management must converge to create solutions that are not only advanced but also inclusive, ethical and environmentally conscious.

IS-EDESM 2025 stands as a dynamic platform that brings together diverse minds from across the globe. By hosting multidisciplinary dialogues, keynote deliberations, technical paper presentations and collaborative discussions, the summit fosters intellectual exchange and collective action. Such gatherings nurture creativity, critical thinking and cross-sector partnerships that are essential for achieving long-term development goals.

The International Conference on Sustainable Advances in Biotechnology & Sciences (IC-SABS-2025), with its theme *"Innovating Life: Interdisciplinary Approaches in Sciences and Biotechnology,"* highlights the transformative role of science in addressing real-world challenges. Today, biotechnology and scientific research are pivotal in areas such as healthcare innovation, food security, environmental sustainability and clean energy. Interdisciplinary collaboration—integrating computational tools, nanotechnology, environmental sciences and management strategies—has become the foundation of meaningful progress.

As a Member of KSVCEM, I take pride in supporting initiatives that promote academic excellence, research innovation and societal development. Conferences like these not only advance scholarly discourse but also inspire young researchers and students to pursue knowledge with purpose and integrity. They serve as catalysts for ideas that can shape a resilient and sustainable future.

I congratulate the organizing committee for their dedication and vision in curating such a prestigious international platform. May this summit lead to fruitful collaborations, impactful research outcomes and lasting professional relationships.

Kunwarani Ruchi Vira (Hon'ble Member of Parliament, Moradabad)
Member, Kunwar Satyavira College of Engineering and Management
Co-Patron IE-EDESM 2025

Resi.: Opp. St. Paul's School, Civil Lines, Moradabad - 244 001, (U.P.), Ph. : 0591 2990100

College Code : 017

Estd.1999

KUNWAR SATYAVIRA

College of Engineering and Management

(Approved by AICTE, New Delhi & Affiliated to Dr. APJ AKTU, Lucknow)
(Formerly VIRA College of Engineering)

Website: www.ksvira.edu.in Email: director@ksvira.edu.in

Message

It is a matter of great honor and pride to convey my sincere greetings on the occasion of the **International Summit on Empowering Development through Engineering, Science & Management towards a Sustainable Future (IS-EDESM 2025)** and the **International Conference on Sustainable Advances in Biotechnology & Sciences (IC-SABS-2025)**.

In today's interconnected world, sustainable development is not merely a concept but a collective responsibility. The summit's theme, *"Bridging Innovation and Sustainability through Engineering, Sciences & Management,"* resonates deeply with the need for balanced growth that harmonizes technological progress with environmental stewardship and social well-being. Such initiatives reinforce the idea that innovation must be aligned with ethical values and long-term sustainability objectives.

IS-EDESM 2025 provides an exceptional platform for global scholars, industry leaders and policymakers to engage in meaningful dialogue and share transformative research. The collaborative spirit fostered by this summit will undoubtedly contribute to actionable strategies and scalable solutions that address pressing global issues.

The focus of IC-SABS-2025 on *"Innovating Life: Interdisciplinary Approaches in Sciences and Biotechnology"* is particularly significant. Scientific and biotechnological advancements are reshaping medicine, agriculture, environmental management and industrial practices. By integrating multiple scientific disciplines and promoting application-driven research, the conference emphasizes solutions that directly impact society—ranging from disease diagnostics and sustainable agriculture to renewable energy and ecological preservation.

As Treasurer of KSVCEM, I firmly believe that investing in knowledge, research and academic collaboration yields the most valuable returns for society. Supporting such international academic endeavors reflects our commitment to fostering innovation, encouraging research excellence and building networks that transcend geographical boundaries.

I commend the organizers for their meticulous planning and visionary leadership in bringing together such a distinguished gathering of intellectuals and professionals. May this summit inspire groundbreaking ideas, strengthen global partnerships and pave the way for a sustainable and prosperous future.

I extend my best wishes for the remarkable success of IS-EDESM 2025 and IC-SABS-2025.

Mrs. Swati Vira Mahajan
Treasurer, Kunwar Satyavira College of Engineering and Management (KSVCEM)
Co-Patron IS-EDESM 2025

Campus: Bijnor City, Bijnor-246701 [U.P.] T: 01342-263514, 263833 Mob: 7830824300 / 7055522100

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Website: www.ksvira.edu.in Email: director@ksvira.edu.in

Message

It gives me immense pleasure to welcome all delegates, scholars, industry experts and students to the *International Summit on Empowering Development through Engineering, Science & Management towards Sustainable Future (IS-EDESM 2025)*, organized at Kunwar Satyavira College of Engineering and Management. This prestigious global platform reflects our institutional commitment to academic excellence, research innovation and sustainable development.

The theme of the summit, **"Bridging Innovation and Sustainability through Engineering, Sciences & Management,"** resonates strongly with the urgent need of our times. Today, the world stands at a critical juncture where emerging technologies, scientific advancements and effective management practices must converge to address complex global challenges such as climate change, resource scarcity, public health crises and sustainable industrial growth. IS-EDESM 2025 has been conceptualized as a multidisciplinary confluence that promotes collaborative thinking and actionable solutions.

The summit's parallel conferences, including the *International Conference on Sustainable Advances in Biotechnology & Sciences (IC-SABS-2025)*, provide a dynamic platform for the exchange of transformative ideas. The focus on **"Innovating Life: Interdisciplinary Approaches in Sciences and Biotechnology"** underlines the significance of integrating biotechnology with computational sciences, nanotechnology, environmental engineering and data-driven methodologies. Such interdisciplinary collaboration is essential for addressing issues related to disease diagnostics, food security, clean energy and ecological sustainability.

At KSVCEM, we firmly believe that research must transcend theoretical exploration and contribute meaningfully to societal progress. Through keynote addresses by eminent global thought leaders, technical paper presentations, panel discussions and workshops, IS-EDESM 2025 aims to inspire young researchers and professionals to pursue innovation with responsibility and sustainability at its core.

I extend my heartfelt appreciation to the organizing committee, reviewers, session chairs and contributors whose dedication has made this summit possible. I am confident that the deliberations and scholarly contributions presented in these proceedings will generate impactful insights and foster long-term academic and industry collaborations.

I wish IS-EDESM 2025 and IC-SABS-2025 grand success and hope that this summit becomes a catalyst for transformative research and sustainable global development.

Prof. (Dr.) Amit Kumar Bansal
Director, KSVCEM
Summit Chair – IS-EDESM 2025

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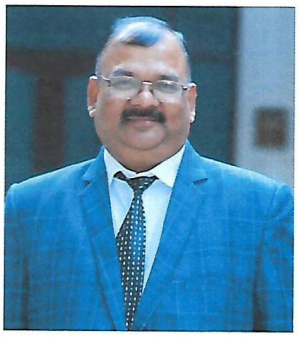

Message

It is a matter of great pride and honor to serve as the Organizing Secretary of the *International Summit on Empowering Development through Engineering, Science & Management towards Sustainable Future (IS-EDESM 2025)* and the *International Conference on Sustainable Advances in Biotechnology & Sciences (IC-SABS-2025)* hosted by Kunwar Satyavira College of Engineering and Management.

This summit has been thoughtfully designed as a vibrant academic ecosystem that brings together academicians, researchers, innovators, policy-makers and students from across the globe. The multidisciplinary framework of IS-EDESM 2025 reflects our belief that sustainable progress can only be achieved when engineering, science and management disciplines collaborate in a meaningful and structured manner.

The conference theme, **"Innovating Life: Interdisciplinary Approaches in Sciences and Biotechnology,"** emphasizes the transformative power of cross-disciplinary research. Today's scientific landscape demands integration—where biotechnology intersects with computational modeling, materials science, nanotechnology and environmental engineering to produce scalable and impactful solutions. The discussions and research presented at IC-SABS-2025 will not only enrich academic discourse but also address real-world challenges such as healthcare innovation, agricultural productivity, renewable energy and environmental protection.

As Organizing Secretary, I have witnessed the collective enthusiasm and dedication of our organizing team, advisory board and volunteers. Their coordinated efforts have ensured a seamless academic platform featuring keynote lectures, technical sessions, panel deliberations and networking opportunities that encourage knowledge exchange and collaborative research initiatives.

I extend my sincere gratitude to all contributors and participants who have enriched this summit with their scholarly work. I am confident that the ideas deliberated during IS-EDESM 2025 will inspire future research directions, strengthen institutional partnerships and contribute significantly to sustainable global advancement.

I wish all participants an intellectually stimulating and rewarding experience.

Prof. (Dr.) Lokesh Kumar Agrawal
Dean, Student Welfare, KSVCEM
Organizing Secretary – IS-EDESM 2025

Campus: Bijnor City, Bijnor-246701 [U.P.] T: 01342-263514, 263833 Mob: 7830824300 / 7055522100

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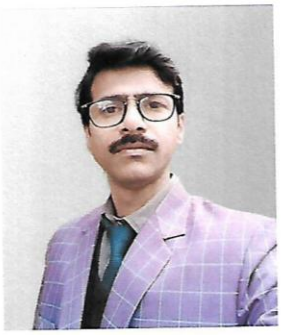

Message

It gives me immense pleasure to extend my heartfelt greetings to all the delegates, researchers, academicians and industry experts participating in the **International Conference on Sustainable Advances in Biotechnology & Sciences (IC-SABS-2025)** organized under the prestigious **International Summit on Empowering Development through Engineering, Science & Management towards a Sustainable Future (IS-EDESM 2025).**

The theme of this year's conference, **"Innovating Life: Interdisciplinary Approaches in Sciences and Biotechnology,"** emphasizes the growing importance of integrating diverse scientific disciplines to address complex global challenges. The convergence of biotechnology with fields such as data science, nanotechnology, environmental science, and computational modeling is revolutionizing how we approach problems in medicine, agriculture, industrial biotechnology and ecological sustainability.

IC-SABS-2025 aims to provide a dynamic academic environment that encourages researchers and practitioners to share their latest findings, innovative methodologies and practical solutions. The conference brings together distinguished keynote speakers, eminent academicians, young researchers and students who are committed to advancing knowledge and fostering collaborations across disciplines and geographical boundaries.

I am confident that the research papers presented in this conference proceedings will contribute significantly to the advancement of scientific knowledge and inspire future research in biotechnology and allied sciences. Such academic gatherings not only enhance scholarly dialogue but also strengthen partnerships between academia, industry and policy-making bodies.

I extend my sincere appreciation to the organizing committee, reviewers, contributors and volunteers whose dedicated efforts have made this conference possible. I also thank all the participants for their valuable contributions and enthusiastic involvement.

I hope that the deliberations and discussions during this conference will lead to innovative ideas, fruitful collaborations and sustainable solutions that benefit society and the global scientific community.

Mr. Manoj Kumar Kushwaha
Dean Academics, Kunwar Satyavira College of Engineering and Management (KSVCEM)
Convener, IS-EDESM 2025

Campus: Bijnor City, Bijnor-246701 [U.P.] T: 01342-263514, 263833 Mob: 7830824300 / 7055522100

राजकीय इंजीनियरिंग कॉलेज,बिजनौर
Rajkiya Engineering College, Bijnor
An Institute of Government of Uttar Pradesh
(AICTE APPROVED)
Jaleelpur Road, Near Eidgah,
Chandpur, Distt- Bijnor,
Uttar Pradesh, India -246725,
Website : www.recb.ac.in
Ph: 01345-221301
Fax 01345-221302

Message

It is a matter of great honor and privilege to welcome distinguished scholars, researchers, academicians and industry professionals to the **International Conference on Sustainable Advances in Biotechnology & Sciences (IC-SABS-2025)**, organized as part of the **International Summit on Empowering Development through Engineering, Science & Management towards a Sustainable Future (IS-EDESM 2025)**.

The rapid advancement of science and technology has opened unprecedented opportunities to address some of the most pressing global challenges of our time. Biotechnology and allied scientific disciplines are at the forefront of innovation, offering transformative solutions in healthcare, agriculture, environmental sustainability and industrial development. The significance of interdisciplinary collaboration has never been greater, as modern scientific challenges demand integrated approaches that combine knowledge from multiple fields.

The theme of IC-SABS-2025, **"Innovating Life: Interdisciplinary Approaches in Sciences and Biotechnology,"** highlights the importance of collaborative research that bridges traditional scientific boundaries. By bringing together experts from biotechnology, life sciences, computational sciences, environmental studies and engineering disciplines, the conference provides a platform for exploring innovative solutions that can contribute to sustainable development and global well-being.

This conference is designed to promote academic excellence and foster meaningful dialogue among participants from diverse backgrounds. Through keynote lectures, technical sessions, and research paper presentations, IC-SABS-2025 aims to facilitate the exchange of ideas, encourage collaborative research and inspire young scholars to pursue innovative scientific inquiry.

The proceedings of this conference reflect the collective efforts of researchers who are dedicated to advancing knowledge and developing practical solutions to real-world challenges. I believe that the contributions presented here will not only enrich the academic community but also support the development of sustainable technologies and practices for the benefit of society.

I would like to express my sincere gratitude to all the authors, reviewers, keynote speakers and organizing committee members for their invaluable contributions in making this conference a success. Their dedication and commitment have played a crucial role in shaping this significant academic event.

I wish all participants a productive and enriching experience and hope that the discussions and collaborations initiated during IC-SABS-2025 will lead to impactful innovations and lasting academic partnerships.

Dr. Ishan Bharadwaj
Head of Department, Information Technology
Rajkiya Engineering College Bijnor
Convener, IS-EDESM 2025

Prof. Dr. Sandeep Poddar
Deputy Vice Chancellor (Research & Innovation)
Lincoln University College, Malaysia
International Advisory Board Member, IS-EDESM 2025

Message

It is with great enthusiasm that I extend my warmest greetings to all participants of the International Summit on Empowering Development through Engineering, Science & Management towards a Sustainable Future (IS-EDESM 2025), scheduled for the 7th and 8th of November, 2025. This summit will be held under three parallel thematic conferences, each dedicated to fostering transformative innovations and sustainable practices across key areas of development. These conferences are International Conference on Empowering Development through Smart Engineering Systems (IC-EDSES-2025), the International Conference on Innovative Business Practices, Digital Transformation of Sustainable Management & Humanities (IC-IBDSMH-2025), and the International Conference on Sustainable Advances in Biotechnology & Sciences (IC-SABS-2025). As a member of the International Advisory Board for this pivotal summit, I am delighted to be part of this distinguished event that will bring together thought leaders, innovators, researchers, and professionals, all united in their mission to shape a sustainable future through collaboration, knowledge exchange, and innovative problem-solving.The challenges we face in the 21st century, from climate change to resource depletion, demand a comprehensive, interdisciplinary approach that bridges the fields of science, engineering, and management. This summit offers an invaluable platform for addressing these complex global challenges by fostering meaningful dialogue and collaboration across industries and disciplines. It provides us with a unique opportunity to exchange ideas, share best practices, and work together to develop solutions that will help us navigate the uncertainties of the future, while driving us towards a more sustainable and resilient tomorrow.At Lincoln University College, Malaysia, we remain committed to fostering global partnerships that enhance education, research, and technological innovation. In collaboration with Kunwar Satyavira College of Engineering & Management and Rajkiya Engineering College, Chandpur, Bijnor, we are united in our mission to advance knowledge that not only drives technological excellence but also contributes positively to social and environmental change. Through collective action and the exchange of expertise, we aim to make tangible progress toward the achievement of the United Nations Sustainable Development Goals (SDGs). I am confident that IS-EDESM 2025 will serve as a catalyst for inspiring conversations, groundbreaking research, and the formation of lasting global networks. These efforts will undoubtedly contribute to the achievement of the SDGs and help us build a sustainable, equitable future for all. I would like to commend the organizers for their vision and unwavering commitment in bringing this summit to life. Their efforts will ensure the success of this essential academic and professional gathering. I wish all participants a fruitful and impactful experience and eagerly look forward to the ideas and solutions that will emerge from this summit.

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KUNWAR SATYAVIRA
College of Engineering and Management
(Approved by AICTE, New Delhi & Affiliated to Dr. APJ AKTU, Lucknow)
(Formerly VIRA College of Engineering)
Website: www.ksvira.edu.in Email: director@ksvira.edu.in

Message from the Editorial Board

It is our privilege to present the conference proceedings of the **International Conference on Sustainable Advances in Biotechnology & Sciences (IC-SABS-2025)**, organized under the umbrella of the **International Summit on Empowering Development through Engineering, Science & Management towards a Sustainable Future (IS-EDESM 2025)**.

The International Summit IS-EDESM 2025 has been envisioned as a multidisciplinary academic platform that brings together distinguished academicians, researchers, scientists, industry experts, policymakers and students from across the globe.

The theme of the summit, **"Bridging Innovation and Sustainability through Engineering, Sciences & Management,"** reflects the urgent need for collaborative and interdisciplinary approaches to address global issues such as environmental sustainability, technological advancement, healthcare innovation and responsible economic growth.

The **International Conference on Sustainable Advances in Biotechnology & Sciences (IC-SABS-2025)** specifically focuses on the transformative role of scientific research and biotechnology in addressing some of the most pressing challenges of our time. In an era marked by emerging diseases, climate change, increasing population demands and limited natural resources, the role of science and biotechnology has become increasingly significant.

We sincerely appreciate the valuable contributions of all authors, reviewers and technical committee members whose dedication and expertise made this publication possible. Their scholarly efforts have significantly contributed to the success of this conference and the advancement of scientific knowledge.

We hope that the research presented in these proceedings will inspire further investigation, encourage interdisciplinary collaboration and support the development of sustainable and innovative solutions for the benefit of society.

The Editorial Board extends its heartfelt gratitude to the organizing committee, keynote speakers, participating institutions and all contributors for their support in making **IS-EDESM 2025** a meaningful and impactful academic endeavor.

We trust that these proceedings will serve as a valuable resource for researchers, academicians and practitioners and will continue to stimulate intellectual discourse and innovation in the years to come.

Editorial Board
IS-EDESM 2025

Campus: Bijnor City, Bijnor-246701 (U.P.) T: 01342-263514, 263833 Mob: 7830824300 / 7055522100

Introduction

The rapid progress of biotechnology and scientific innovation has transformed the modern world by offering solutions to pressing challenges in healthcare, agriculture, environment, industry, and sustainability. Emerging technologies such as genetic engineering, bioinformatics, nanotechnology, artificial intelligence in life sciences, and green innovations are shaping the future of global development.

The **International Conference on Sustainable Advances in Biotechnology & Sciences (IC-SABS-2025)** was organized to provide a common platform for discussing these transformative developments. The conference emphasized interdisciplinary research and collaboration to address real-world problems through science and innovation.

This proceeding captures the essence of the conference through a collection of peer-reviewed research papers, case studies, and technical insights contributed by experts from diverse fields. It reflects current trends, challenges, and opportunities in the scientific community.

Learning Objectives

This proceeding aims to achieve the following objectives:

1. To disseminate recent research findings in biotechnology and scientific sciences.
2. To promote interdisciplinary collaboration among researchers and professionals.
3. To explore sustainable technological solutions for global challenges.
4. To encourage innovation in healthcare, agriculture, environment, and industrial sectors.
5. To provide a platform for knowledge exchange among academicians, students, and industry experts.
6. To inspire future research in emerging frontiers of science and biotechnology.
7. To strengthen networks for international scientific cooperation.

ANTIBACTERIAL PROPERTY OF E.COLI ON HERBS

Ms. Shaily Rajput[1]

Department of Master of Business Administration, Swami Vivekanand Subharti University Meerut

Ms. Shivangi Bhardwaj[2]

Cloud Computing in IT training institute, DUCAT - Gurugram, Haryana

Abstract

The rise of antibiotic-resistant bacteria has necessitated the exploration of alternative anti microbial agents, including herbal remedies. This study investigates the antibacterial properties of various herbs against Escherichia coli (E. coli), a common Gram-negative bacterium responsible for numerous infections. We selected a range of medicinal herbs known for their traditional use in treating infections and subjected them to in vitro antibacterial assays. The herbs tested included neem (Azadiractha indica), tulsi (Oscimum teniflorum) and ginger (Zingiber officinale).

Methanolic and aqueous extracts of these herbs were prepared and their antibacterial activity was evaluated using the agar well diffusion method and minimum inhibitory concentration (MIC) assays. The results indicated that all tested herbs exhibited varying degrees of antibacterial activity against. coli, with garlic and oregano demonstrating the most potent effects. Neem extract showed the largest zone of inhibition (15 mm) and the lowest MIC value (0.5 mg/mL), suggesting a strong antibacterial effect.

Keywords: Antibacterial, Zone of inhibition, Medicinal plants, Infectious disease.

Introduction

A medicinal plant is the gift of nature. Natural products derived from plants for the treatment of disease. Medicinal plants have been used in healthcare since time immortal. The role,

contributions, and usefulness of medicinal plants in tackling diseases of public health importance, with particular emphasis on the current strategic approaches to disease prevention. Medicinal plants play vital roles indica as prevention and their promotion and use fit into all exist in prevention strategies. A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs.

Urinary tract infection (UTI), a prevalent disease in India, also ranks among the most common infections in developing countries. The rapid emergence of antibiotic-resistant uro pathogenic Escherichia coli (UPECs), the leading etiologic agent of UTI, in the last few years, led to an upsurge in the health care cost. Therefore, then action word widespread occurrences of highly virulent MDR UPEC together with the limited availability of therapeutics highlighted the urgent need for promotion and invention of alternative therapeutics, searchforwhichhadalreadybeenstarted.Moreover,investigationofseveral mechanisms of UPEC infection and the search for potential drug targets might help to design newer therapeutics

MATERIALS AND METHODS

This process began with the determination of the acid value from free fatty acids to identify the necessary steps of the process. The second stage involved drying to remove water from the Fatty Acid Methyl Esters (FAME). The final stage focused on analyzing the physical properties to determine the contents and quality of FAME.

Source of Microorganism

Escherichia coli (E. coli) is a gram-negative, rod-shaped bacterium commonly found in the intestines of humans and animals. It is an essential component of the gut micro biota and plays a significant role in maintaining intestinal health. The preparation of cultural media is a critical step in microbiological research, as it provides the necessary nutrients and suitable environment for the growth and isolation of *Escherichia coli* (E. coli).

Staining

Gram staining is a differential staining technique that classifies bacteria into two major groups—Gram-positive and Gram-negative—based on structural differences in their cell walls. *E. coli* is a Gram-negative bacterium, and this test is used to confirm its Gram reaction.

Preparation of Plant Extract

The preparation of plant extracts involves several steps to ensure that bioactive compounds are efficiently extracted from the plant materials. This section outlines the procedures for preparing extracts from the leaves of *Azadirachta indica* (Neem), *Zingiber officinale* (Ginger), and *Ocimum tenuiflorum* (Tulsi).

At the bottom will be presence of extract of plants from leaves.

Preparation of Diluents

A series of sterile test tubes or dilution bottles, each containing 9 ml of sterile saline or phosphate-buffered saline (PBS), were prepared. Each test tube was labelled according to the dilution factors, such as 10^{-1}, 10^{-2}, 10^{-3}, and so on, up to the desired dilution level.

Initial Sample Dilution

Using a micropipette, 1 ml of the original *E. coli* sample was transferred into the first test tube containing 9 ml of diluents, resulting in a 10^{-1} dilution. The tube was vortexes thoroughly to ensure proper mixing of the sample.

Subsequent Dilutions

Using a new sterile pipette tip, 1 ml from the 10^{-1} dilution was transferred into the next test tube containing 9 ml of diluents to create a 10^{-2} dilution. This process was repeated as necessary to achieve further dilutions.

Plating the Dilutions

Sterile nutrient agar plates were labelled with their corresponding dilution factors. Using a micropipette, 100 µl of each dilution was transferred onto the surface of the labelled agar plates and spread evenly for uniform colony distribution.

Incubation

The lids of the agar plates were closed, and the plates were placed upside down (agar side up) in an incubator. The plates were incubated at 37°C for 24–48 hours to allow bacterial growth.

RESULTS

EFFECT OF HERBS ON BACTERIA

Summary

This study investigated the antibacterial properties of plant extracts from Azadirachta indica (Neem), Zingier officinale (Ginger), and Ocimum tenuiflorum (Tulsi) against Escherichia coli (E. coli). The research was conducted to explore natural alternatives to conventional antibiotics and to understand the efficacy of these herbal extracts in inhibiting bacterial growth. E.coli was successfully isolated from the provided sample using Nutrient Agar and MacConkeyAgarplates.ThebacterialcoloniesexhibitedtypicalcharacteristicsofE.coli, including pink colonies on Mac Conkey Agar. Gram staining confirmed the identity of E.coli as Gram-negative, rod-shaped bacteria.

Extracts were prepared from the leaves of Neem, Ginger, and Tulsi using aqueous and ethanol extraction methods. The extracts were concentrated and stored for antibacterial testing.

The antibacterial activity of the extracts was evaluated using the well diffusion method. AgarplatesinoculatedwithE.coliwereusedtoassessthezonesofinhibitionaroundwells filled with each plant extract.

Results indicated varying degrees of antibacterial activity, with each extract showing some level of inhibition against E. coli.

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'Cordycepin upregulation during in-vitro cultivation of *cordyceps militaris*'

Shivani Singh[1]

Dr. Ramesh Joshi[2] Gunjan Tiwari[2],

Surbhi Dhaka[1]

[1] *Department of Biotechnology, KunwarSatyavira College of Engineering & Management, Bijnor, India*

[2] *Sharda School of Basic Sciences & Research, Sharda University, Greater Noida, India*

[3]*Department of Biotechnology, KunwarSatyavira College of Engineering & Management, Bijnor, India*

Abstract

Cordycepin (3′-deoxyadenosine) is a major bioactive compound of *Cordyceps militaris*, widely valued for its pharmacological properties, including anticancer, antioxidant, immunomodulatory and anti-inflammatory activities. However, its natural yield varies considerably due to environmental and physiological constraints, creating a need for optimized in-vitro cultivation strategies to enhance cordycepin biosynthesis. The present study investigates the upregulation of cordycepin during controlled in-vitro cultivation of *C. militaris* by evaluating the influence of nutrient composition, carbon–nitrogen ratio, pH, light regimes, and supplementation with metabolic precursors. Solid and liquid culture systems were standardized, and cordycepin accumulation was quantified using spectrophotometric and chromatographic assays. The results demonstrate that optimized media enriched with yeast extract, adenine precursors, and balanced nitrogen sources significantly enhanced mycelial biomass and cordycepin content. Light exposure at specific wavelengths further stimulated secondary metabolite production. Overall, the study highlights that targeted manipulation of culture conditions markedly improves

cordycepin yields, offering an efficient and scalable platform for biotechnological and pharmaceutical applications.

Keywords: Cordycepin; Cordyceps militaris; Carbon sources; Nitrogen sources.

Introduction;

Cordyceps militaris (L.) Link is an entomopathogenic fungus,widely used as a traditional medicinal mushroom due to various bioactive metabolites activity since ancient times in Asia,this fungus belonging to the family Clavicipitaceae and a species of Cordyceps genus.Locally known as “keeda Jhadi’’ and it is a highly-valued, edible mushroom reported to possess important properties such as immunomodulatory,antioxidant,anti-tumor,anti-inflammatory and anti-hypertensive etc.(Chen et al., 2017) This fungus contains medicinally useful bioactive metabolites,including adenosine pentostin,carotenoids and cordycepin (3’-deoxyadenosine) etc.(Chen,B.X et al.,2018a).Cordycepin(3`-deoxyadenosine), one of nucleoside analogues, was first isolated from the medicinal mushroom Cordyceps militaris. the difference between cordycepin and adenosine is the lack of the 3`-hydroxyl group in cordycepin and not the “3` position of the ribose part’’(Zheng et al., 2011 and Tuli et al., 2014) Previous studies on *in vitro* cultivation of *C. militaris*reported that degeneration of this fungus *is* due to environmental and genetic variations, but is not associated with a specific geographical condition (Chen, X et al.,2019a.). Previous studies have also reported that accumulation of cellular reactive oxygen species (ROS) and other super oxides in mycelia could be another reason for the degeneration of fungal growth (Jiao et al., 2018.). A recent study has also showed that the cultivation of *C. militaris* strains for growth and cordycepin production was rightly dependent on preferable carbon and nitrogen source (Wongsa et al., 2020). This finding suggests that the design of cultivation medium is crucial for growth and cordycepin production. We propose that optimization of growth conditions and

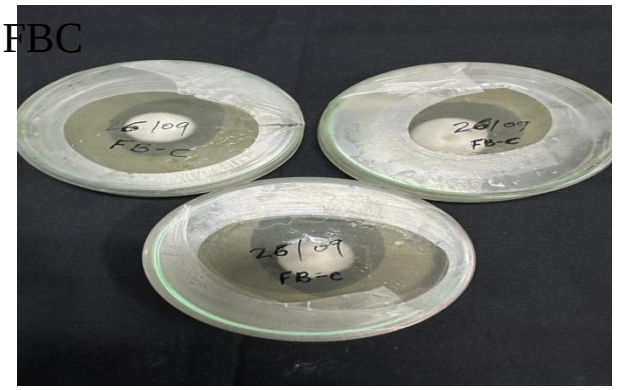
supplementing useful constituents into culture medium can effectively delay its rate of degeneration delivering more bioavailability of its medicinal ingredients. In addition, its appropriate preservation can maintain fungal viability and reduce fruiting body production (Liu et al., 2018b). In previous work, cordycepin has been synthesized by chemical (Abdulla et al.,2023; Olatunji et al., 2018) and microbial fermentation using *C. militaris* (Turk et al., 2022; Turk et al., 2021) or *Aspergillus nidulans* (Wu et al., 2019; Zhang et al., 2019). However, Fermentation takes too long time and is difficult to overcome problem of contamination to achieve large scale production to reduce production cost (Guo et al., 2020; Jo et al. 2020; Karol et al., 2021). Though *C. militaris* can be grown in culture but its large-scale production is still a challenge. The proposed investigation may provide useful information that can help to maintain the quality of *C. militaris*, which is important for its large-scale cultivation in the biotechnology industries. This study will provide a system-level approach for enhancing *C. militaris* growth towards an efficient fungal cell factory for the increased production of cordycepin and enhance the growth of *C.militaris.* Therefore, the major objective of this study was to improve cordycepin production and enhance the growth of *C.militaris* in submerged culture. Among the various *C. militaris*, using two different strain name as (FBC & JR-18) both strain with high yields of cordycepin production per cell mass was selected, andthe culture conditions such as temperature, different –different concentration of ingridient, and shaking speed were fundamentally investigated. in addition, a medium composed of different carbon and nitrogen sources was prepared and the efective composition for cordycepin production and enhance the growth of *C.militaris* was determined based on experimental results. Investigation of secondary metabolite production through changes in concentration medium components requires a number of trials. It is possible to understand more clear and simple correlations by the experiments. Thus, a fundamental study of *C. militaris* culture under submerged conditions would bebeneficial for scale-up in industrial processes.

.Methods& Material

2.1.Collectio of strain;*C.militaris* FBC and JR-18 were purchased from the Visheshwar Agro Products from the Dehradun .C.*militaris* FBC and JR-18 was mutaed from wild type *C.militaris* by artificial cultivation of wild *Cordyceps* at solid state with various substrate medium have been studied for commercial use.Especially nutritional requirement,environmental conditions and inoculum preparation were investigated for the cultivation.Both strain were inoculated on a PDA(Potato dextrose agar) and MgSO4 (Magnesium sulfate) plate at 20°C in dark room for 7-10 days.

2.2 .Preperatio of Mycelium;The stock culture of different strains (FBC & JR-18) of *C. militaris* mycelium shall be maintained on agar slants containing 3.9% (PDA) potato dextrose agar and further experiments will be conducted to maintain the stock culture on 0.05% MgSO4 and 2% glucose, 2% peptone, 0.2% KH2PO4, and 0.3% MgSO4. The inoculated slants will be incubated at 20℃ in the dark for 7-10 days and then will be stored at 4℃.

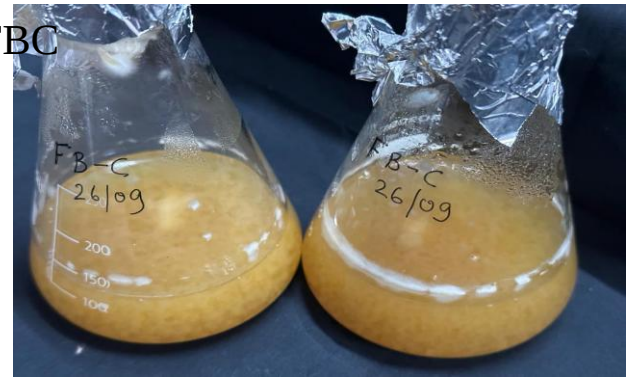
FBC



JR-18

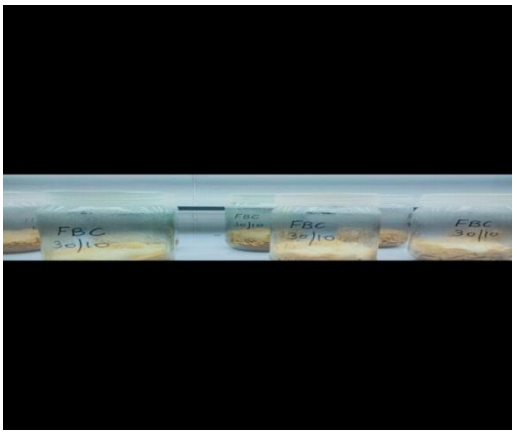
2.2.2. ***Preperatio of Liquid media ;***The mycelium from agar slants stock will be inoculated on liquid medium in 250ml conical flask for multiplication of spawn. The liquid medium will be comprised of 2.4% Potato dextrose broth (PDB), 0.5% Peptone, 0.3% Yeast extract, 0.1% KH2PO4, 0.05% MgSO4.7H2O, 50mg/L Vitamin B1(Thiamine). Liquid medium vessels will be incubated on a rotary shaker at the speed of 130-150RPM for 15 days at 20℃ in dark room.

FBC



JR-18

2.2.3.Substrate Medium For Mycelium Multiplication;The substrate medium will be comprised of 3.0% Glucose, 0.5%Peptone, 0.3%Yeast Extract, 0.1% KH_2PO_4, 0.1% Tri-ammonia Citrate, 0.05% $MgSO_4.7H_2O$, 50mg/L Vitamin B1 (Thiamine), 10mg/L Vitamin B12 (Cyanocobalamin) and brown rice 36gm in 63 ml of nutrient medium in a 300 mL cylindrical glass bottle and will be autoclaved.After the completion of spawn formation, 5-10ml of the spawn suspension will be transferred to substrate media will be incubated at 21–23℃ with the air humidity above 70% and in the dark for 15-20 days for base cultivation. After the complete spread of *C. militaris* mycelia on the medium surface, they were subjected to an alternating light–dark cycle of 12 h of light and 12 h of darkness for 30 days at 21–23℃ to stimulate fruiting body growth. The cultivation environment and growth status will be examined regularly. After 30-60 days, *C. militaris* fruiting bodies will be harvested, vacuum freeze-dried, weighed, collected, ground into powder, and stored at -20℃ for further analysis.



2.2.4. Optimization of solvent-solvent ratio (v/v) *of nutrient concentration of substrate medium On growth of fruiting bodies of C.militaris & Cordyceps militaris.*

1200 /200		*1.*	*2.*	*3.*	*4.*	*6.*	*7.*	*8.*	*9.*
SN.	*Cnctn. Gm/L*	*Glucose*	*Peptone*	*Yeast extract*	*KH2PO4*	*C6H17N3O7*	*MgSO4*	*Vit.B1*	*Vit.B12*
A	*1200ml*	*3%*	*0.5%*	*0.3%*	*0.1%*	*0.1%*	*0.05%*	*5%*	*1%*
B	*200ml 10%Cmp*	*0.3%*	*0.05%*	*0.03%*	*0.01%*	*0.01%*	*0.005%*	*0.5%*	*0.6%*
C	*20%mp*	*0.06%*	*0.1%*	*0.06%*	*0.02%*	*0.02%*	*0.01%*	*1%*	*1.2%*
D	*200ml 30%cmp*	*0.9%*	*0.15%*	*0.09%*	*0.03%*	*0.03%*	*0.015%*	*1.5%*	*1.8%*
E	*200ml 40%cmp*	*1.2%*	*0.2%*	*0.12%*	*0.04%*	*0.04%*	*0.02%*	*2%*	*2.4%*

2.3. Determination of Cordycepin and Growth of *Cordyceps Militaris*

Methanolic, ethanolic and aqueous extracts of different stages fruiting body of C.

militaris will be prepared to quantify Cordycepin. Standards of a cordycepin (both from Sigma-Aldrich; USA) will be prepared at 1 mg/mL and then diluted to obtain the desired concentrations (0.39 µg/mL, 0.78 µg/mL, 1.36 µg/mL, 3.12 µg/mL, 6.25 µg/mL, 12.5 µg/mL, 25 µg/mL, 50 µg/mL, 100 µg/mL and 200 µg/mL).The crude extracts will be prepared from mycelia and fruiting bodies of different developmental statges by extracting with deionized water, incubating at 50°C for 3 hr and passing through a 0.45 µm membrane filter. The cordycepin contents will be analyzed using high-performance liquid chromatography, (HPLC; Waters Corporation; USA) by comparing to the standard compounds. This method will be adapted from Xia et al. (2017). An aliquot of 1 µL was analyzed using a reversed-phase C18 column (4.6 mm × 250 mm, 5 µm particle size). The conditions used an isocratic 95:5 (0.2% of formic acid-to-methanol), flow rate of 0.2 mL/min and a column temperature 30°C. The adenosine and cordycepin will be monitored and quantified at 260 nm. The metabolite production rate of each generation (expressed as µg/g dry weight/day) will be determined by subtracting the amount of cordycepin in mycelia after growing at day 30 with day 1, and divided by cultivation time (total 30 days)(Stanberry et al.,)

3. RESULTS& DISCUSSION;

3.1.1 Mycelial Growth Response on Different Culture Media; Significant variation in the radial growth of *Cordyceps militaris* was observed across the tested media. PDA and SDAY supported the fastest mycelial expansion, with mean radial growth of **7.8 ± 0.24 cm** and **7.4 ± 0.31 cm**, respectively, after 14 days. Minimal growth was recorded on CzapekDox Agar (**4.1 ± 0.28 cm**). Mycelia grown on enriched SDAY exhibited dense, cottony white to orange pigmentation, indicating improved biomass accumulation.

3.1.2. Effect of Carbon and Nitrogen Sources; Among the carbon sources, **glucose (2%)** yielded the highest mycelial dry weight (**1.82 g/100 mL**), followed by sucrose

(**1.57 g/100 mL**). For nitrogen supplementation, **yeast extract (1%)** produced the maximum biomass (**2.04 g/100 mL**), outperforming peptone and ammonium salts.

3.1.3. Influence of Light Regimes on Fruiting Body Development; Light exposure significantly influenced pigmentation and fruiting. Cultures exposed to **12 h light/12 h dark** photoperiod produced well-developed orange stomata with an average stripe length of **3.6 ± 0.18 cm**. Continuous darkness resulted in poor pigmentation and malformed fruiting bodies.

3.1.4. Cordycepin Quantification in Different Treatments; Cordycepin concentration varied significantly in response to nutrient enrichment and light treatment. Maximum cordycepin content (**247.3 ± 5.9 mg/L**) was recorded in SDAY supplemented with glucose and yeast extract under 12 h photoperiod. Minimal content (**98.6 ± 4.7 mg/L**) was found in unsupplemented Czapek Dox broth. HPLC analysis confirmed a 1.8–2.5-fold increase in cordycepin content in optimized cultures.

3.1.5. Antioxidant Activity of Extracted Cordycepin; Cordycepin extracted from optimized cultures showed strong antioxidant activity, with **DPPH radical scavenging capacity of 72.4%**, significantly higher than extracts from control cultures (**48.7%**). FRAP values also indicated enhanced reducing power, confirming improved bioactivity.

3.2DISCUSSION

The present investigation demonstrates that optimized in vitro conditions can markedly enhance the vegetative growth, fruiting potential, and cordycepin biosynthesis in *Cordyceps militaris*. Media composition played a crucial role in determining both mycelial morphology and metabolite production. PDA and SDAY offered higher nutrient availability, supporting rapid mycelial colonization. This agrees with previous studies reporting that nutrient-rich substrates promote vigorous

fungal growth and secondary metabolite synthesis.

Carbon and nitrogen supplementation significantly influenced biomass and cordycepin levels. Glucose, being a readily metabolizable sugar, promoted higher ATP generation and faster mycelial proliferation. Similarly, yeast extract, rich in amino acids and vitamins, acted as a strong inducer for metabolite pathways, resulting in elevated cordycepin accumulation. The findings support earlier evidence that nitrogen-rich environments upregulate metabolic genes responsible for nucleoside analog synthesis in *C. militaris*.Light is another critical factor in developmental regulation. The 12 h light/12 h dark cycle enhanced carotenoid formation and stimulated stromatal differentiation, correlating with higher cordycepin yield. Light likely acts as a signaling cue influencing gene clusters associated with secondary metabolite biosynthesis, including cordycepin-related pathways. The significant improvement in antioxidant activity of cordycepin obtained from optimized cultures indicates that enhanced metabolite biosynthesis is directly linked to improved functional properties. Higher DPPH and FRsAP values reflect increased nucleoside analog concentration and possibly the presence of co-metabolites such as adenosine derivatives.

Overall, the study highlights that carefully manipulated in vitro culture conditions substantially enhance both growth and metabolite production in *Cordyceps militaris*. The combined effect of nutrient enrichment and controlled photoperiod led to a 2-fold increase in cordycepin content, indicating strong potential for commercial-scale production. These results contribute to understanding metabolic regulation in medicinal fungi and pave the way for optimizing large-scale bioreactor cultivation for cordycepin overproduction.

Conclusion

The present in vitro study clearly demonstrates that strategic optimization of culture

conditions can significantly enhance the growth, development, and cordycepin biosynthesis in *Cordyceps militaris* (L.) Link. Nutrient-rich media, particularly SDAY supplemented with glucose and yeast extract, proved highly effective in promoting vigorous mycelial proliferation and increasing biomass. Similarly, controlled environmental factors, especially the 12 h light–12 h dark photoperiod, played a vital role in stimulating proper stromatal formation and pigmentation, leading to improved physiological development.

HPLC-based quantification confirmed a substantial up regulation of cordycepin content under optimized conditions, showing nearly a two-fold increase compared to untreated controls. The enhanced antioxidant activity of cordycepin extracted from optimized cultures further validated the improvement in metabolite quality. These findings highlight that manipulating carbon and nitrogen sources, along with regulated illumination, provides a reliable approach for boosting cordycepin production in vitro.Overall, the study establishes an efficient and reproducible framework for maximizing both growth performance and secondary metabolite output in *Cordyceps militaris*. The insights gained not only offer a foundation for large-scale commercial cultivation but also contribute to the broader understanding of metabolic regulation in medicinal fungi. Future research may focus on molecular pathway analysis and bioreactor-based optimization to further enhance cordycepin overproduction.

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Analysis of Microbial Fauna Ram Ganga and Ganga River

Vipin Kumar

Department of Biotechnology Kunwar Satyavira College of Engineering &Management

Bijnor (246701) UP. India

Abstract

This study investigates the microbial diversity and water quality of two prominent river systems in northern India: The Ramganga River at Moradabad and the Ganges River at Haridwar. Water samples were collected from selected sites along both rivers and subjected to microbiological analysis using standard culture-based and biochemical identification techniques. The primary objective was to identify and compare the microbial communities present in these water bodies, assess potential pathogenic contamination, and evaluate the implications for public health and environmental sustainability. Results indicated the presence of a diverse range of bacteria, including coliforms, Escherichia coli, Pseudomonas spp., and Bacillus spp., with notably higher microbial loads in the Ramganga samples, suggesting increased anthropogenic and industrial pollution. The presence of fecal indicator bacteria in both rivers highlights concerns regarding untreated sewage discharge and the need for better wastewater management. The findings underscore the importance of regular microbial monitoring to safeguard water resources, especially in regions with significant religious, agricultural, and domestic usage like Haridwar. Recommendations for improving water quality and microbial safety are also discussed.

Keywords: Microbial diversity, Ramganga River, Ganga River, Haridwar, water qualityassessment, coliforms, *Escherichia coli*, *Pseudomonas spp.*, *Bacillus spp.*, fecal contamination,

Introduction

Pollution of a river first affects its chemical quality and then systematically destroys the community disrupting the delicate food web. Diverse uses of the rivers are seriously impaired due to pollution and even the polluters like industry suffer due to increased pollution of the rivers. River pollution has several dimensions and effective monitoring and control of river pollution requires the expertise from various disciplines [1].Pollution of river is a global problem. In India it is reported that about 70% of the available water is polluted. The chief source of pollution is identified as sewage constituting 84 to 92 percent of the waste water.

The indiscriminate and large-scale deforestation and over grazing in the watershed areas of river basins have caused soil erosion resulting in considerable silting of dams and shrinkage of river flows. This leads to the flooding of the rivers at the time of excessive rains [2]. The disposal of waste leads to contamination of river and lakes chronically affecting the flora and fauna. According to surveys carried out on selected stretches of important rivers, it has been found that most of the rivers are grossly polluted. The domestic sewage discharged from a population of about 2 millions gives rise to numerous water-borne diseases like typhoid, cholera, dysentery, poliomyelitis and cysticercosis, thereby affecting the human health and deterioration of the water quality [3]. Water is the most precious resource, essential to sustain the life on earth. The Ganga River is one of the most utilized rivers in the world, due to the abundant availability of water throughout the year; it has played an important role in the development of Indian civilization and economy. The water of river Ganga is frequently used for drinking, cooking and bathing purposes due to ancient knowledge that Ganges water does not putrefy, even after a long period of storage; Water has been used from time immemorial for remedial purposes. Most religious beliefs involve some ceremonial use of Holy water and in the India the water of river Ganga

is treated with such reverence. Under the continuous Saraswati- Indus civilization going back to 7500 BC, the river Ganga is mentioned in Rigveda [4].

Outbreaks of acute diarrheal disease have been identified as causes of fatal disease dating back as far as the Sanskrit literature and during Hippocratic times. Increased urbanization and industrialization in the basin, has resulted in polluting the river, since the river has been preferred waste disposal sites for industrial and domestic effluents. Arbitrary and hysterical discharge of industrial and urban wastes into the environmental sink has become an issue of major global concern [5].

Ganga, the mighty Indian River originates from the snowed peaks of Himalayas, is the lifeline of millions of Indians. From its source to its entry in to the Bay of Bengal, it travels a distance of around 2525 Kms. The river with its well-knit tributaries drains the Ganga Basinwhich encompasses an area of more than a million square kilometers. (1060,000 sq km) spread over four countries- India, Nepal, Bangladesh and China. Hardwar is a city in Northern India on the bank of the Ganga River north east of Delhi. It is a Hindu pilgrimage center. Hardwar lies along the Ganga River at the boundary between the Indo-gangetic plain (South) and the Himalayan foothills (North). The water supply of the Ganga system is partly dependent on the rains brought by the monsoon winds from July to October as well as on the flow from melting Himalayan glaciers in the hot season from April to June. The religious importance of Ganga may exceed than that of any other river in the world. For this study, the water samples were collected from two spots. Sampling station A (Neelkanth).

Sampling station is situated in the north of Haridwar**.** Sampling station.B (Ramganga) sampling station is situated in the north of Moradabad. Accurate and timely information on the quality of water is necessary to shape a sound public policy and to implement the water quality improvement programmes efficiently. One of the most effective ways to communicate information on water quality trends is with indices. Water quality index (WQI) is commonly used for the detection and evaluation of

water pollution and may be defined as “a rating reflecting the composite influence of different quality parameters on the overall quality of water.” [6]The wastewater pollutants are harmful to environment and public health.The biological decomposition of organics could result in fish kills and foul odours. Waterborne diseases are also eliminated through proper wastewater treatment. There are many pollutantsthat could exhibit toxic effects on aquatic life and the public.The wastewater treatment is removal of contaminants from water in order to decrease the possibility of detrimental in part on the ecosystem including humans[7].High turbidity can inhibit the effects of disinfection against microorganisms and enable bacterial growth. Drinking water should be colourless, since drinkingwater coloration may be due to the presence of coloured organic matter. Organic substances cause water odour, though odours may result from many factors, including biological activity and industrial pollution also microbial pathogens cause health hazards[8]. Water covers 78% of the earth’s surface, yet the water available for human use is limited. Water is a very essential substance for all living organisms, as it happens to be an important constituent in human body and play very important role in all metabolic process inside the body. This is the age of quality consciousness as we always insist on the quality of many things that we use or own. The quality of surface as well as ground water quality is constantly changing because of environmental degradation and anthropogenic activities which contribute in water pollution[9]. The availability of safe potable water demand will be a challenging task in near future [10]. Water quality factor is directly interrelated with the physical chemical and biological characteristics of its surrounding environment [11-12]. . Safe drinking water is an important issue for human health point concern. Contamination in drinking water may lead to serious health hazards which may cause diseases viz typhoid, cholera, jaundice etc[13]. In our country, many researches are continuously going on for assessment of pollution status of water bodies[14-15]. River Bhagirathi and river Alaknanda originate in Garhwal Himalayas and join at Devprayag to form River Ganga. River Ganga traverses through Uttarakhand, Uttar

Pradesh, Bihar, Jharkhand and West Bengal and thereafter enters Bangladesh. The important tributaries of Ganga are the Yamuna, the Kali, the Ramganga, the Ghaghra, the Gandak, the Kosi, and the Sone. The most culturally significant hotspot of the river is at Haridwar where according to Hindu mythology it is said to have descended from the heavens. The holy city of Haridwar is located in the north Indian state of Uttaranchal at a distance of 214 km from Delhi at the foothills of Shivalik. The distance from Rishikesh to Hardwar is about 28.3 km. Haridwar extends from latitude 29°58′ in the north to longitude 78°13′ in the east. The city is situated at a height of almost 300 m above sea level and the temperature usually hovers around 40 °C during summers. Winters see the mercury dipping to as low as 6 °C.

The river has been the focus of national and international intervention and study for past several decades to identify and establish causes and impact of anthropogenic activities on river water quality. Ganga river basin, which was comparatively free from anthropocentric activities until the 1940s, became a disposal site for agricultural, industrial and sewage wastes after independence of India in 1947[16].

Aim and objectives

AIM: -Analysis of Microbial Fauna from Ramganga and Ganga River

Objectives

- To assess Antibiotic sensitivity test.
- To assess the turbidity of Ramganga and Haridwar water.
- To assess the pH of Ramganga and Haridwar water.
- Gram ‘s staining.
- Endospore staining test.
- Catalase test.

Material and Method

Sample collection was Ganga water from Haridwar and Ramganga water from

Moradabad characterization of microbes in Ganga water sample from Haridwar and Moradabad

Nutrient agar media

Nutrient agar is used as a general-purpose medium for the growth of a wide variety of non-fastidious microorganisms. It consists of peptone, beef extract and agar. This relatively simple formulation provides the nutrients necessary for the replication of a large number of non-fastidious microorganisms. Nutrient Agar/broth is used for the cultivation and maintenance of non-fastidious organisms as well as enumeration of organisms in water, sewage, dairy products, feces and other materials.

Composition of nutrient agar: -

- Peptone –0.5g
- Beef extract –0.3g
- Agar – 1.5g
- NaCl – 0.5g

We use the glassware and many types of instruments as Petriplates, beakar, soil, measuring cylinder, autoclave, laminar air flow, incubator, pH meter, micropipette, tip, cotton, foil paper, tissue paper, and much type of other materials. Firstly, we make the agar solution.

Beef extract is an aqueous extract of lean beef tissues. It contains water-soluble substances of animal tissue, which include carbohydrates, organic nitrogen compounds, water soluble vitamins, and salts.

Peptone is made by digesting proteinaceous materials e.g., meat, casein, gelatin, using acids or enzymes. Peptone is the principal source of organic nitrogen and may contain carbohydrates or vitamins. Depending up on the nature of protein and method of digestion, peptones differ in their constituents, differing in their ability to support the growth of bacteria.

Agar is a complex carbohydrate obtained from certain marine algae. It is used as a solidifying agent for media and does not have any nutritive value. Agar gels when the

temperature of media reaches 45°C and melts when the temperature reaches 95 °C.

4.1 Gram's staining kit

4.2 Antibiotic disc

4.3 Turbidimeter

4.4 Hydrogen peroxide powder

4.5 Malachite Green

4.6 pH meter

Gram's staining

Gram staining or Gram stain, also called Gram's Method, is a method of staining used to differentiate bacterial species into two large groups (gram-positive and gram-negative). The name comes from the Danish bacteriologist Hans Christian Gram, who developed the technique. Gram staining differentiates bacteria by the chemical and physical properties of their cell walls by detecting peptidoglycan, which is present in the cell wall of Gram-positive bacteria. Gram-positive bacteria retain the crystal violet dye, and thus are stained violet, while the Gram-negative bacteria do not; after washing, a counter stain is added (commonly safranin or fuchsine) that will stain these Gram-negative bacteria a pink color. It should be noted that both Gram-positive bacteria and Gram-negative bacteria pick up the counter stain. The counter stain, however, is unseen on Gram-positive bacteria because of the darker crystal violet stain.

The differences in cell wall composition of Gram positive and Gram-negative bacteria accounts for the Gram staining differences. Gram positive cell wall contain thick layer of **peptidoglycan** with numerous teichoic acid cross linking which resists the decolorization.

In aqueous solutions crystal violet dissociates into CV+ and Cl – ions that penetrate through the wall and membrane of both Gram-positive and Gram-negative cells. The CV+ interacts with negatively charged components of bacterial cells, staining the cells purple.

When added, iodine (I- or I3-) interacts with CV+ to form large crystal violet iodine

(CV-I) complexes within the cytoplasm and outer layers of the cell.

The decolorizing agent, (ethanol or an ethanol and acetone solution), interacts with the lipids of the membranes of both gram-positive and gram-negative bacteria.

The outer membrane of the Gram-negative cell (**lipopolysaccharide layer**) is lost from the cell, leaving the peptidoglycan layer exposed. Gram-negative cells have thin layers of peptidoglycan, one to three layers deep with a slightly different structure than the peptidoglycan of gram-positive cells. With ethanol treatment, gram-negative cell walls become leaky and allow the large CV-I complexes to be washed from the cell.

The highly cross-linked and **multi-layered peptidoglycan** of the gram-positive cell is dehydrated by the addition of ethanol. The multi-layered nature of the peptidoglycan along with the **dehydration** from the ethanol treatment traps the large CV-I complexes within the cell.

After decolorization, the gram-positive cell remains purple in color, whereas the gram-negative cell loses the purple color and is only revealed when the counter stain, the positively charged dye **safranin**, is added.

Materials Required:

1. Clean glass slides

2. Inoculating loop

3. Bunsen burner

4. Bibulous paper

5. Microscope

6. Lens paper and lens cleaner

7. Immersion oil

8. Distilled water

9.18-to-24-hour cultures of organisms

Reagents:

1. Primary Stain - Crystal Violet
2. Mordant - Grams Iodine
3. Decolorizer - Ethyl Alcohol
4. Secondary Stain - Safranin

Procedure:

Preparation of the glass microscopic slide

Grease or oil free slides are essential for the preparation of microbial smears. Grease or oil from the fingers on the slides is removed by washing the slides with soap and water. Wipe the slides with spirit or alcohol. After cleaning, dry the slides and place them on laboratory towels until ready for use.

Labeling of the slides

Drawing a circle on the underside of the slide using a glassware-marking pen may be helpful to clearly designate the area in which you will prepare the smear. You may also label the slide with the initials of the name of the organism on the edge of the slide. Care should be taken that the label should not be in contact with the staining reagents.

Preparation of the smear

Bacterial plate cultures: With a sterile cooled loop, place a drop of sterile water or saline solution on the slide. Sterilize and cool the loop again and pick up a very small sample of a bacterial colony and gently stir into the drop of water/saline on the slide to create an emulsion.

Heat Fixing: -

Heat fixing kills the bacteria in the smear, firmly adheres the smear to the slide, and allows the sample to more readily take up stains.

- Allow the smear to air dry.
- After the smear has air-dried, hold the slide at one end and pass the entire slide through the flame of a Bunsen burner two to three times with the smear-side up.
- Now the smear is ready to be stained.

Antibiotic sensitivity test:

The introduction of various antimicrobials for treating variety of infections showed the necessity of performing antimicrobial susceptibility testing as a routine procedure in all microbiology laboratories. In laboratories it can be made available by using antibiotic disk which will diffuse slowly into the medium where the suspected organism is grown. The basic principle of the antibiotic susceptibility testing has been used in microbiology laboratories over 80 years. Various chemical agents such as antiseptics, disinfectants, and antibiotics are employed to combat with the microbial growth. Among these, antibiotics are generally defined as the substances produced by the microorganism such as Penicillium, which has the ability to kill or inhibit the growth of other microorganisms, mainly bacteria. Antimicrobial susceptibility tests (ASTs) basically measure the ability of an antibiotic or other antimicrobial agent to inhibit the in vitro microbial growth.

There are many different procedures that microbiologists use to study the effects of various antimicrobial agents in treating an infection caused by different microorganisms. Mueller Hinton Agar is considered as best for the routine susceptibility testing since it is has batch-to-batch reproducibility, low concentration of inhibitors of sulphonamide, trimethoprim and tetracyclines and produce

satisfactory results for most of the non-fastidious pathogens. Fastidious organisms which require specific growth supplements need different media to grow for studying the susceptibility patterns. The Kirby Bauer test is a qualitative assay whereby disks of filter paper are impregnated with a single concentration of different antibiotics or any chemicals that will diffuse from the disk into the agar. The selected antibiotic disks are placed on the surface of an agar plate which has already been inoculated with test bacteria. During the incubation period, the antibiotics/chemicals diffuse outward from the disks into the agar. This will create a concentration gradient in the agar which depends on the solubility of the chemical and its molecular size. The absence of growth of the organism around the antibiotic disks indicates that, the respected organism is susceptible to that antibiotic and the presence of growth around the antibiotic disk indicates the organism is resistant to that particular antibiotic. This area of no growth around the disk is known as a zone of inhibition, which is uniformly circular with a confluent lawn of growth in the media.

Turbidity

Turbidity is the measure of the relative clarity of water. Turbidity water is caused by suspended and colloidal matter such as clay, silt, organic and inorganic matter, and microscopic organisms. Turbidity should not be confused with color, since darkly colored water can still be clear and not turbid. Turbid water may be the result of soil erosion, urban runoff algal blooms, and bottom sediment disturbances which can be caused by boat traffic and abundant bottom feeders.

Turbidity Procedure

The water testing kit container is used to perform the turbidity test.

- Remove the backing from the secchi disk icon sticker.

- Adhere sticker on the inside bottom of the large white jar (kit container) Position the sticker slightly off center.
- Fill the jar to the turbidity fill line located on the outside kit label.
- Hold the Turbidity Chart on the top edge of the jar. Looking down into the jar, compare the appearance of the secchi disk icon in the jar to the chart. Record the result as Turbidity in JTU.

Catalase test: -

This test demonstrates the presence of catalase, an enzyme that catalyses the release of oxygen from hydrogen peroxide (H_2O_2). It is used to differentiate those bacteria that produce an enzyme catalase, such as *staphylococci*, from non-catalase producing bacteria such as *streptococci*. Normally 3% H_2O_2 is used for the routine culture while 15% H_2O_2 is used for detection of catalase in anaerobes.

Principle of catalase Test

The enzyme catalase mediates the breakdown of hydrogen peroxide into oxygen and water. The presence of the enzyme in a bacterial isolate is evident when a small inoculum is introduced into hydrogen peroxide, and the rapid elaboration of oxygen bubbles occurs. The lack of catalase is evident by a lack of or weak bubble production. The culture should not be more than 24 hours old.

Bacteria thereby protect themselves from the lethal effect of Hydrogen peroxide which is accumulated as an end product of aerobic carbohydrate metabolism.

Slide Method

1. Use a loop or sterile wooden stick to transfer a small amount of colony growth in the surface of a clean, dry glass slide.
2. Place a drop of 3% H_2O_2 in the glass slide.

3. Observe for the evolution of oxygen bubbles.

Endospore staining: -

Endospore staining is a technique used inbacteriology to identify the presence of endospores in a bacterial sample, which can be useful for classifying bacteria. Within bacteria, endospores are quite protective structures used to survive extreme conditions, but this protective nature makes them difficult to stain using normal techniques. Special techniques for endospore staining include the Schaeffer–Fulton stain and the Moeller stain. A good stain to use for spore staining is malachite green. It takes a long time for the spores to stain due to their density, so time acts as the mordant when doing this differential stain; the slide with the bacterium should be soaked in malachite green for at least 30 minutes. Water acts as the decolorizer. A counterstain to differentiate the vegetative cells is 0.5% safranin. Types of endospores that could be identified are free endospores, central endospores, central and swollen endospores, and subterminal endospores. One obstacle of this stain is if staining *Mycobacterium* because due to its thick, wax coats, some cells will stain green, looking positive for spores although this particular bacterium does not produce.

***Procedure:* -**

1. Prepare smears of organisms to be tested for endospores.
2. Heat fix the smears.
3. Cover the smears with a piece of absorbent paper cut to fit the slide and place the slide on wire gauze on a ring stand.
4. Saturate the paper with malachite green and holding the Bunsen burner in the hand heat the slide until steam can be seen rising from the surface. Remove the heat and reheat the slide as needed to keep the slide steaming for about three minutes. As the paper begins to dry add a drop or two of malachite green to keep it moist, but don't

add so much at one time that the temperature is appreciably reduced. DO NOT OVERHEAT. The process is steaming and not baking.

5. Remove the paper with tweezers and rinse the slide thoroughly with tap water.
6. Drain the slide and counterstain 45 seconds with 0.5% safranin.
7. Wash, blot, and examine.
8. The vegetative cells will appear red and the spores will appear green.

Measurement of pH: -

Materials/Apparatus Required: - Water sample, pH meter, 100 ml beakers, distilled water in wash bottle, soft tissue.

Procedure: -

Rinse the beaker with sample water. Fill the beaker with 70-80 ml of sample water. Rinse the electrode of the pH meter with distilled water and blot dry with a soft tissue. Calibrate the pH meter. Put the electrode into the sample water. Record the pH value.

Results and Discussion: -

Gram's staining: -

(A)

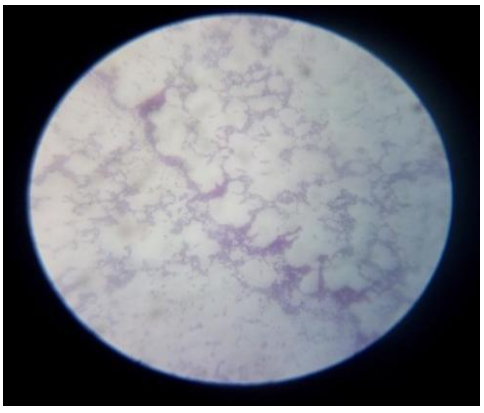

Figure1.A: -Gram's staining test

Figure 1 A suggest the presence of gram-positive bacteria in Ganga water sample. It means that these particular microbes bear the tough thick layer of peptidoglycan outside of cell membrane which give the test of Gram's staining.

Antibiotic sensitivity test: -

(B)

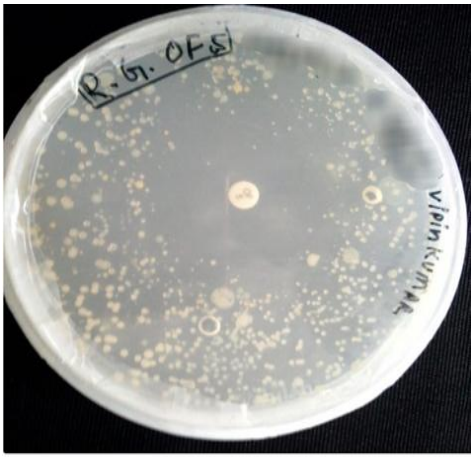

Figure1 (B): - antibiotic sensitivity test from Ramganga water

Figure 1B shows the antibiotic sensitivity assay from Ramganga water sample, which suggest that antibiotic OFS (Ofloxacin) displayed larger zone of clearance, which suggest its greater infectivity towards microbes in Ramganga water.

(C)

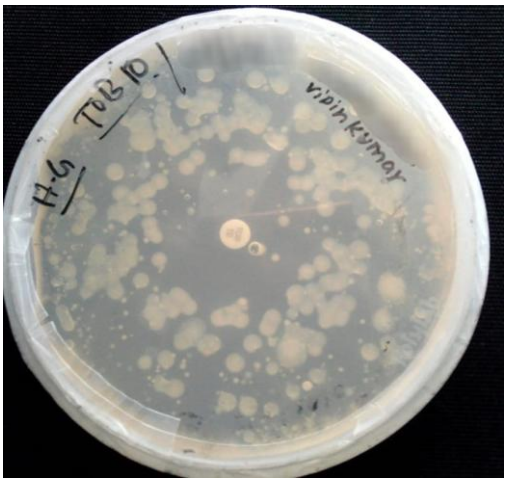

Figure2 (C): - antibiotic sensitivity test from Haridwar water

Figure 2 C shows the antibiotic sensitivity assay from Haridwar water using TOB (Tobramycin) antibiotic which has not shown much effect on the microbes, it means

these microbes show much resistance against this kind of antibiotic.

(D)

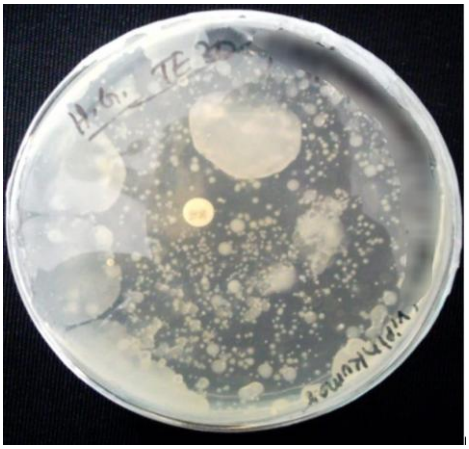

Figure 3(D): -antibiotic resistance test sensitivity from Haridwar Ganga water

Figure 3 D suggest another antibiotic sensitivity assay tested on Haridwar water using different antibiotic as compared to Figure 2C, this result suggested that almost all the microbes has shown quite large resistance towards the given TE (Tetracycline) antibiotic.

Endospore staining test: -

Observe the bacteria under 40X (oil immersion) total magnification.

Results: The vegetative cells will appear pink/red and the spores will appear green.

(E)

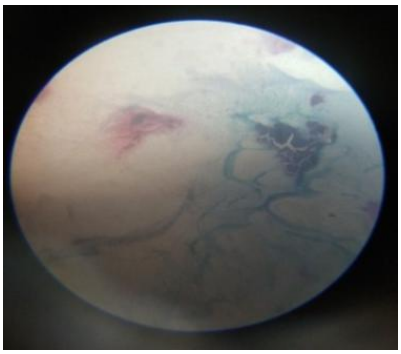

Figure 3(E): - Endospore staining test (Haridwar Ganga water)

(F)

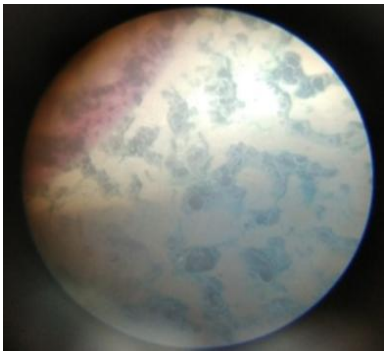

Figure 3(F): - Endospore staining test (Ramganga water)

Comparing figure 3 E and Figure 3 F which is a endospore staining test carried out on Haridwar and Ramganga water sample respectively. It shows that green cells suggest presence of endospore and red cells suggest presence of vegetative cell (endospore is tougher than vegetative cell). Lot of endospore has been reported from Haridwar water as compared to Ramganga water sample

S.NO	Ramganga water turbidity value in Nephelometer turbidity unit (NTU)	Haridwar Ganga water turbidity value in Nephelometer turbidity unit (NTU)
1.	TU=007.8 NTU	TU=001.6 NTU

Turbidity: -

Turbidity result shows that Ramganga water is much more turbid than Haridwar water, which suggest that Ramganga water is cloudier and mudy as compared to Haridwar water which is still clear that can be used for devotional purpose. But still Haridwar water is turbid which is not good for human health and therefore care must be taken to reduce its turbidity.

Catalase test: -

(A)

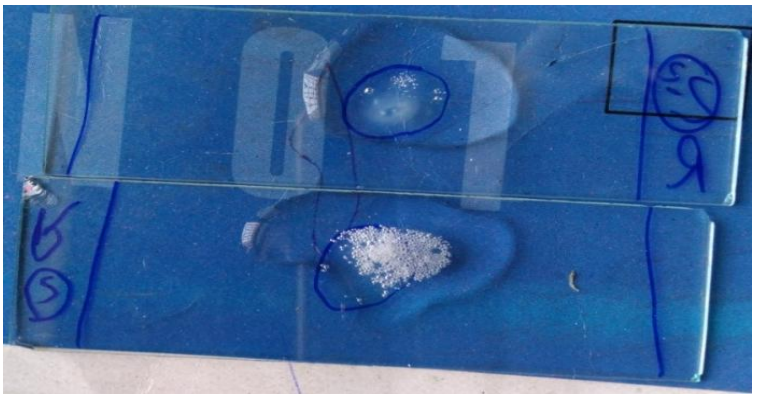

Figure 4(G):-Ramganga water (aerobic)

(B)

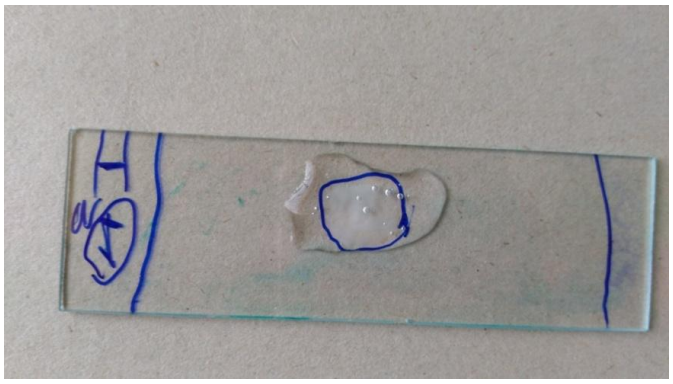

Figure 5(H): -Haridwar Ganga water (anaerobic)

Comparing the catalase assay from figure 4 G and figure 5H, shows that lot of oxygen bubble has been reported from Ramganga water as compared to Haridwar water sample which suggest that lot of anaerobic bacteria resides in Haridwar water than

Ramganga water

pH Measure: -

S.no.	Ramganga water (pH value)	Haridwar Ganga water (pH value)
1.	7.38	6.83

After comparing the acidity of both the water sample, pH value approximately fall in the range of neutral value on the pH scale.

CONCLUSION

To sum up, there is diversity of microbes residing in both Haridwar and Ramganga water. Although, Haridwar Ganga water is treated as a holy water right from early civilization, quality of its water in present scenario is getting deteriorated day by day due to presence of infectious microbes, which has been added to Ganga water from time to time. From the experimental data, it is clear that lot of anaerobic bacteria is being reported in Haridwar water as compared to Ramganga water, which may be due to the discharge of lot of organic wastes coming from household, factories, municipal corporation etc. located near Ganga River. It is surprised to report microbes bearing endospore in river Ganga from both Haridwar and Ramganga, which is a mode of survival for microbes under stress condition. Varieties of gram-positive bacteria have been reported from Haridwar water as compared to Ramganga water. Antibiotic resistance assay clearly suggests that Haridwar water bearing lot of antibiotic resistance bacteria as compared to Ramganga water, which is not good with respect to holiness of Haridwar Ganga water because in Hindu religion Ganga water is used for treatment of various diseases, which could not be beneficial for treatment purposes. Last but not the least, it is expected that in future Indian Government should take a positive mandatory step towards cleaning of River Ganga so that its holiness will be retained forever.

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THE ROLE OF LIN28/LET-7 MICRORNAs IN CANCER THERAPEUTICS

HaiderIqbal[1*], AbulVafa[2], Dr.SaimaWajid[3]

[1,2,3]Department of Biotechnology,Jamia Hamdard,NewDelhi-110062*

ABSTRACT

A highly conserved RNA-binding protein that is essential for many biological processes, such as metabolism, cancer progression, and pluripotency, is encoded by the LIN28 gene. By preventing the synthesis of let-7, a tumor-suppressive microRNA, LIN28 promotes the development of tumors and causes the overexpression of carcinogenic factors in a variety of human cancers. The connection between LIN28 and let-7 is examined in this review, with a focus on their involvement in the biology of cancer, the mechanisms by which LIN28 affects microRNA processing, and the possible therapeutic consequences of addressing this interaction. The discussion includes the structural characteristics of LIN28 and its isoforms, LIN28Aand LIN28B, as wellas how theyregulate gene expressionand cellular metabolism. The paper also looks at the link between LIN28 and the carcinogenic microRNA miR-21, highlighting the importance of these molecular interactions in of cancer development and treatment strategies.

Keywords: RNA-binding protein, gene regulation, therapeutic targets, LIN28, let-7, microRNA, cancer, oncogene, tumor suppressor, miR-21.

INTRODUCTION

The lin28 gene encodes the RNA-binding protein LIN28, which plays a vital role in pluripotency, metabolism, cellular reprogramming, and development due to its high evolutionary conservation [1]. It is recognized as an oncogene that promotes tumor growth and metastasis in several human cancers [56].

Studies report that nearly 15% of 527 primary human malignancies—including cancers of the kidney, lung, brain, ovary, breast, prostate, and other organs—show overexpression of LIN28 [3]. Initially identified in Caenorhabditis elegans as a heterochronic gene [4], LIN28 in mammals has two paralogs, LIN28A and LIN28B. Both interact with let-7, a microRNA (miRNA) family known for its tumor-suppressive functions [11]. While LIN28 regulates non-coding RNAs in cancer,let-7 acts to suppress tumor development, and their deregulated expression has been linked to multiple solid and hematologic malignancies [80]. This review primarily focuses on the LIN28/let-7 interaction in cancer biology. The correlation between high miR-21 and low let-7 levels often signifies poor prognosis in various cancers [4]. Notably, when let-7b is overexpressed, it downregulates 838 genes that contain let-7 binding sites in their 3′untranslated regions [8, 46]. LIN28 is unique among RNA-binding proteins for its ability to selectively bind miRNA, while transcriptionfactors suchas Oct4, SOX2, and Nanog regulate pluripotencyand reprogramming instemcells [3]. Moreover, LIN28 enhances the expression of SOX2 and Oct4 in cancer cells, reinforcing its role in tumorigenesis [3]. Furthermore, LIN28 facilitates communication between the let-7 family and the miR- 17/92 clusters, particularly in lung and colorectal cancers. Elevated expression of the miR-17/92 cluster accelerates lymphoma development in mice, whereas the tumor suppressor FHIT, located on chromosome 3p14.2, is frequently deleted in cancer cells [7]. Interestingly, the let-7 and miR-17/92 clusters exhibit opposing regulatoryrelationships, as miRNAs are often amplified twice in FHIT-positive clusters [7].

Human cells contain two LIN28 genes — LIN28A and LIN28B — which, despite their high homology, are rarely expressed together within the same cell type [3]. Both isoforms share conserved regions such as the Cold Shock

Domain (CSD) and the Zinc Knuckle Domain (ZKD) .Overexpression of these genes inhibits the biogenesis of let-7 microRNA (miRNA) by blocking its maturation. Inthe cytoplasm, LIN28A interacts with terminaluridylyl transferase enzymes (TUT4/TUT7) to add uridine residues to pre-let-7, preventing its processing by Dicer. In the nucleus, LIN28B performs a similar function by attaching short oligo-uridine stretches to pre-let-7, leading to its degradation [12]. The reduction of mature let-7 levels coincides with elevated LIN28A/B expression in various tumors, suggesting their oncogenic role in promoting malignant transformation [13].

Overexpression of let-7 miRNA, conversely, suppresses LIN28 activity and interferes with key signaling pathways, including RAS, C-myc, insulin/IGFR, and mTOR. Research indicates that let-7 downregulates multiple transcription factors associated with cancer proliferation. Inflammation-induced NF-κB signaling also stimulates LIN28A/B expression, while β-catenin and STAT3 activation during epithelial-to- mesenchymal transition (EMT) further increase LIN28B transcription [9]. Moreover, SOX2 interacts with the promoter regions of LIN28 to enhance its transcriptional activity, aided by histone acetyl-transferase complexes that loosen chromatin and elevate LIN28A expression.

Structurally, the CSD resides at the N-terminus of LIN28, while tandem $Cys_2HisCys$-type zinc knuckles (ZKs) occupy the C-terminus. The biochemical interaction between LIN28 and pre-let-7 is defined by conserved sequence motifs — specifically, 5′-GGGAG-3′ and 5′-GXGAY-3′ — located within the terminal loopofpre-let-7[10].These motifs provide the primarybinding sites for LIN28, enabling tight regulationof miRNA maturation. The biogenesis of let-7 closely parallels that of other miRNAs.

Initially, RNA polymerase II transcribes canonical let-7 genes to form primary miRNAs (pri-miRNAs), which are subsequently processed into precursor miRNAs (pre-miRNAs). This multi-step transcriptional control highlights the complexity of let-7 regulation and underscores its essential role in maintaining cellular homeostasis and preventing uncontrolled tumor growth.

UNRAVELING THE ONCOGENIC AND REGULATORY ROLES OF MIRNA-21AND TRUB1 IN CANCER AND RNA BIOLOGY

The let-7 miRNAundergoes a complex biogenesis process similar to other miRNAs. Drosha, anRNase III– like enzyme, functions alongside DGCR8 to process primary miRNAs (pri-miRNAs) into precursormiRNAs (pre-miRNAs) within the nucleus. These pre-miRNAs, approximately 60–70 nucleotides long, are later exported to the cytoplasm for further maturation. Mature let-7 miRNAs, in conjunction with Ago proteins, attachto the3′untranslated regions(3′-UTRs) oftarget mRNAsand formpart ofthe RNA-Induced Silencing Complex (RISC), leading to mRNA degradation or translational repression [14].

Given the regulatory importance of the LIN28/let-7 axis in cancer progression, researchers have developed therapeutic inhibitors to disrupt this interaction. Small-molecule inhibitors can modify the binding affinity between LIN28 and let-7, restoring the biogenesis of let-7 miRNA and potentially reversing oncogenic effects [14]. Several chemical compounds have been identified that inhibit let-7 processing by interfering with the LIN28–let-7 complex. These inhibitors function as chemicalprobes that provide insight into RNA- protein interactions, making them valuable in studying RNA regulatory networks [15].

Researchers have already identified at least six small molecules that specifically bind to LIN28, altering its ability to recognize and inhibit let-7 precursors. This breakthrough opens new opportunities for designing RNA-binding protein (RBP)–targeted drugs, an emerging strategy in cancer therapy [16],[17],[18],[19],[26],[33]. Since RBPs play vital roles in post-transcriptional gene regulation, small- molecule inhibitors that target LIN28 may serve both as research tools and as therapeutic leads against LIN28-related disorders [15].

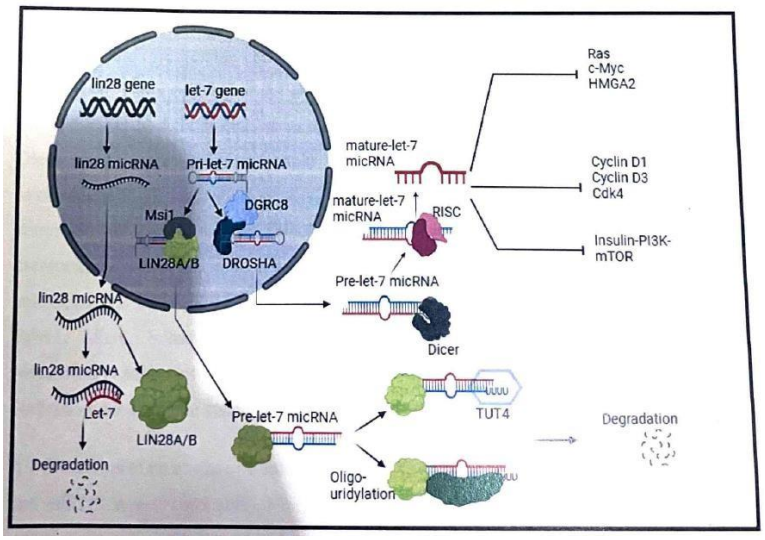

Figure1 several pathways interact withthe*LIN28/let-7*axis, influencing cellular proliferation and contributing to oncogenesis. The diagram illustrates the steps of *let-7* maturation, showing the involvement of LIN28A/B, DGCR8, Drosha, Dicer, and TUT4, as well as downstream targets including Ras, c-Myc, HMGA2, Cyclin D1/D3, Cdk4, and the insulin–PI3K–mTOR pathway.

1. STRUCTUREOFLIN28ANDLET-7

Human LIN28A and LIN28B genes encode RNA-binding proteins (RBPs) consisting of approximately 209 and 250 amino acids, respectively, with molecular weights of 23 kDa and 27 kDa [4]. These two proteins share a high degree of structural similarity and contain three conserved domains: a Zinc-Knuckle Domain (ZKD), a Cysteine–Histidine–Cysteine (CCHC) motif, and a Cold-Shock Domain (CSD). The CSD, composed of roughly 70 amino acids, forms a five-stranded, antiparallel β-barrel structure that can bind to single-stranded RNA or DNA molecules, typically within regions that lack secondary structure [15].

The ZKD region includes two tandem CCHC-type zinc knuckles, which are responsible for recognizing conserved GGAG/GGUG motifs present in mRNAs and miRNAs [16]. Research indicates that LIN28B is frequently expressed in various cell lines, with notably higher expression in tumor cells [84]. The presenceof a GGG motif in pre-let-7g is crucial for let-7 maturation; however, LIN28 binding at this site blocks its processing, preventing Dicer from cleaving pre-let-7 into mature let-7 miRNA intermediates [85]. This inhibition reflects LIN28's critical regulatory control over miRNA biogenesis.

Reactivation of LIN28A expression has been experimentally linked to enhanced tissue regeneration. It promotes anagen phase activation in hair follicles and accelerates cartilage and bone repair in mesenchymal tissues after injury. Additionally, LIN28A modulates cellular metabolism by binding to and increasing the translation of mRNAs encoding metabolic enzymes such as Pfkp, Pdha1, Idh3b, Sdha, Ndufb3, and Ndufb8 [91]. These enzymes collectively enhance oxidative metabolism, supporting the establishment of a robust embryonic bioenergetic state. Structurally, LIN28 recognizes a G-quartet (G4) motif common between its mRNA and miRNA targets.

Homologs of LIN28 have been identified across multiple species, including Drosophila, Ciona, Xenopus, mice, and humans, all sharing the conserved CSD and CCHC-type zinc finger motifs typical of the Caenorhabditis elegans lin-28 gene [4]. These domains also resemble those found in retroviral zinc finger proteins, reflecting evolutionary conservation in RNA-binding architecture.

The LIN28A and LIN28B genes in humans share about 40–45% sequence similarity with Caenorhabditis elegans LIN-28, despite differences in gene structure and chromosomal organization. Interestingly, the human LIN28 gene appears unique, with only one copy present in the genome. To confirm this, researchers sequenced two complementary DNA (cDNA) clones covering the open reading frame (ORF) and the 3′ untranslated region (UTR). Using the mRNA consensus sequence, they identified a locus on chromosome 1 corresponding to this gene. Human LIN28 comprises four exons and introns within the ORF, along with a 2.7-kb 3′ UTR [20]. Comparisons with other loci on chromosomes 2 and 20 revealed about 90% nucleotide similarity to the LIN28 ORF and its 3′ UTR, indicating evolutionary conservation with some divergence in genomic organization.

Experimental activation of LIN28A in mouse embryonic stem cells (ESCs) led to elevated activity in the threonine and S-adenosyl-methionine (Thr–SAM) pathways, both central to cellular metabolism and differentiation [79]. This indicates that LIN28 homologs are significant regulators of glucose metabolismand energy balance, functioning within a highly interconnected network of metabolic pathways. In C. elegans, the lethal-7 (let-7) gene was initially discovered as a pivotal regulator of embryonic development. The let-7 miRNA is a 22-nucleotide, highly conserved molecule present across diverse animal species [21]. The let-7familyinC.eleganscomprises nine members: mir-

793, mir-794, mir-795, mir-1821, mir-241,mir- 265, mir-248, mir-84, and mir-241, all of which regulate the timing of developmental transitions during the larval stage. The major target genes of let-7 in C. elegans include hbl-1, lin-41, and lin-42, which control differentiation timing [21].

In mammals, let-7 targets the mRNAs of several oncogenes, such as HMGA2, MYC, and RAS [71]. Its expression increases during celldifferentiation, where let-7 promotes blastocyst adhesion and supports early embryonic growth. Conversely, LIN28 suppresses let-7 during early development to allow organogenesis to proceed. After birth, let-7 expression progressively rises across various tissues, acting as a "stop" signal for uncontrolled proliferation. In the hypothalamus, let-7a and let-7b levels increase gradually after birth, influencing postnatal development and puberty onset [82].

The Zinc Knuckle Domain(ZKD) ofLIN28 and the pre-let-7 microRNAcooperatestructurallyto locatethe N-terminal segment of TUT4 (also known as NTUT4), facilitating the formation of a stable ZKD complex. This interaction promotes oligouridylation, a post-transcriptional modification that contributes to TUT4's processivity during pre-let-7 regulation. The tripartite NTUT4:pre-let-7:LIN28 complex is vital for this process. Consequently, certain inhibitors targeting the atypical tandem CCHC-type ZKD motif in LIN28 could serve as potential therapeutic agents by obstructing LIN28's binding affinity to pre-let-7 [56].

Mutations within let-7 can alter the terminal loop conformation of pre-let-7, particularly affecting how the Cold Shock Domain (CSD) of LIN28 interacts with it. This structural change facilitates LIN28 ZKD's binding tothe conserved GGAG motif, essentialforregulatoryfunction[60].In C.elegans,

mutations in let- 7 cause abnormal developmental timing, disrupting the larva-to-adult transition [21]. In both human and animal cell lines,let-7 plays a centralrole in cellproliferation and differentiation, reinforcing its function as a tumor suppressor miRNA [57]. The let-7 miRNA family is highly conserved and includes 14 members in mice and 13 members in humans, such as let-7a-1, let-7a-2, let-7a-3, let-7b, let-7c, let-7d, let-7e, let-7f-1,let-7f-2, let-7g, let-7i, mir-98, and 202 [19]. Among these, let-7a exhibits the highest degreeofconservation across species, including humans and C. elegans. In animals, let-7 expression peaks during central nervous system (CNS) development and embryogenesis. However, let-7 family members are typically absent in mouse or human embryonic stem cells, consistent with their role in differentiation rather than pluripotency [19].

The regulatory mechanism of let-7 involves “seed sequence” recognition, wherein a short, conserved region—positions 2–7 (or 2–8) from the 5′ end—binds to target mRNAs. While perfect complementarity is not always required, the seed sequence must maintain exact base pairing for effective gene silencing [11]. Additionally, LIN28 regulates mRNA splicing through its control of splicing factors such as TIA-1, TDP- 43, FUS/TLS, and hnRNP F. By binding to mRNA transcripts, LIN28 influences the production of spliced protein variants. Elevated LIN28 levels in somatic cells enhance protein synthesis from these transcripts, leading to significant shifts in alternative splicing patterns [31].

2. UNRAVELING THE ONCOGENIC AND REGULATORY ROLES OF MIRNA-21AND TRUB 1IN CANCER AND RNA BIOLOGY

The oncogenic microRNA miR-21 is among the most prominent oncomiRs, known to upregulate multiple genes implicated in cancer progression [34]. MiR-21 acts as a post-transcriptional regulator, promoting proliferation, invasion, and metastasis by repressing tumor-suppressor genes and modulating pathways associated with apoptosis and cell cycle regulation. The relationship between miR-21, LIN28, and let-7 highlights a regulatory feedback loop in which elevated miR-21 suppresses let-7 expression, indirectly amplifying oncogenic LIN28 activity. The enzyme TRUB1 (TruB Pseudouridine Synthase 1) and its associated partners Trim71 and METTL1 are involved in RNA modification and maturation processes that influence miRNA stability and function. Aberrant expression of these factors disrupts the balance between oncogenicandtumor-suppressive miRNAs, contributingto malignant transformationandtumorprogression. Throughthese interconnected pathways; miR-21 and TRUB1 exemplify how dys regulation of RNA-binding and modifying enzymes plays a crucial role in cancer and RNA biology.

The oncogenic microRNA miR-21 is closelyassociated with cellproliferationand invasion during all stages of carcinogenesis. A comprehensive miRNome analysis of 540 tumor samples revealed that miR-21 is the most frequently upregulated miRNA across various solid human carcinomas, particularly in lung cancer[34]. Elevated miR-21 expression has also been observed in ovarian, thyroid, liver, and severalother tumor types. The carcinogenic potential of miR-21 largely results from its suppression of tumor-suppressor genes such as PTEN and PDCD4, which are key inhibitors of uncontrolled cell growth. Furthermore, miR-21 interferes with the tumor-suppressive activity of let-7 miRNAs by disrupting their ability to inhibit oncogenic targets. Experimental studies revealed that loss of MIR21 results in

reduced expression of three major oncogenic let-7-5p target proteins: IGF2BP1, LIN28B, and HMGA2 [25]. These three proteins collectively establish an oncogenic triangle, which counteracts let-7's tumor-suppressive role, thereby enabling cancer cell proliferation and survival.

Because ofitsdiagnostic and prognostic significance, miR-21 isrecognized asa biomarker in multipletypes of gastrointestinal cancers, including colorectal cancer (CRC), pancreatic carcinoma (PC), gastric cancer (GC), and esophageal cancer (EC) [35]. Additionally, studies have identified 95 miR-21 target genes in nasopharyngeal carcinoma (NPC), providing new insights into the molecular mechanisms underlying NPC pathogenesis and suggesting improved diagnostic strategies [36]. TRUB1 (TruB pseudouridine synthase 1) plays a fundamental role in the process of mRNA pseudouridylation, which modifies RNA structure and stability. This modification, through the addition of pseudouridine (Ψ), enhances RNA's hydrogen bonding and stabilizes its secondary structure. Historically, pseudouridylation was studied in tRNA and rRNA, but recent evidence suggests that it also influences mRNA localization, stability, and translation by altering the binding affinity of RNA-binding proteins (RBPs) [28]. This mechanism resembles the functions of N6-methyladenosine (m6A) modifications in post-transcriptional gene regulation.

Importantly, TruB1 contributes to the maturation of let-7 miRNA, not through catalytic activity but by enhancing the interaction between primary let-7 (pri-let-7) transcripts and the microprocessor complex DGCR8 [27]. This non-catalytic promotion of let-7 maturation has been validated in CRISPR-Cas9 and RNAi-KD functionalscreens, whichdemonstratethe criticalrole ofTRUB1 in maintaining let-7expression, particularly in systems where loss of function proves detrimental. Experimental approaches have alsoutilized an SV-40

promoter within pLuc2 plasmids, designed with a let-7 sensor reporter. The let-7a target site is incorporated in the 3′ UTR of the luciferase gene, creating a system where luciferase activitydecreases in the presence of let-7 miRNA. This innovative designenables researchers to quantitativelytrack let-7 expression levels, providing an efficient assay for functional miRNA studies [29].

Researchers have demonstrated that TruB1 regulates let-7 biogenesis through its pseudouridine synthase activity. Studies in Escherichia coli have identified crucialamino acid residues in TruB—namelyD48, D90, and K64—which are essential for its enzymatic function and RNA-binding capacity [27]. Mutation of these conserved residues resulted in two variants: mt1 (lacking catalytic activity) and mt2 (exhibiting diminished RNA-binding ability). Both mutants displayed a complete loss of pseudouridylation activity when testedwith a tRNA^Phe substrate, confirming the importance of these residues for TruB1's function [27].

The complex interaction between LIN28A and miRNA precursors underscores the intricacies of miRNA maturation control. Among these, onlya subset of pre-let-7 molecules are successfully uridylated by TUT4, making them susceptible to degradation by exonuclease Dis3L2 [31]. Experimental findings indicate that blocking the ubiquitination pathway reduces TUT4 stability and impairs its ability to uridylate pre-let-7a-1, demonstrating tha tubiquitinationis essential fo rmaintaining TUT4 functionality[29].Anothercrucial player, TRIM71, influences let-7 activity in two distinct ways: (1) it regulates the degradation of pre-let-7 throughinteractions with LIN28 and TUT4, and (2) it modulates the maturationand stabilityof let-7targets. TRIM71's association with the RISC

effector protein AGO2 further highlights its complex involvement in miRNA regulation, emphasizing its role in the post-transcriptional silencing mechanism [32].

Similarly, METTL1 (methyltransferase-like 1) is vital for the m^7G (N7-methylguanosine) modification of tRNAs and miRNAs, influencing their maturation and stability. This enzyme affects cell metabolism and differentiation by modifying RNA structures in ways that alter processing efficiency. The discovery that G- quadruplex (G4) structures within miRNAs—especially those overlapping with DROSHA cleavage sites— can impede miRNAprocessing underscores the role of METTL1-mediated m^7G methylation in facilitating pre- to pre-miRNA conversion [30]. The m^7G pathway is thus a promising therapeutic target forcontrolling miRNA dynamics and developing treatments for miRNA-linked diseases. Cellular metabolism plays a pivotal role in cancer development, and cancer cells often undergo metabolic reprogramming to sustain rapid proliferation. This phenomenon, known as the Warburg effect, is characterized by enhanced aerobic glycolysis, enabling cancer cells to generate both energy and biosynthetic precursors [92]. ThePI3K-Akt-mTOR signaling pathway is central to this metabolic adaptation. The interaction between LIN28 and let-7 significantly impacts glucose metabolism and tumor progression, as LIN28 reactivation promotes metabolic reprogramming that supports tumor growth. Therefore, understanding the metabolic functions of LIN28 is crucial, as it governs key processes of self-renewal, differentiation, and energy metabolism in cancer [92].

1. SEVERALLIN28/LET-7PATHWAY-MEDIATEDCANCERTHERAPEUTIC STRATEGIES

Targeting LIN28 and its associated proteins has emerged as a promising strategy for cancer therapy, given their pivotalroles in regulating cellproliferation, differentiation, and tumor growth. Therapeutic approaches that inhibit LIN28 expression or activity have shown potential in restoring let-7 levels, thereby suppressing oncogenesis and aiding tissue repair and regeneration [39]. However, comprehensive studies are still required to validate the safety and efficacy of these interventions before clinical application. The 26S proteasomal degradation pathway plays a vital role in maintaining LIN28A protein homeostasis through ubiquitination. Recent findings demonstrate that USP28, a deubiquitinating enzyme, specifically stabilizes LIN28A by removing ubiquitin tags, thus preventing its proteasomal degradation [37]. This stabilization significantly extends the half-life of LIN28A, promoting cancer cell proliferation and migration. Experimental research further established that USP28-mediated LIN28A stabilization contributes to increased cancerous cell viability and invasiveness [37].

Using CRISPR/Cas9 genome editing, two sgRNAs targeting exon 2 of the USP28 gene were designed to study the functional consequences of USP28 deletion. Results from T7E1 assays confirmed successful gene displacement, and subsequent experiments in NCCIT cells—conducted both with and without puromycin selection—revealed that the knockout of USP28 led to a marked reduction in LIN28A protein production [37]. Further investigationshavedemonstratedthat inmultiple humancancer lineages, let-7directlybindsto the 3′-UTR of PD-L1 mRNA, repressing its translation. However, LIN28A interferes with this regulatory mechanism, thereby enhancing PD-L1 expression and allowing tumor cells to evade immune detection. In contrast, the compound C1632, a small-molecule inhibitor of LIN28, has been shown to restore let-7activity, reduce PD-L1 expression, and

inhibit tumor growth both in vitro and in vivo [38], [53]. These findings suggest that LIN28-targeted therapies may not onlycontrol tumor proliferation but also enhance the efficacy of immune therapies by reactivating immune surveillance mechanisms.

Another promising therapeutic agent is Nb-S2A4, a nanobody inhibitor that selectively suppresses TUT4 enzyme activity, thereby preventing the uridylation of pre-let-7 miRNAs. This nanobody was developed using a functional epitope selection platform, which identified the LLI fragment—the least useful yet structurally important region of TUT4—as an optimal target for inhibition. The LLI fragment of TUT4 is essential for mono- and oligo-uridylation processes that degrade pre-let-7, but it is not required for general mRNA uridylation. By targeting this specific domain, Nb-S2A4 effectively blocks the LIN28:pre-let- 7: TUT asepathway, therebyrestoring let-7biogenesisandreestablishing itstumor-suppressive function [40].

Collectively, these findings demonstrate that disrupting LIN28/let-7 pathway interactions—whether through USP28 inhibition, LIN28 blockade, or TUT4 targeting—can significantly reduce cancer cell viability and enhance therapeutic outcomes. Such interventions represent a multifaceted approachto combating cancer by reinstating the natural let-7 tumor-suppressor axis.

2. THE EFFECT OF THE LIN28/LET-7 INTER ACTION ON THE IMMUNE RESPONSE

LIN28 functions as an oncogenic regulator primarily by blocking the maturation of the tumor-suppressive microRNA let-7. This inhibition alters the expressionofnumerous downstreamgenes that are either directly or indirectly controlled by let-7, including MYC, HMGA2, and components of

the PI3K–mTOR signaling pathway [41]. Studies have shown that LIN28 stimulates tumor cell proliferation by driving the G0/G1phase progression of the cell cycle. To achieve this, let-7 expression must be suppressed, leading to the upregulation of key cell cycle–related proteins such as Cyclin D1/D2, CDC25A, CDK34, and CDK6 [47].

The proto-oncogene MYC enhances oncogenic signaling by activating the miR-17–92 cluster, a group of miRNAs linked to cancer progression. In certain malignant lymphomas, this cluster is amplified at chromosomal locus 13q31, promoting tumorigenesis through the downregulation of tumor-suppressivegenes such as TP53, E2F1, and PTEN (phosphatase and tensin homolog) [47]. Reduced expression of these tumor suppressors activates the PI3K/AKT pathway, preventing apoptosis and allowing cancer cells to survive. Furthermore, MYC can recruit histone deacetylases (HDACs) to repress the transcription ofmultiple miRNAs possessing tumor-suppressive properties [41].

Elevated levels of HMGA2 and LIN28 proteins are essential for embryonic stem cells (ESCs) to transition into epiblast-like cell types. This transformation involves complex regulatory mechanisms that include a protein complex of HMGA2 and the transcription factor Otx2. The LIN28–let-7–HMGA2 axis increases cellular HMGA2 levels, facilitating stem cell differentiation [43]. Moreover, IGFBP1 (insulin-like growth factor-binding protein 1) binds to mRNAs encoding HMGA2 and LIN28, enhancing their translation. As a downstream target of HMGA2, IGFBP1 further promotes HMGA2 expression, thereby strengthening the feedback mechanism. In cancers of the stomach, breast, prostate, and tongue, aberrant activation of the Wnt/β-catenin signaling pathwayupregulates HMGA2, correlating with

increased tumor aggressiveness and epithelial–mesenchymal transition (EMT) [43].

The LIN28/let-7 regulatory network encompasses LIN28A/B, let-7 miRNA, c-MYC, NF-κB, and IL-6, forming a complex feedback loop that tightly controls oncogenic signaling. let-7 acts as a key effector by inhibiting the translation of LIN28A/B, thus limiting the production of these proteins. However, once LIN28A/Bexpressionisrestored,it releases let-7-mediatedsuppression, generatinga positive feedbackloop that reinforces c-MYC transcriptional activity. Concurrently, NF-κB enhances LIN28B expression, while LIN28B suppresses let-7 maturation, leading to increased IL-6 production. Elevated IL-6, in turn, activates NF-κB, sustaining this loop and fostering a pro-inflammatory, tumor-promoting environment [45].

Overall, these interactions illustrate the intricate molecular network that links LIN28, let-7, and immune modulation. Through these regulatory feedback mechanisms, LIN28 not only drives oncogenesis but also influences immune evasion, chronic inflammation, and tumor microenvironment remodeling—making it a key target for cancer immunotherapy.

3. CONCLUSION

The LIN28 gene encodes an RNA-binding protein that plays a crucial role in multiple biological processes, including cellular metabolism, differentiation, and tumor growth. It is notably overexpressed in approximately 15% of primary human cancers, contributing to oncogenesis through the inhibition of let-7 microRNA biogenesis. The LIN28/let-7 regulatory axis is central to cancer biology, as elevated LIN28levels lead to reduced let-7 expression, thereby promoting the expression of oncogenes such as RAS and MYC that

drive tumorigenesis. Therapeutically, disrupting the LIN28–let-7 interaction with small-molecule inhibitorshasdemonstratedpotentialinrestoringlet-7 levels and suppressing cancer cell proliferation. These findings underscore the importance of targeting the LIN28/let-7 pathway as a promising novelstrategy in oncology, offering opportunities for the development of targeted cancer therapies that restore tumor-suppressive mechanisms and impede malignant progression.

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Nanotechnology methods to isolate DNA

Khushi kumari, Insiya tathir, Samiya Shakeel

Kunwar Satyavira College of Engineering and Management

Abstract

Deoxyribonucleic Acid (DNA) is unique to each individual and will remain unchanged for a lifetime as it follows the rules of the Mendelian lineage. However DNA analysis is widely accepted technique for individual identification.The past years have witnessed a rapid development of DNA nanotechnology in nanomaterials science with a central focus on programmable material construction on the nanoscale.An efficient method is therefore highly desirable for analytical/preparative purification of DNA conjugated nano objects and their DNA assemblies.The correct isolation of nucleic acid from various cell is an important preliminary step before many biochemical and diagnostic process such as cloning, sequencing ,replication, hybridization, and complementary DNA (cDNA) synthesis.Extracting and isolating DNA from ancient bone samples and mutilated body parts is an even more difficult and challenging task for the forensic scientists.

Key words: Deoxyribonucleic Acid, Mendelian Lineage, Complementary DNA, cloning, sequencing, replication, hybridization.

Introduction

Personal Identification using DNATraditionally, human remains have been identified individuually or forensically based on fingerprint,otontology or bone evidence ,facial features, scars, marks or other special features. In many cases, available methods have not proven effective because the amount of destruction or destruction of debris or debris is very high.

Disasters terrorist attacks,traffic accidents, wars, fires, explosions, plane crashes highly, decomposed body or severly burnt and other harrowing events individual identification or forensic identification become very tough for investigation man from skeletal remains.During decomposition human bodies undergo a series of multiple changes and the rate of

decomposition may vary due to factors like monsoon, microorganisms growth, and insect and wild scavengers.During this time the soft tissues are often destroyed and may have no dental history. Some of conditions Deoxyribo Nucleic Acid (DNA) can prove to be the most very very essential and useful source of identification or a good another source of identification purposes.Deoxyribo Nucleic Acid (DNA) is responsible for genetic inheritance, so the discovery of dual helix structure of DNA in 193 had a major impact And in all fields of science ,including forensic investigations led to significant changes. This understanding is the foundation for the development of new techniques and methods it is based on the individual DNA sequence.

DNA isolation is one of the most crucial steps in molecular biology, genetics, forensic analysis, and clinical diagnostics. The purity and integrity of extracted DNA directly influence the success of downstream applications such as PCR amplification, cloning, sequencing, and gene expression studies.Traditional DNA extraction techniques—including phenol-chloroform extraction, ethanol precipitation, and silica spin column methods—have been widely used for decades. However, these techniques are often time-consuming, labor-intensive, and involve toxic chemicals, leading to partial DNA loss or degradation.

Nanotechnology introduces various nanomaterials that can selectively interact with DNA molecules through electrostatic forces, hydrogen bonding, and π–π interactions. The negatively charged phosphate backbone of DNA allows it to bind with positively charged or functionalized nanoparticles.

Future Prospects

- The future of DNA isolation lies in the integration of nanotechnology, microfluidics, and artificial intelligence.
- Microfluidic lab-on-chip systems can automate DNA extraction, reducing human error.
- AI-driven design can optimize nanoparticle structure for maximum DNA affinity.
- Biodegradable nanomaterials will ensure environmental safety.

- CRISPR-nanocomplexes could enable selective capture of target DNA sequences for diagnostic and therapeutic use.

- As these technologies mature, nanotechnology-based DNA isolation is expected to become the global standard for molecular biology and precision medicine.

Body

These nanomaterials not only simplify the extraction process but also reduce the need for centrifugation and organic reagents. The most commonly used nanomaterials for DNA isolation include magnetic nanoparticles, silica nanoparticles, and gold nanoparticles, each having specific mechanisms and advantages.In recent years, nanotechnology has provided innovative solutions to these challenges. Nanomaterials possess unique physical and chemical properties due to their nanoscale size (1–100 nm) and large surface-to-volume ratio, allowing efficient binding and purification of biomolecules like DNA. Nanotechnology-based methods enable rapid, efficient, and solvent-free DNA isolation suitable for both laboratory and field applications.

THREE NANOTECHNOLOGY METHODS USED TO ISOLATE DNA THAT CAN BE ADDED TO REVIEW PAPER

1-: MAGNETIC NANOPARTICALE (based DNA isolation)

Magnetic nano particles (MNPs) have revolutionized DNA Isolation by enabling fast, efficient, and selective extraction of DNA from diverse biological source. these nanoparticles, typically composed of iron oxide and coated with functional materials like silica, chitosan, or polyethyleneimine, possess unique magnetic properties that facilitate easy manipulation through external magnetic fields. The DNA extraction process begins with the lysis of cells in the sample to release DNA functionalised magnetic nanoparticles are then introduced to the lysate, where they exhibit high affinity for nucleic acids due to electrostatic induction, hydrogen bonding,or ligand receptor compatibility once bound, the DNA-nanoparticles

complexes are separated from the rest of the sample using a magnet, and several wash steps ensure the removal of proteins, salts, and others contaminants. The final DNA is eluted from the nanoparticles using a suitable buffer for downstream analyses like PCR, sequencing, or genotyping. This method is advantageous because it eliminates the need for centrifugations and hazardous organic reagents, such as phenolchloroform,promoting environmental safety and operational convenience. The approach achieves high purity and yield, even from challenging samples like blood, saliva or plant tissues, and is suitable for high throughput automation and clinical diagnostics. Optimisation is studies show that silica coated FE3O4 MNPs can achieve absorption efficiencies near 88% and elution efficiencies close to 98% Providing excellent recovery and minimal sample loss.

2:- GOLD NANOPARTICLES-ASSISTED DNA EXTRACTION

Gold nanoparticles (AuNPs) Have emerged as versatile nanoplatforms in DNA isolation due to their superior surface chemistry, biocompatibility, and tunable size and shape. AuNps tends to be functionalised with ligands, such as thiol or amine- containing molecules, which facilitates strong binding to DNA And ensure selectively over other biomolecules. In a typical protocol, a cell lysate is mixed with functionalized AuNps, enabling DNA to attach firmly to the nanoparticles surface through covalent or non covalent interactions. Separation can then be achieved through centrifugation or filtration, concentrating the AuNPs-DNA complexes. Subsequent washing steps remove extraneous substances, and elution buffers disrupt the DNA-nanoprtical linkage, releasing pure DNA for analytical purpose. This strategy not only streamlines DNA extraction but also allows integration with biosensors for real time detection and quantification of DNA. AuNPs based isolation is particularly advantageous for low abundance samples and forensic applications, where sensitivity and specificity are critical. Furthermore, the method is adaptable for multiplexed extraction processes and can be scaled for automation. The key strengths are its high selectively, compatibility with micro fluidic devices, and potential for downstream integration with electrochemical or optical biosensors, which further expand its applications in genomics, diagnostics, and environmental monitoring.

3:- Silica COATED NANOPARTICLE DNA PURIFICATION

Silica coated nanoparticles have become a standard in nanotechnology based DNA isolation, leveraging their high affinity for nucleic acid under chaotropic salt conditions. These nanoparticles, commonly constructed from a magnetic core enveloped in a salica shell, support efficient binding and purification processes. During extraction, the sample undergoes cell lysis, and the released DNA is exposed to the silica coated nanoparticles in the presence of a chaotropic agent (like guanidinium thiocyante), which promotes rapid and selective adsorption of DNA onto the nanoparticles surface. The resulting DNA bead complexes are isolated through magnetic separation, washed with appropriate buffers to remove contaminants, and finally eluted with a low Salt buffer. The silica surface promotes nucleic acid binding through dehydration and hydrogen bond formations, outperforming conventional organic extraction methods by avoiding exposure to toxic reagents. This technique has demonstrated outstanding yields and purity across a variety of samples types, including blood, saliva, plants,and microbial cultures. It's advantages include minimal hands on time, scalability for automation,and suitability for clinical and forensic uses where reproducibility and DNA integrity are paramount. Silica coated nanoparticles purification represents an eco friendly, rapid,and robust solution for modern molecular biology workflows, particularly in clinical diagnostics and environmental genomics.

Conclusion

Nanotechnology has revolutionized DNA isolation by providing efficient, rapid, and eco-friendly alternatives to traditional extraction techniques. The use of magnetic, silica, and gold nanoparticles demonstrates how nanoscale materials can drastically improve DNA binding, purification, and recovery processes.Magnetic nanoparticles simplify separation through magnet-based handling, silica nanoparticles ensure high binding efficiency with minimal contamination, and gold nanoparticles offer multifunctionality with sensing capabilities.

Although cost, large-scale synthesis, and standardization remain challenges, the integration of these nanomaterials with microfluidic, biosensing, and lab-on-chip technologies represents

the future of molecular diagnostics. In summary, nanotechnology-based DNA isolation methods are transforming biological research and clinical diagnostics into faster, cleaner, and more accurate processes.

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Biopesticides: A Sustainable Approach to Pest Management – A Review

Lavish Choudhary[1], Prashansha[1], Prerna Ahlawat[1]

[1] *Kunwar Satyavira College of Engineering & Management, Bijnor*

Abstract

In order to manage agricultural pests, Biopesticides are biologically based substances made from natural resources like microbes, plants, and certain minerals. Biopesticides are target-specific, biodegradable, and environmentally benign in contrast to traditional chemical pesticides. The kinds, modes of action, manufacturing processes, uses, benefits, drawbacks, and potential future developments of Biopesticides are highlighted in this study. Biopesticides are sustainable, safe, eco-friendly approach in pest control. Their function in integrated pest control and sustainable agriculture is highlighted in the conversation.

Keywords: Biopesticides, Microbes, Sustainable.

Introduction

Biopesticides are natural substances made from plants, microbes, or biologically active substances that are used to control agricultural pests and illnesses with the least amount of negative environmental effect. They are essential to programs for Integrated Pest Management (IPM) and sustainable agriculture [1]

By infecting pests or producing toxins, bacteria, fungus, viruses, and nematodes are examples of microbial biopesticides. For example, fungi such as *Beauveria bassiana* infect and kill insects, whereas *Bacillus thuringiensis* (Bt) generates proteins that are fatal to some insect larvae [2]

Naturally occurring compounds including plant extracts, pheromones, and growth regulators that disrupt the physiology or behavior of pests make up biochemical biopesticides. Common examples that lessen dependency on artificial chemicals include pyrethrins, insect sex pheromones, and neem-based treatments (3,4).

Genetically modified plants that generate pesticidal compounds, like Bt cotton and Bt maize, are known as plant-incorporated protectants (PIPs). These plants offer natural resistance against certain pests [5].

Beneficial creatures that naturally control pest populations include parasitoids (*Trichogramma* spp.), predatory insects, and entomopathogenic nematodes [6].

All things considered, biopesticides offer sustainable, target-specific, and eco-friendly pest management options. Their creation promotes biodiversity and soil health while meeting the increasing demand for residue-free crops and less chemical pesticide use [2]

Classification of Biopesticides

Depending on their composition, biopesticides are divided into various groups. Like Microbial Biopesticides, Biochemical, Plant incorporated protectents etc.

Microbial Biopesticides

To manage agricultural pests in an ecologically friendly manner, microbial Biopesticides are biological control agents made from live microorganisms such bacteria, fungus, viruses, and nematodes. Beauveria bassiana, a fungal pathogen of several insect pests, and *Bacillus thuringiensis* (Bt), which generates insecticidal toxins, are two of the most often cited examples (Bioprotection Portal, 2023; Kumar et al., 2024). Beneficial insects and non-target animals are mainly unaffected by these

compounds since they are extremely specialized to their target organisms (Bioprotection Portal, 2023).

Microbial biopesticides' biodegradability, low levels of harmful residues, and suitability for integrated pest management (IPM) initiatives are some of its main benefits [1]. Additionally, they support sustainable agriculture by decreasing the need for synthetic pesticides, which lowers chemical pollution and the emergence of insect population resistance.

Despite these advantages, a number of obstacles prevent their widespread use. According to Kumar et al. (2024), problems such formulation stability, short shelf life, restricted host range, and uneven field performance continue to exist. Furthermore, to guarantee safe and efficient usage, regulatory obstacles and other non-target consequences need to be addressed [7].

All things considered, microbial biopesticides are a viable and environmentally benign substitute for conventional pest control in contemporary agriculture.

a. Biochemical Biopesticides

Naturally occurring substances known as biochemical biopesticides work by preventing pests from reproducing, growing, or behaving in certain ways (U.S. Environmental Protection Agency, n.d.). These include compounds that interfere with the life cycles of pests rather than killing them directly, such as insect sex pheromones, plant extracts, essential oils, and insect growth regulators [1] To lower the rate of pest reproduction, pheromone-based traps, for instance, are frequently employed to stop mating in insect populations [4].

Because they break down quickly and leave less residues than synthetic chemical pesticides, these biopesticides are often safer for both people and the environment [3].

They are also excellent parts of integrated pest management (IPM) programs because of their great specificity to target pests [5]. However, environmental conditions including temperature, humidity, and sunshine can affect their effectiveness, frequently requiring

Regulan application [1]

Though further study is needed to improve their stability and efficacy, biochemical biopesticides are generally important for sustainable agriculture since they provide ecologically benign substitutes for traditional pesticides [4].

b. Plant incorporated protectants (PIPs)

Plants may withstand some pests by producing pesticidal compounds called Plant-Incorporated Protectants (PIPs) from inserted genetic material (U.S. Environmental Protection Agency [EPA], n.d.). These compounds are produced by the use of recombinant DNA technology to introduce genes encoding pest-resistance characteristics, including those from *Bacillus thuringiensis* (Bt), into the plant genome [1] The resultant plants can reduce the requirement for external pesticide treatments by producing insecticidal proteins that target certain pests [5]

PIPs provide a number of benefits, such as less environmental effect, increased agricultural output, and decreased use of chemical pesticides [2] Furthermore, these protective compounds offer systemic and long-term pest control since they are produced inside plant tissues [1] The development of pest resistance, gene flow to wild relatives, and regulatory obstacles pertaining to ecological dangers and biosafety, however, continue to be issues [5].

While requiring ongoing monitoring and careful application, Plant-Incorporated Protectants are an important development in agricultural biotechnology that helps ensure food security and sustainable pest control (EPA, n.d.).

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c. Other Biopesticides

According to the U.S. Environmental Protection Agency [EPA], n.d., "other biopesticides" are naturally occurring pest control agents that do not fit into the primary classifications of microbial, biochemical, or plant-incorporated protectants. These include beneficial creatures that biologically regulate pest populations, such as parasitoids, entomopathogenic nematodes, and predatory insects [3]. For example, because of their parasitic and insecticidal properties, nematodes belonging to the genera *Steinernema* and *Heterorhabditis* are employed to combat soil-dwelling insect pests [6].

Additional instances include botanically derived formulations and microbial consortia, which mix many biological agents to provide synergistic effects on pest reduction [4]. By lowering reliance on chemical pesticides and protecting beneficial creatures, these biopesticides support ecological balance and integrated pest management (IPM) [1] However, restrictions including their shorter shelf life, increased production costs, and susceptibility to environmental conditions may prevent them from being widely used [2]

All things considered, these alternative biopesticides improve the sustainability of contemporary agriculture by providing a variety of ecologically friendly pest control techniques that reduce hazards to human health and the environment (EPA, n.d.).

2. Mechanism of action

Microbial biopesticides work by infecting, producing toxins, or competing with other organisms. For instance, the cytolytic (Cyt) and crystal (Cry) proteins produced by *Bacillus thuringiensis* (Bt) attach to certain receptors in insect midguts, resulting in cell lysis and pore formation [1] According to Sharma et al. (2024), fungi such as

Beauveria bassiana infect pests by piercing their cuticle and multiplying inside their body, which finally results in death.

Instead of killing pests directly, biochemical biopesticides work by interfering with physiological or behavioral processes. Growth regulators prevent molting or reproduction, pheromones disrupt insect mating behavior, and essential oils serve as repellents or feeding deterrents [3,4]

Transgenes, like the Bt toxin genes that are introduced into agricultural plants, encode pesticidal proteins that are expressed by plant-incorporated protectants (PIPs). The expressed proteins cause cell rupture and death when they attach to the gut receptors of pests that consume these plants [5].

Other biopesticides work by biologically preying on or parasitizing organisms, such as beneficial insects and entomopathogenic nematodes. Nematodes cause septicemia and mortality in their pest hosts by releasing symbiotic bacteria (Xenorhabdus or Photorhabdus) [5].

All things considered, these biopesticides provide species-specific, eco-friendly pest management methods that lessen reliance on artificial chemicals and improve agricultural sustainability [1]

3. Production and formulation

Certain bacteria, such as *Bacillus thuringiensis* or *Beauveria bassiana*, cultivated on nutrient-rich medium, are used in fermentation procedures to create microbial biopesticides [1] To improve shelf life and field stability, the resulting spores, cells, or metabolites are separated, dried, and combined with carriers such talc or starch [2] Depending on the target pest and application technique, formulations might be wettable powders, dusts, granules, or liquid suspensions [4].

Microbial biopesticides are produced by fermentation processes using certain bacteria, such as Beauveria bassiana or Bacillus thuringiensis, that are grown on nutrient-rich media [1] The resultant spores, cells, or metabolites are separated, dried, and mixed with carriers like talc or starch to increase shelf life and field stability [2] Formulations may be wettable powders, dusts, granules, or liquid solutions, depending on the target pest and application method [4].

Through the use of gene gun techniques or *Agrobacterium tumefaciens*-mediated transformation, genes encoding pesticidal proteins—like Bt Cry toxins—are introduced into plant genomes to create plant-incorporated protectants (PIPs)[5]. The resultant transgenic plants innately express pesticidal proteins without additionalformulation.

In lab bioreactors or artificial medium, other biopesticides, such as beneficial insects and entomopathogenic nematodes, are mass-cultured [5]. To preserve viability during storage and transportation, they are made with inert materials, gels, or protective carriers [1]

4. Application in agriculture

In agriculture, microbial biopesticides are widely used to control nematodes, plant diseases, and insect pests. While fungal agents like *Beauveria bassiana* and *Metarhizium anisopliae* are used to combat soil and foliar insects, species like *Bacillus thuringiensis* (Bt) are employed to manage lepidopteran pests in crops including cotton, maize, and vegetables [1,2]. To provide biological control and enhance plant health, bacterial and fungal compositions are usually sprayed on soil or crop leaves.

Biochemical biopesticides can be applied as growth regulators, attractants, or repellents. Aphids, whiteflies, and moths are among the pests that are frequently managed with plant-derived essential oils, neem-based formulations, and pheromone traps. [3,4] To reduce reliance on chemical pesticides and their negative effects on the environment, they are included into Integrated Pest Management (IPM) systems.

Without the need for frequent pesticide applications, plant-incorporated protectants (PIPs), like Bt cotton and Bt maize, offer ongoing defense against target pests like bollworms and corn borers by expressing insecticidal proteins inside plant tissues [5].

In order to promote ecological pest regulation, other biopesticides are released in agricultural fields, such as beneficial insects (e.g., Trichogramma spp.) and entomopathogenic nematodes (*Steinernema* spp., *Heterorhabditis* spp.), which parasitize
or prey on insect pests. [6,8]

Overall, by lowering chemical inputs, safeguarding beneficial species, and increasing crop output, biopesticides are essential to sustainable agriculture [2]

5. Advantages of Biopesticides

By producing toxins and causing biological infection, microbial biopesticides are used extensively in agriculture to manage nematodes, plant diseases, and insect pests. While fungal species like *Beauveria bassiana* and *Metarhizium anisopliae* are used to combat aphids, beetles, and soil-borne pests, Bacillus thuringiensis (Bt) formulations are sprayed to cotton, maize, and vegetable crops to control lepidopteran larvae [8,2]. These microorganisms are used as seed treatments, foliar sprays, or soil drenches to improve agricultural production and pest management.

Insect pheromones, pyrethrins, and neem oil are examples of biochemical biopesticides that are used to deter or interfere with insect mating [3]. They are essential parts of Integrated Pest Management (IPM) strategies and are particularly effective against moths,
aphids, and whiteflies [4].

By expressing insecticidal proteins inside the plant tissues, plant-incorporated protectants (PIPs), such as Bt cotton and Bt maize, offer season-long pest resistance [5]. These crops increase yield stability and lessen the requirement for external pesticide sprays.

To naturally control pest populations by predation or parasitism, other biopesticides are introduced in agricultural areas, such as beneficial parasitoids (*Trichogramma* spp.) and entomopathogenic nematodes (*Steinernema* spp., *Heterorhabditis* spp.) [6,8].

All things considered, biopesticides offer targeted, sustainable, and ecologically safe pest management options that promote organic and eco-friendly farming [2]

6. Limitations of Biopesticides

Narrow host specificity, slower action than chemical pesticides, and vulnerability to environmental variables including humidity, temperature, and UV light are some of the drawbacks of microbial biopesticides. These elements may require more frequent treatments and decrease field efficacy [8,2]. Additionally, to preserve microbial viability, manufacturing and storage must be handled carefully.

Plant extracts and pheromones are examples of biochemical biopesticides that frequently break down quickly in the presence of sunshine or precipitation, reducing

their residual effectiveness. Environmental factors and pest density might affect how effective they are, and the extraction and purifying procedures involved in large-scale manufacturing can be costly [3,4]

Over time, plant-incorporated protectants (PIPs), like Bt crops, may cause insect resistance to emerge, necessitating resistance management techniques. Adoption may also be constrained by regulatory limitations and public concerns about genetically modified crops [5].

Other biopesticides, such as beneficial insects and entomopathogenic nematodes, have drawbacks such a limited shelf life, high susceptibility to environmental factors, and possible challenges with bulk manufacturing and field release. Ecological compatibility with the agricultural system may also be a determining factor in their field establishment [6,8].

In summary, while biopesticides offer sustainable and eco-friendly pest control, their field efficiency can be constrained by environmental, biological, and regulatory factors, requiring careful management for optimal results [2]

7. Conclusion

An eco-friendly and sustainable substitute for traditional chemical pesticides are biopesticides. Microbial, biochemical, plant-incorporated protectants (PIPs), and other biological agents are some of their varied categories that offer targeted pest management while reducing threats to the environment and human health [8,2]. While biochemical biopesticides, such as neem extracts and insect pheromones, alter pest behavior and lessen crop damage, microbial biopesticides, such as Bacillus thuringiensis and entomopathogenic fungi, provide efficient control against certain pests [3,4]. PIPs reduce the need for external pesticide treatments by allowing crops to develop their own protective agents, such as Bt cotton and Bt maize [5]. Furthermore,

entomopathogenic nematodes and predatory insects are examples of beneficial species that enhance natural pest suppression [5].

Biopesticides have drawbacks despite their benefits, including as sensitivity to the environment, delayed action, increased expense, and regulatory difficulties. Many of these limitations should be addressed by developments in formulation technologies, RNAi-based treatments, and AI-assisted strain identification, which will increase uptake and effectiveness [2] All things considered, biopesticides are crucial to integrated pest management methods because they enhance biodiversity, lower chemical residues, and promote sustainable crop protection—all of which are important aspects of modern agriculture.

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Production of Biodiesel based on Waste Cooking Oil (WCO)

Ms. Shivangi Bhardwaj

Cloud Computing in IT training institute, DUCAT - Gurugram, Haryana

Mrs. Sangeeta Sharma

Department of Biotechnology, Kunwar Satyavira College of Engineering & Management, Bijnor

Ms. Shaily Rajput

Department of Master of Business Administration, Swami Vivekanand Subharti University Meerut

Abstract

The most effective solution for managing waste cooking oil is its utilization for industrial purposes, particularly for biodiesel production. In this project, waste cooking oil was collected from a chip and cracker factory in Johor and processed into bio diesel using a pilot-scale continuous production plant .The resulting biodiesel was analyzed in a laboratory for quality and performance. It was then blended with diesel fuel to produce B5 and B10 biodiesel grades. The production process adhered to the American bio diesel standard ASTMD6751.This initiative allowed the company to repurpose its waste cooking oil, reducing disposal costs and contributing to environmental conservation. Additionally, converting waste into a valuable energy source not only promotes sustainability but also encourages staff awareness and engagement in environmental protection and alternative energy solutions, thus supporting the long-term viability of the operation.

Keyword:-waste cooking oil, pilot plant and Biodiesel.

Introduction

Biodiesel is an alternative diesel fuel derived from vegetable oils or animal fats.

The main components of vegetable oil sand animal fats are triglycerides or also known as ester of fatty acid attached to glycerol. One of the main driving force for biodiesel widespread is the greenhouse gas emission (CO2 being the major one). The term waste cooking oil (WCO) refers to vegetable oil has been in food production and which is no longer viable for its intended use. WCO arises from many different sources, including domestic, commercial and industrial. WCO is a potentially problematic waste stream which requires proper management. The disposal of WCO can be problematic when disposed incorrectly.

MATERIALSANDMETHODS

This process started from free fatty acid to determine acid value and step of process. Second process is drying to remove water in FAME and last process is properties physical todetermine content SOF FAME.

A. Feed stock Waste Cooking Oil

It is recognized that the production of waste cooking oil will be the function of the frying temperature and length of use as well as the material used for frying. In this experiment, WCO were collected from a local Factory in Bijnor, which produced chip cracker as well as continental foods.

B. Free Fatty Acids

The acid value of the waste cooking oil was determined in order to estimate the free fatty acid content and give an idea of how much acid catalyst and methanol would be needed to push the acid esterification chemical towards methyl ester production.

C. Processing in Biodiesel

Biodiesel has two main stage process is esterification and tranesterification process. Separation was used to separate two layer between catalyze and oil. Washing process

to produce the neutral biodiesel and remove catalyzes glycerol, soap and methanol.

Esterification

Sulphuric acid (95 – 98%) is used by 1% in esterification process depend from waste cooking. In these experiments the sulphuric acid was first mixed with methanol before adding to the waste cooking oil. After adding them ethanol/ sulphuric acid and waste cooking oil the agitator speed were used to mix the solvents until they became murky. This was then heated to about 60°C for 2hours.At higher temperature or a faster stirring rate may push the acidic esterification equation to convert free fatty acid to methyl ester.

1) Separation

Separation needed 3 hour togetther to pmethanol and bottom oil layers of the biodiesel.Two layers could clearly be seen in the successful basic esterification biodiesel.

2) Trans esterification

The amount of catalyst had an impact in the conversion of esters during the Trans esterification process. Thereactionwascarriedoutusing1%ofcatalystconcentration.Before tran esterification process Sodium hydroxide was first mixed with methanol together in one container before adding to the waste cooking oil.

3) Separation2

Trans esterification process and any methanol evaporation the resultant biodiesels were left to lie for at least 8hours.Separations were used to separate the top(methyl ester) and bottom(glycerol) layersofthe biodiesel samples. Two layers could clearly be seen in the successful basic Trans esterification biodiesel samples.

Result

- At the top will be presence methanol and at the bottom show triglycerin.
- involvesthereactionofalcohol(suchasmethanol)withfattyacidsascatalyzed
- to reduce the levels of FFA in the low-cost feedstock to an acceptable range
- Reaction between acids and alcohols in the presence of strong acid catalyst
- Produce ester and water
- freefattyacidofmaterialmustbelessthan2%

Summary

The biodiesel was prepared from waste cooking oil sample collected from a local factory in Bijnor.The biodiesel was characterized for its physical and fuel properties using ASTM standard methods for biodiesel fuel quality assurance. The composition offinal biodiesel was determined by physical properties such as density, viscosity, flash point, water content and acid value. From the tests, the flash point was found to be 97^0C, water and sediment was 0.02%, total acid number was 0.29 mg KOH/g, viscosity at 40^0C was 4.2 mm2/sec and density 0.82g/cm. Out

of 5 properties tested, all of them met the ASTM criteria for fuel standard. Production of biodiesel from waste cooking oils for diesel substitute is particularly important because the increasing cost of oil extracted from petroleum source and also it is good for environment.Waste cooking oil can be an important source for bio diesel production inMalaysia it is ready available and environment.

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"Molecular Biology of Withanolide Biosynthesis in *Withaniasomnifera*: Pathways, Genes, and Regulatory Mechanisms"

Nishi Chandra and Pushpendra Kumar

Division of Microbial and Environmental Biotechnology

College of Biotechnology, Sardar Vallabh Bhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India, 250110

Abstract

Withaniasomnifera (Ashwagandha) is a medicinal plant with pharmacological significance that is widely utilized in Ayurveda and modern medicine for its adaptogenic, anti-inflammatory, and neuroprotective qualities. The plant's medicinal potential is due to bioactive steroidal lactones, withanolides, and glycosylated derivatives (withanosides). Discoveries in genomes, transcriptomics, and metabolomics have revealed the substances' biosynthesis processes, regulatory mechanisms, and molecular targets.

Keyword:- Ayurveda, adaptogenic, metabolomics, transcriptomics and molecular targets.

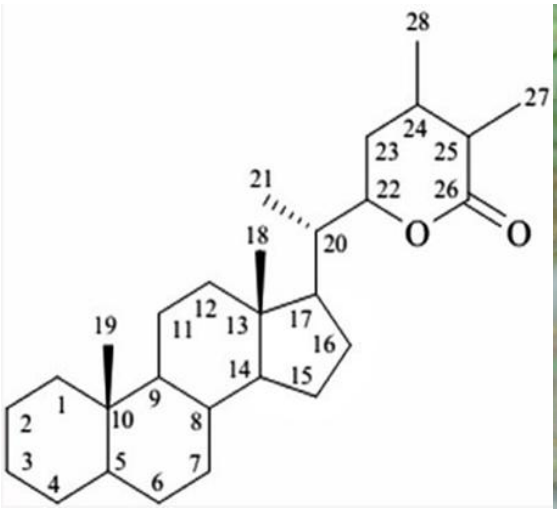
Introduction

Ashwagandha, or Indian Ginseng (*Withaniasomnifera*; Solanaceae family), is a highly regarded and significant medicinal plant utilized in the Indian system of medicine. For thousands of years, this medicinal herb has been used as Rasayana (tonic) and/or churna (powder). It has been used for its many health benefits, including memory enhancement, nervous system enhancement, sexual and reproductive balance, increased body resilience to stress, immunomodulatory effect, antioxidant activity, and more. Withanolides (triterpenic lactones such as withaferin A, withanolide A, withanolide B, withastromonolide, withanone), alkaloids (tropine, causcohygrine), phenolics, saponins, etc., are the main secondary metabolites that have been found to be present and have particular biological activities within the body (Rohan Sarkar *et al*., 2023). Twelve-deoxywithastromonolide (WD), withanolide A

(WLA), and withaferin A (WA) make up the majority of the many withanolides that are present. To date, the structures of twelve alkaloids, thirty-five withanolides, and a number of sitoindosides have been determined.

Bioactive Compounds in Ashwagandha

Withanolides are a particular type of steroidal lactone. More than 130 withanolides are found in 15 genera within the Solanaceae family. The greatest number of withanolides with a broad range of functional groups is produced by the plant *W. somnifera*. To create withanolides, a 28-carbon natural steroidal lactone is built upon an erostane skeleton. Lactone rings with six or five members are formed in this skeleton by the oxidation of C-22 and C-26. The basic structure, which consists of the 22 hydroxy ergostane-26-oic acid 26, 22-lactones, is referred to as the withanolide skeleton. Amyloid beta aggregation, which is believed to exacerbate Alzheimer's disease, can be stopped by both withanolide A and withanolide B. The additional hydroxyl group on the C-20 atom of withanolide A sets it apart from withanolide B. At a concentration of 0.13–0.31% dry weight, withaferin A, also known as 4β,27-dihydroxy-1-oxo-5β, 6β-epoxywitha-2 24-dienolide, was first discovered in a South Asian variety of W. somnifera leaves. The quantitative study of Indian chemotypes of W. somnifera using TLC densitometry and HPLC analysis revealed that withaferin A was present in leaves at a concentration of 1.6%, while it was present in roots and stems at very low amounts. Another withanolide that is present in considerable quantities in ashwagandha extracts is withanone, which is believed to be the cause of many of the plant's therapeutic benefits, including its anti-oxidant, anti-amyloid, and anti-inflammatory qualities (19 and 3 mg/g dry weight in leaves and roots, respectively) (Valentina Lerose *et al*., 2024).



The basic structure of withanolide	*Withaniasomnifera*(Ashwagandha)

The withanolide group comes in a variety of forms, such as witanone, withaferin A, withanolides A-Y, and withanopherin A. Important pharmacological characteristics of withanolides include their anti-inflammatory, neuroprotective, adaptogenic, and anti-cancer effects. Other withanolide glycosides, such as sitoindoside IX and X, and steroidal saponins, such as sitoindoside VII and VIII, have a glucose moiety at position C-27.

Biosynthetic pathway of withanolides

The vital upstream metabolic process of isoprenogenesis is incorporated into withanolide biosynthesis, which uses isoprenoid as a precursor. Isoprenogenesis is known to be triggered by two different and independent pathways: mevalonic acid (MVA) and methylerythritol phosphate (MEP; often referred to as the deoxyxylulose route, or DOXP). These activities, which occur in the cytosol and plastid, respectively, culminate in the synthesis of 24-methylene cholesterol, a 30-carbon molecule (triterpenoids) (Niha Dhar *et al.*, 2015).

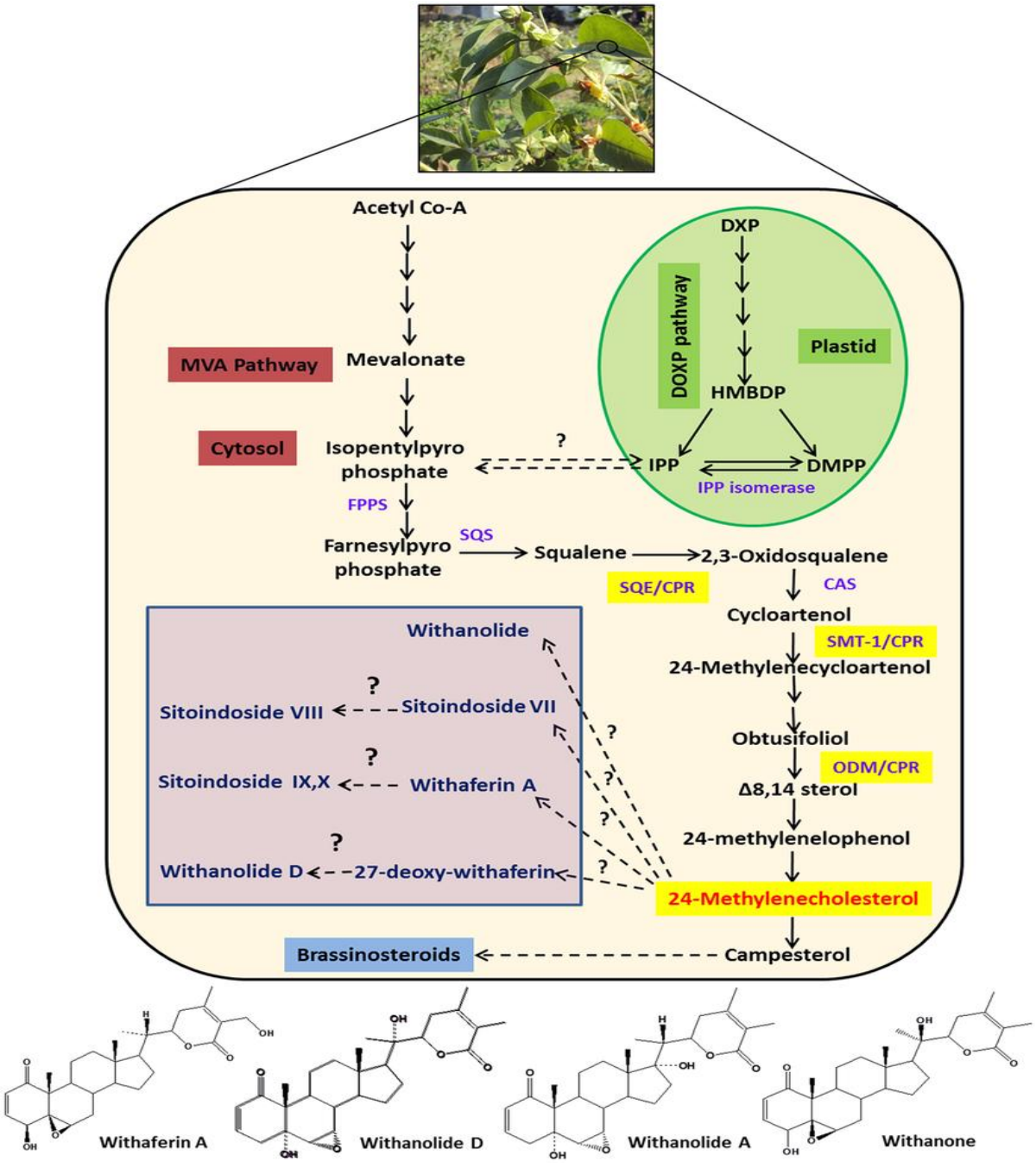
Mevalonate Pathway (Cytosolic)

The MVA route involves seven terpenoid biosynthesis-related enzymes, including DMAPP and IPP. HMG-CoA reductase (HMGR) is necessary for mevalonate biosynthesis in order to create HMG-CoA following acetyl-CoA condensation. Mevalonate is converted to IPP via phosphorylation and decarboxylation processes involving the enzymes mevalonate kinase,

phosphomevalonate kinase, and mevalonate diphosphate decarboxylase. After that, isopentenyl diphosphate isomerase and IPP combine to form DMAPP.

Methylerythritol Phosphate Pathway (Plastidial)

The MEP route is a metabolic process that converts precursors like pyruvate and glyceraldehyde 3-phosphate into 1-deoxy-Dxylulose 5-phosphate (DXP). After that, it is transformed into MEP, which is later altered by a variety of enzymes into 1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate. Finally, the branching of HMBPP to IPP and DMAPP is catalyzed by a single enzyme known as (E)-4-hydroxy-3-methyl but-2-enyl diphosphate reductase (HDR). Plasmid-localized isopentenyl diphosphate isomerase aids in substrate optimization bycatalyzing IPP isomerization. The main building block for triterpenoids, farnesyl pyrophosphate (FPP), is produced by farnesyl diphosphate synthase (FPPS).



Putative biosynthetic pathway of withanolides (Satiander Rana *et al.*, 2013)

FPP serves as a substrate for several branching pathways that produce compounds that are

essential for plant growth and development as well as having potential medical uses. Squalene is a metabolic step for the biosynthesis of other triterpenoids, whereas cycloartenol is produced by the enzyme cycloartenol synthase. The vital step 24-methylenecholestrol, which is biosynthesised from cycloartenol, is part of the metabolic route that leads to the biosynthesis of withanolides.

Genes involved in biosynthesis of withanolides (Pandey V., *et al*., 2017)

Genes involved in biosynthesis of withanolides are Δ14-sterol reductase (EC 1.3.1.70), 1-deoxy-D-xylulose-5-phosphate reducto-isomerase/reductase (DXR; EC 1.1.1.267), 1-deoxy-D-xylulose-5-phosphate synthase (DXS; EC 2.2.1.7), 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase (MEcPP synthase, IspF, EC 4.6.1.12), 2-C-methyl-D-erythritol 4-phosphate cytidylyl transferase (EC 2.7.7.60), 3-hydroxy-3-methylglutaryl-coenzymeA reductase (HMGR; EC 1.1.1.34), 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase (EC 2.7.1.148), 4-hydroxy-3-methylbut-2-enyldiphosphate reductase (EC 1.17.1.2), 4-hydroxy-3-methylbut-2-enyldiphosphate synthase (HMB-PPS, IspG, EC 1.17.7.1), acetyl-CoA acetyltransferase (ACT, EC 2.3.1.9), C-5-sterol desaturase (C5SD, EC 1.14.19.20), cycloartenol C-24 methyltransferase (EC 2.1.1.142), cycloartenol synthase (CAS; EC 5.4.99.8), cycloeucalenol cycloisomerase (EC 5.5.1.9), cytochrome-P450s reductase (CPR, EC 1.6.2.4), farnesyl diphosphate synthase (FPPS, EC 2.5.1.10), geranyl diphosphate synthase (GPPS, EC 2.5.1.1), geranyl-geranyl diphosphate synthase (GGPPS, EC 2.5.1.29), glycosyltransferases (GT, EC 2.4.-), hydroxymethyl glutaryl-CoA synthase (HMGS, EC 2.3.3.10), isopentenyl diphosphate isomerase (IPPI, EC 5.3.3.2), methyltransferase (MT, EC 2.1.1.), mevalonate diphosphosphate decarboxylase (EC 4.1.1.33), mevalonate kinase (MVAK, EC 2.7.1.36), obtusifoliol 14-demethylase (EC 1.14.13.70), phosphomevalonate kinase (EC 2.7.4.2), squalene synthase (SQS, EC 2.5.1.21), squalene monooxygenase/epoxidase (SQE, 1.14.14.17), sterol Δ7 reductase (DWF, EC 1.3.1.21), etc.

Biosynthetic Pathways of The Enzymes in *WithaniaSomnifera* with their Molecular Functions and Roles in Withanolide Biosynthesis

Enzyme	Pathway	Function	Expression	Correlation with

			Pattern	**witanolides**
3-Hydroxy-3-methylglutaryl Coenzyme A Reductase (HMGR)	MVA	Rate-limiting enzyme converting HMG-CoA to mevalonate (precursor of IPP/DMAPP).	Highest expression in young leaves and 30-day-old roots. - Declines in roots after 45 days.	Positive correlation with withanolide A accumulation in roots. Enhanced expression under stress/salicylate elicitation
1-deoxy-D-xylulose-5-phosphate Synthase (DXS)	MEP	Catalyzes pyruvate + glyceraldehyde-3-phosphate → DXP (first MEP step).	Elevated in young leaves compared to roots. Highest in NMITLI-118/135 chemotypes	Linked to higher withanolide D and WS-3 accumulation in chemotypes
1-deoxy-D-xylulose-5-phosphate Reductoisomerase (DXR)	MEP	Converts DXP to MEP (committed step for MEP-derived isoprenoids).	Uniform expression across chemotypes. Higher in leaves than roots	Contributes ~30% of isoprenoid precursors for withanolides
Farnesyl Diphosphate Synthase (FPPS)	MVA\MEP	Synthesizes FPP (C15) from IPP/DMAPP, a precursor for triterpenoids.	Higher expression in roots than leaves. - Peaks at 45–60 days in roots	Strong correlation with withanolide A (roots) and withaferin A (leaves). - Enhances

				substrate flux to withanolides
Squalene Synthase (SQS)	Phytosterol / Withanolide biosynthesis	Catalyzes condensation of two farnesyl diphosphate (FPP) molecules to form squalene, an early step in phytosterol and withanolide biosynthesis.	Higher expression in leaves, correlating with elevated withanolide production; inducible by methyl jasmonate (MJ), salicylic acid (SA), and 2,4-D.	Key regulatory enzyme directing carbon flux toward phytosterols, brassinosteroids, withanolides, and triterpenoids; target for pathway intensification.
Squalene Epoxidase (SQE)	Sterol / Withanolide biosynthesis	Catalyzes stereospecific epoxidation of squalene to 2,3-oxidosqualene, a rate-limiting step in sterol and withanolide biosynthesis.	Maximum expression in withanolide-rich leaves; expression enhanced by elicitors; promoter contains multiple cis-regulatory elements.	Rate-limiting enzyme controlling flux into cyclic triterpenoids; important target for genetic manipulation to enhance withanolide production.
Cytochrome P450 Monooxygenases (P450s)	Catalyze regio- and stereo-specific	Heme-thiolate enzymes catalyzing regio- and stereo-	Tissue-specific expression: *WsCYP98A* highest in stalk; *WsCYP76A*	Catalyze key biosynthetic steps modifying withanolide

(e.g., WsCYP71B35, WsCYP98A, WsCYP76A, CYP88C7, CYP88C8, CYP88C10)	oxidative modification (hydroxylation, epoxidation, ketone formation) on withanolide skeleton.	specific oxidation reactions (hydroxylations, epoxidations, etc.) essential for functionalizing withanolide core structures and other secondary metabolites.	in roots; others mostly in leaves. Expression varies among chemotypes.	skeletons; e.g., *WsCYP71B35* involved in withanolide A biosynthesis; *WsCYP93Id* hydroxylates withaferin A.
Cytochrome P450 Reductases (CPRs)	Electron donors transferring electrons from NADPH to P450 monooxygenases enabling catalytic activity.	Diflavin enzymes transferring electrons from NADPH to P450 monooxygenases, enabling their catalytic activity. Two paralogs identified with distinct expression profiles.	*WsCPR1* constitutively expressed; *WsCPR2* inducible, especially in leaves with high withanolide content.	*WsCPR2* supports increased reductive demand of P450s during withanolide biosynthesis and stress; essential for efficient P450 function.
Sterol Glucosyltransf		Catalyze glycosylation of	Ubiquitously expressed in	Final step in glycosylation of

erases (SGTs) (e.g., SGTL1, SGTL2, SGTL3)		sterols by transferring glucose from UDP-glucose to hydroxyl groups, forming glycosterols/glycowithanolides.	roots, leaves, and other tissues; expression induced by environmental stresses.	withanolides forming glycowithanolides; involved in stress responses; silencing alters withanolide profiles and reduces biotic tolerance.
UDP-Glycosyltransferases (UGTs) WsGT4 and WsGT6	Catalyze glycosylation of withanolides and sterols, transferring glucose from UDP-glucose to acceptor molecules.	Catalyze glycosylation of withanolides forming withanosides; transfer glucose or galactose to specific withanolide substrates.	Highest expression in leaves; induced by methyl jasmonate (MJ); silencing reduces withanosides, overexpression enhances them.	Directly involved in biosynthesis of withanosides (glycosylated withanolides); modulate defense responses against pathogens.
DIMINUTO/DWARF1 (DWF1)	Catalyzes isomerization and reduction of 24-methylene	Multifunctional enzyme catalyzing isomerization and reduction of 24-methylene	Highest transcript levels in leaves; expression increases upon elicitor treatments (MJ, SA, 2,4-D).	Regulates metabolic flux toward withanolide and brassinolidebiosynthesis; mutations

	cholesterol to campesterol and sitosterol.	cholesterol to campesterol and sitosterol; key in phytosterol and withanolide precursor biosynthesis.		cause dwarf phenotype due to impaired sterol biosynthesis; expression correlates with withanolide accumulation.

Molecular Regulation and Gene Expression

Higher levels of withanolide content in young leaves and reproductive phases are linked to tissue-specific and developmentally controlled gene expression of key enzymes, such as HMGR, DXS, DXR, SQS, and SQE (Pandey V. *et al*., 2017). Salicylic acid (SA), methyl jasmonate (MJ), and 2,4-dichlorophenoxyacetic acid (2,4-D) are examples of environmental factors and elicitors that affect expression by triggering biosynthetic genes and encouraging metabolite accumulation (Ahmad Z. *et al*., 2024).*Bacillus thuringinesis*, *Streptomyces* sp., *Pseudomonas* sp., *Aspergillus terreus*, and *Penicillium* sp. are endophytic microbes associated with *W. somnifera* that can influence metabolite profiles and gene expression, suggesting a role in natural biotic elicitation.Recent genome sequencing has discovered clusters of biosynthetic genes that encode enzymes involved in withanolide production, such as cytochrome P450s and 2-oxoglutarate-dependent dioxygenases, which can be used to reconstitute pathways in heterologous systems (Reynolds, E. *et al*., 2024).

Conclusion

The formation of important natural compounds called withanolides is thought to be responsible for the medicinal properties of ashwagandha (*Withaniasomnifera*). The molecular biology of withanolide formation in *Withaniasomnifera* includes intricate metabolic pathways that include MVA and MEP precursor routes, significant enzymatic steps catalyzed by HMGR, SQS, SQE, DWF1, cytochrome P450s, and glycosyltransferases. Gene expression,

which can be utilized to boost metabolite production, is strictly regulated by developmental, tissue-specific, and environmental factors.

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Algae-Grass Biopaper with Okra Gel as a Natural Binder: A Sustainable Solution for Eco-friendly Packaging

Sadaf Khan[1]

[1]*Department of Biotechnology, KunwarSatyavira College of Engineering & Management, Bijnor, Uttar Pradesh 246701, India*

ABSTRACT

This research explores a sustainable approach to biodegradable packaging by fabricating bio-paper using locally sourced algae and grass fibers, bound with naturally extracted okra mucilage. Systematic experimentation assessed the influence of varying algae: grass ratios and okra gel concentrations on the mechanical and biodegradation properties of the resulting sheets. The optimal formulation featured a 70:30 algae to grass ratio with 10% okra gel binder, dried via oven at 50°C, and demonstrated tensile strength of 3.5 MPa, moderate water absorption, and over 70% biodegradation within 30 days in soil. The developed bio-paper underscores potential as an eco-friendly, low-cost alternative for sustainable packaging solutions, aligning with circular economy principles.

Keywords: Biodegradable packaging, algae fibers, grass fibers, okra gel, bio-paper, sustainable materials, compostability.

INTRODUCTION

Plastic pollution and deforestation driven by conventional packaging have created an urgent need for sustainable, biodegradable alternatives. While paper remains a widely used packaging material, its production and end-of-life impacts—particularly when combined with synthetic adhesives and coatings—pose environmental challenges. Recent advances in bio-based materials point toward renewable, non-wood fibers and plant-based binders as viable

routes to reduce ecological footprints while preserving functional performance. This study explores the fabrication of a biodegradable wrapping material from locally available algae and grass fibers, bound with okra mucilage gel. Algae and grasses offer renewable, non-competitive feedstocks with rapid growth and minimal land use, while okra gel provides a natural, non-toxic binding mechanism that can replace conventional synthetic adhesives. By employing simple, low-energy processing and traditional sheet-forming techniques, the work aims to produce a flexible, durable bio-paper capable of wrapping packaging needs with complete or near-complete biodegradability under typical composting or soil conditions.The central objective is to (i) evaluate how algae-to-grass fiber ratios and okra gel binder content influence mechanical strength, flexibility, and barrier properties, (ii) identify an optimal formulation that balances performance with rapid biodegradability, and (iii) compare the resulting bio-paper against conventional handmade paper as a relevant benchmark. The anticipated outcome is a sustainable, cost-effective packaging material suitable for small-scale production and community-scale adoption, contributing to circular economy goals and reduced environmental impact.

MATERIALS AND METHODS

1. Equipments used:

Autoclave, Laminar Air Flow Cabinet, Weighing Balance, Binocular Electronic Microscope, Incubator, Hot Air Oven, Compound Microscope, Centrifuge, pH Meter, Refrigerator, Water Bath.

Glass Wares:

Conical Flasks, Beakers, Measuring Cylinders, Petri Plates, Test Tubes, Micropipettes, Glass Rods.

Others:

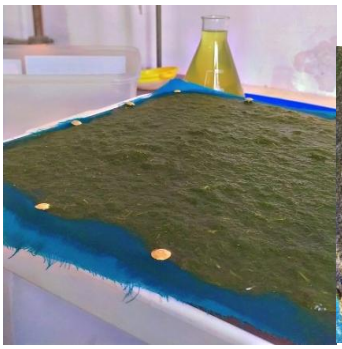
Inoculating Loop, Bunsen Burner, Forceps, Whatman Filter Paper, Mask, Gloves, Micropipette Tips, Spatula, Cotton Cloth (for sheet couching).

2.Raw Material Collection and PreparationAlgae: Sargassumwightii was collected from coastal waters, thoroughly washed, and boiled in distilled water for 20 minutes to soften cell walls (Kandasamy&Pugazhenthi, 2019). The boiled algae were blended with water to produce a viscous slurry.Grass: Cynodondactylon was collected from college lawns, chopped, boiled in 1% NaOH solution for 40 minutes to remove lignin, washed, and ground into fibers (Ghaffar et al., 2019).Okra Gel: Fresh okra pods were sliced, boiled in distilled water, strained through muslin cloth, and stored at 4°C for use within 48 hours (Ghosh & Das, 2020).

Okra Gel Extraction Pulp Formation

3.Experimental Design: A factorial design varying algae:grass ratios (100:0; 70:30; 50:50; 30:70; 0:100), okra gel concentrations (5%; 10%; 15%; 20%), and drying methods (sun-drying, oven at 50°C, air-drying) was adopted to identify optimal formulations.

4.Sheet Formation and Processing: Pulp blends were mixed with specified okra gel percentages, cast onto molds, pressed, and dried under selected methods. The sheets were kept at ambient conditions during curing.



Sheet Formation

Sun Drying

5.Characterization Tests: Mechanical strength (tensile testing, ASTM D882), water absorption, biodegradability (soil burial for 30 days), and microstructural analyses (SEM) were conducted with triplicate samples for statistical validity.

RESULTS

Mechanical Properties: The 70:30 algae:grass sheet with 10% okra gel exhibited an average tensile strength of 3.5 MPa, elongation of 4.2%, and tear resistance of 35 N, comparable to conventional handmade paper .

TABLE I. MECHANICAL PROPERTIES OF BIO-PAPER

Formulation	Tensile Strength(MPa)	Elongation (%)	Water Absorption (%)	Biodegradation (%) (30days)
70:30+10%	3.5	4.2	12	72
50:50+10%	3.2	3.8	14	65
30:70+10%	2.7	3.5	17	80

Water and Biodegradability: Sheets demonstrated moderate water uptake (~12%), which is

sufficient for wrapping purposes. Soil burial tests indicated over 70% mass loss within 30 days, confirming rapid biodegradability comparable to natural organic materials (Figure 1).

Microstructural Observations: SEM images revealed good fiber–binder adhesion with minimal porosity, indicating strong interfiber bonding facilitated by okra mucilage.

DISCUSSION: Optimized formulations balanced mechanical strength with biodegradability, with 70:30 algae:grass ratio and 10% okra gel providing the best properties. The natural binder enhanced sheet cohesiveness, while the low-energy drying processes aligned with sustainability goals. The material's rapid biodegradation, low water absorption, and comparable strength suggest its viability as an eco-friendly packaging substitute, especially for food wrapping applications (Ghosh & Das, 2020; Lopez et al., 2020).

CONCLUSION: This study successfully developed a biodegradable bio-paper from algae and grass fibers bound with okra mucilage, suitable for eco-friendly packaging. The optimal formulation demonstrated good mechanical properties, environmental compatibility, and rapid compostability, offering a promising sustainable alternative to plastics and conventional paper.

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Decomposition of crude oil by using agricultural waste and Inorganic fertilizer

Mrs. Sangeeta Sharma

[1]*Department of Biotechnology Kunwar Satyavira College of Engineering & Management Bijnor (246701) UP. India*

Mrs.Aanchal Bhardwaj

Department of Bio-Technology, K. S. Vira College of Engineering & Management, Bijnor (U.P.), India

ABSTRACT

Crude oil pollution is one of the most serious environmental issues today, and its cleanup process is considered particularly challenging. Within one to four weeks, oil-degrading bacteria can break down approximately 60% of crude oil, leaving about 40% remaining. In this study, soil was contaminated with 150 ml of crude oil and treated with 150 g of poultry manure and 75 g of NPK fertilizer. An herbal plant, Indian Tulsi, was planted in each pot. The results showed that this treatment reduced total soil hydrocarbons by approximately 87%. The combination of organic and inorganic fertilizers proved effective in restoring crude oil-contaminated soils. Crude oil contamination poses a significant threat to soil health and the environment, with remediation often being complex and costly. This study investigates the effectiveness of agricultural waste (poultry manure) combined with inorganic fertilizer (NPK) in enhancing the biodegradation of crude oil in contaminated soil. Soil samples were artificially polluted with 150 ml of crude oil and amended with 150 g of poultry manure and 75 g of NPK fertilizer. Indian Tulsi (Ocimum tenuiflorum) was planted in each treatment pot to assess phytoremediation support. Over a treatment period, results showed a substantial reduction in total petroleum hydrocarbons (TPH), with up to 87% degradation observed. The findings demonstrate that the synergistic use of organic and inorganic amendments significantly accelerates crude oil decomposition, offering a cost-effective and environmentally friendly approach to soil restoration.

Keyword: - Bacteria, Poultry Manure, Bioremediation, NPK fertilizer, organic fertilizer,

soil restoration and Ocimum tenuiflorum.

INTRODUCTION

The hassle of environment pollutants has assumed an exceptional percentage in lots of components of the world. Pollution caused by petroleum and its side stream product is the most prevalent problem for nearby area of refinery. Since commercial exploration of petroleum has big contribution in growth of any country, a petroleum industry has continuously grown in. However this industry has led to the pollution of land and water in different ways. Petroleum is a complex mixture of aliphatic, naphthenic and, aromatic hydrocarbons, and smaller proportions of heteroatom compounds, such as sulphur, nitrogen, and oxygen. Crude oil also having traces amount of organ metallic complexes containing nickel and vanadium. These organ metallic compounds are problematic during crude oil refining because it had adverse effect on various catalyst that used for refining of crude. Invariably, oil spillage damages the soil, water and both plants and animals. Its pollution renders soils unproductiveness for years after spillage, also reducing the growth performance of plants by deteriorating fertility of soil.

Materials & Methods

The microorganism used in this study was isolated from crude oil–polluted and crude oil–treated soil collected from the Bijnor region. For the preparation of cultural media and nutrient supplements, various materials were utilized, including agar, sterile distilled water, sodium chloride, glucose peptone agar, peptone, ethanol, beef extract, lactose peptone agar, starch, and sodium hypochlorite. The organic nutrient supplement, dried poultry manure, was obtained from Krishna Fertilizer, Bijnor. The dried poultry manure contained 33 mg of nitrogen and 1.6 mg of phosphorus per gram. To simulate conditions of a major oil spill, twenty-five liters of Bonny light crude oil was uniformly poured on each treatment plot, including the control. The plots were left undisturbed for three days, after which the top 3 cm of soil, containing some oil, was manually removed to simulate emergency clean-up conditions. The soil samples were then tilled to ensure proper mixing of nutrients using a long

stainless-steel spoon for each sample. After the zero weeks, samples were taken for analysis. The setups were mixed at weekly intervals under ambient environmental conditions, and soil samples were collected every four weeks for triplicate analyses.

The isolation process involved serial dilution and the pour plate technique. One gram of soil sample was added to 9 ml of sterile distilled water in a sterile test tube and shaken thoroughly using a vortex machine. This dilution process was repeated nine times to obtain a serially diluted sample. A 100 µl portion of the sample suspension was transferred to sterilized Petri plates containing 25 ml of melted nutrient agar medium and incubated at 37°C for 24–72 hours. After incubation, visible bacterial colonies appeared on the plates. A selected mucoid colony was further inoculated using an inoculating loop and streaked onto nutrient agar media plates by the streak plate method to obtain a pure bacterial culture.

Morphological and biochemical characterization included Gram staining, which involves staining with crystal violet, decolorization, and counterstaining with safranin. Due to differences in the thickness of the peptidoglycan layer, Gram-positive bacteria retain the crystal violet stain during decolorization, while Gram-negative bacteria lose it and are counterstained by safranin. In the isolation and identification process, the total heterotrophic bacterial count in the control was initially low during the adaptation period but showed rapid multiplication, peaking at day 84, followed by a decline at day 112.

Physico-chemical analyses were carried out after the soil samples were dried, ground to a fine powder, and sieved through a 2 mm mesh. Particle size distribution was determined using the hydrometer method, and total nitrogen was measured by the micro-Kjeldahl method. The soil suspension was shaken and centrifuged at 5000 rpm for 10 minutes. The supernatant was collected in clean, oil-free flasks, and 50 ml of hexane was added to the solid residue, followed by separation after shaking. The water, acetone, and hexane layers were mixed in a separating funnel and extracted using hexane. The extract was collected in pre-weighed beakers and left to evaporate under a fume hood overnight. The residue was then weighed along with the solvent blank beaker, and the difference was calculated to determine the total hydrocarbon content.

Result

The Fungal isolates from an oil polluted soil sample. The Fungus genera: Trichoderma and Cladosporium (Figure 2.1 & 2.2) are major hydrocarbon utilizers; these were similar to those obtained in the present study.

The potential of various treatment options for the bioredmediation of crude oil polluted soils seems to hold the most immediate solution especially for use in areas that would be adversely affected by physical or other removal methods. In this study, the reduction of oil in the treated samples is evident, polluted samples supplemented with fertilizer and manure proved to be the best option during the 112 days study period (Graph 3.1).

Microscopic view of some microorganism

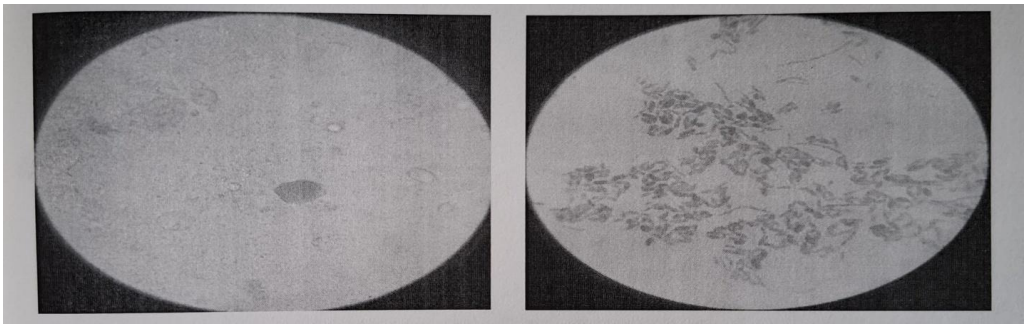
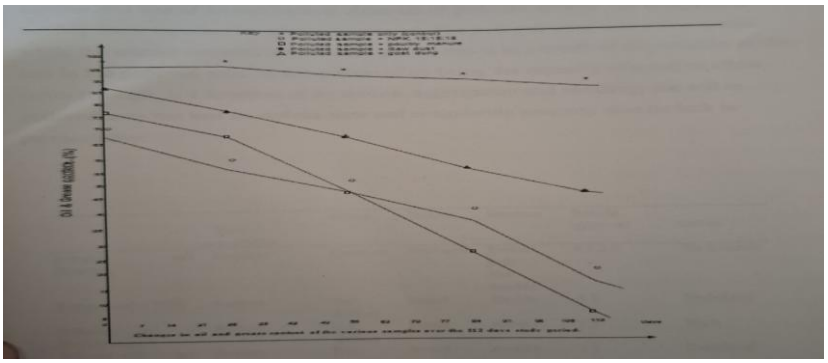


Fig No. 2.1 Trichoderma **Fig No. 2.2 Cladosporium**



Graph No.3.1 change in oil and grease content of various samples over the 112 days study period

Summary

The data presented in this research were limited to laboratory experiments. The option in the protocols have been included after due consideration of technical limitation in the laboratory bearing in mind that results from field demonstration might vary. We recognize other sustainable environmental benefits from the treatment option such as replacement of mineral fertilizer with the use of products from composting. The use of different compost feedstock was investigated in this research. The use of manure yielded the greatest degree of bioremediation in this study; it is also a cheap method to use.

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CAR-T: A Tool for of Cell-Based Immunotherapies

Shubham Saini,

Department of Biotechnology, Kunwar Satyavira College of engineering and management

Bijnor 246701

Abstract:

Cell-based Immunotherapies allow the body's immune system to specifically destroy cancerous cells, they have revolutionised the treatment of cancer. Chimeric antigen receptor T-cell (CAR-T) treatment is one of them that have shown remarkable success, especially in haematologic malignancies. In order to target tumours, CAR T-cell therapy expands patient T-cells ex vivo and re-infuses them with antibody-derived receptors connected to signalling domains. However, issues including cytokine release syndrome, limited efficacy in solid tumours, and high production costs have spurred innovation in the direction of alternative and next-generation cell platforms. This study outlines the development of CAR-T technology, talks about important obstacles and engineering approaches, and looks at new modalities that could be used in personalised cancer immunotherapy in the future, such as CAR-NK, CAR-M, and other modified immune cells.

Keywords: CAR-T, Immunotherapy, Cancer, T-Cell, Personalised Immunotherapy

Introduction

Chimeric antigen receptor T-cell (CAR-T) therapy is at the vanguard of cancer immunotherapy, which has quickly developed into one of the most revolutionary areas of contemporary medicine. In contrast to traditional therapies like radiation or

chemotherapy, CAR-T therapy uses the patient's own immune system by reprogramming T cells to identify and eliminate cancerous cells with exceptional accuracy.[1] Several FDA-approved products and long-lasting remissions in otherwise refractory cancers have resulted from CAR-T therapy's unparalleled effectiveness in treating haematological malignancies by fusing concepts of immunology, genetic engineering, and synthetic biology.[1,2]

However, despite its groundbreaking effects, CART treatment still has a lot of obstacles to overcome. In solid tumours, which account for most human malignancies, its efficacy is hampered by tumour heterogeneity, antigen escape, and the immunosuppressive milieu. Furthermore, concerns about accessibility, manufacturing complexity, and toxicity underscore the necessity of ongoing innovation. Researchers are creating next-generation platforms, such as dual-targeted and armoured CAR-T cells, CAR natural killer (NK) cells, and CAR macrophages, in addition to innovative delivery methods and combination approaches, to get over these obstacles.[3]

The principles of CAR-T treatment, its drawbacks, and the approaches being taken to broaden its application beyond blood malignancies are all covered in this review. It highlights how CAR-based immunotherapies are transforming oncology and opening the door to safer, scalable, and universal treatments by looking at recent developments and potential future paths.[4]

Fundamentals of CAR-T cell therapy

In CAR-T cell therapy patient's own immune system is used to specifically target cancerous cells, Chimeric antigen receptor T-cell (CAR-T) therapy is a revolutionary approach to cancer immunotherapy. T cells from the patient's peripheral blood are first isolated as part of the treatment. These cells are then genetically modified to produce synthetic receptors called CARs, which are able to recognise tumor-associated antigens without the need for the major histocompatibility complex

(MHC). [1,7]

Four domains usually make up a CAR construct:
(i) an extracellular single chain variable fragment (scFv) produced by antibodies to recognise antigens, (ii) a hinge/spacer region,

intracellular signalling motifs, and (iii) a transmembrane domain.

In order to improve persistence and cytotoxicity, later generations of CARs added one (second generation) or two (third generation) co-stimulatory domains, such as CD28 or 4 1BB, to the original generation's CD3ζ signalling chain [2,3]. Cytokine receptor fragments or cytokine inducing modules are further integrated into fourth and fifth generation CARs, allowing for enhanced tumour microenvironment modulation, proliferation, and survival [2].

Numerous FDA-approved products are the result of CAR T therapy's outstanding clinical success in treating haematological malignancies, especially B cell acute lymphoblastic leukaemia, non-Hodgkin's lymphoma, and multiple myeloma [3,7]. However, antigen heterogeneity, the absence of tumor-specific antigens, physical barriers like dense extracellular matrix, and the immunosuppressive tumour microenvironment make its application to solid tumours difficult [2,3]. Dual targeted or tandem CARs, armoured CARs that secrete cytokines, and combination therapies with immune checkpoint inhibitors are some methods to get past these obstacles [2,4]. Other immune effectors, like CAR NK cells and CAR macrophages (CAR M), are being investigated in addition to T cells. While CAR M therapy uses the innate phagocytic and antigen-presenting capabilities of macrophages to reshape the tumour microenvironment [5], CAR NK cells provide "off the shelf" potential with a lower risk of graft versus host disease [3].

In conclusion, CAR T therapy is a prime example of how immunology and genetic engineering can work together. Unlocking its full potential against solid tumours requires constant advancements in receptor design, delivery, and combination strategies, even though its fundamentals in blood cancers are well established.

Limitations of CAR-T therapy

1. Heterogeneity of Tumours and Antigen Escape: Antigen escape, in which tumour cells lose or downregulate the targeted antigen, is one of the most urgent problems, as it can result in relapse. Because solid tumours exhibit high antigen heterogeneity, this is especially problematic. When tumour cells adapt or express low levels of the target, traditional CAR T cells—which are made to recognise a single antigen—may not be effective [2,3]. Clinical validation is still ongoing, but dual targeted or tandem CARs are being developed to address this issue [4].

2. Ineffectiveness in Solid Tumours: Solid tumours have biological and physical barriers, in contrast to haematologic cancers. CAR T infiltration is impeded by cancer-associated fibroblasts, aberrant vasculature, and dense extracellular matrix. Furthermore, CAR T persistence and cytotoxicity are further hampered by the immunosuppressive tumour microenvironment (TME), which is abundant in regulatory T cells, myeloid-derived suppressor cells, and inhibitory cytokines like TGF β [2,3].

3. Toxicity On Target, Off Tumour The majority of tumour associated antigens (TAAs) are expressed by both normal tissues and malignant cells. Severe tissue damage may result from CAR T recognition of these common antigens. For instance, because EGFR and HER2 are expressed in healthy tissues, targeting them has resulted in lethal toxicities in early trials [2,7].

4. Serious Side Effects of Treatment Two potentially fatal side effects of CAR T therapy are Immune Effector Cell Associated Neurotoxicity Syndrome (ICANS) and Cytokine Release Syndrome (CRS). ICANS presents as neurological dysfunction, whereas CRS is caused by a massive release of cytokines (IL 6, IFN γ, and TNF α). Neurotoxicity is still difficult to manage even though medications like tocilizumab (anti-IL 6R) reduce CRS [2,7].

Issues with Accessibility and Manufacturing Autologous T cell collection, ex vivo engineering, and expansion are necessary for the highly customised CAR T therapy. This procedure restricts accessibility because it is expensive, time-consuming, and technically complex. Rapidly advancing patients might not make this within the manufacturing window [1,7].

Relapse and Limited Persistence Relapse may result from CAR T cells' inability to maintain long-term efficacy, even after initial responses are obtained. Reduced durability is caused by T cell exhaustion, inhibitory signalling pathways (e.g., PD 1/PD L1), and memory formation failure [3].

Extending CAR-T to Solid Tumors

Although chimeric antigen receptor T cell (CAR T) therapy has shown impressive results in treating haematological malignancies, it is still very difficult to apply this effectiveness to solid tumours. Since solid tumours make up more than 90% of human cancers, this extension is a crucial immunotherapy frontier [2,3].

Important Obstacles in Solid Tumours Solid tumours pose different challenges than blood cancers. First, since many tumour associated antigens (TAAs) are also expressed on normal tissues, the absence of truly tumour specific antigens (TSAs) raises the risk of "on target, off tumour" toxicity [2,7]. Second, cancer cells can evade

CAR T recognition due to antigen heterogeneity both within and between tumours [2,3]. Third, CAR T cells are exhausted by the tumour microenvironment (TME), which is highly immunosuppressive and rich in regulatory T cells, myeloid-derived suppressor cells, and inhibitory cytokines like TGF β and IL 10 [2,3]. Lastly, CAR T trafficking and infiltration are restricted by physical barriers like aberrant vasculature and dense extracellular matrix [3].

Developing Techniques to Get Past Obstacles Dual targeted, bispecific, and tandem CARs (TanCARs), which recognise multiple antigens at once and lessen the chance of tumour evasion, are being developed by researchers to combat antigen escape [2,4]. By requiring dual antigen recognition prior to activation, logic gated CARs and synNotch systems further improve specificity while reducing off-tumor toxicity [2]. Another goal is to improve infiltration. Improved homing to tumours secreting corresponding chemokines has been shown by CAR T cells engineered with chemokine receptors like CCR2b or CXCR2 [3]. Early trials have also demonstrated promise for local or regional delivery, such as hepatic artery infusion for liver cancers or intracranial infusion for glioblastoma [3].

Armoured CARs (TRUCKs) are engineered to release cytokines such as IL 12, IL 15, or IL 18, which attract and stimulate endogenous immune cells in order to combat the immunosuppressive TME [2, 3]. Furthermore, preclinical and early clinical research has shown that checkpoint blockade combinations—such as anti-PD 1 with CAR T—have synergistic effects [2].

Prospects for the Future Other effectors, like CAR NK cells and CAR macrophages (CAR M), are being investigated in addition to T cells. While CAR M cells take advantage of macrophages' capacity to infiltrate tumours and alter the TME [5], CAR NK cells provide innate tumour killing ability and reduced toxicity [3].

Next generation Cell platforms

The development of next-generation cellular platforms aimed at overcoming present constraints and expanding therapeutic reach has been spurred by the remarkable success of CAR T therapy in haematological malignancies. These platforms include natural killer (NK) cells, macrophages, and engineered stem cell-derived immune cells in addition to traditional T cells.

1.1. **CAR-NK Cells** CAR NK cells combine the natural ability of NK cells to kill tumours with the accuracy of CAR engineering. Unlike T cells, NK cells do not require HLA compatibility, reducing the risk of graft versus host disease and enabling “off the shelf” allogeneic therapies [3]. Additionally, they are less toxic, with a lower frequency of neurotoxicity and cytokine release syndrome (CRS). Their poor in vivo persistence, however, continues to be a problem, leading to tactics like cytokine arming (IL 15 expression) to improve survival [3].

1.2. **CAR-Macrophages (CAR-M)** Macrophages have powerful phagocytic and antigen-presenting abilities and are prevalent in the tumour microenvironment. Utilising these characteristics, CAR M therapy remodels the immunosuppressive microenvironment and directly engulfs tumour cells [5]. The feasibility and safety of early clinical trials (such as CT 0508, which targets HER2) have been demonstrated, and macrophages have outperformed T cells in tumour infiltration. CAR M design is being further improved by developments in synthetic biology and biomaterial-assisted gene delivery [5].

1.3. **Dual-Targeted and Armored CAR-T Cells** Cells Dual targeted CARs (e.g., CD19/CD20, BCMA/CD19) to stop antigen escape [4] and armoured CARs designed to release cytokines (IL 12, IL 18) or withstand inhibitory signals like

TGF β [2] are examples of innovations within the T cell platform itself. In solid tumours, these changes are intended to increase efficacy, specificity, and persistence.

1.4. **Stem Cell–Derived Platforms** Induced pluripotent stem cells (iPSCs) are being investigated as renewable resources for producing CAR T, CAR NK, and CAR M cells in large quantities, providing standardised, commercially available products [5].

Clinical Landscape and Future Directions

With several FDA-approved products that target CD19 (such as tisagenlecleucel and axicabtagene ciloleucel) and BCMA (such as idecabtagene vicleucel and ciltacabtagene autoleucel), CAR T therapy has solidified its place in the treatment of haematological malignancies [1,3,7]. These treatments have shown long-lasting remissions in multiple myeloma, lymphomas, and leukaemias that have relapsed or refractory, improving outcomes for patients who had few other options. By advancing CAR T earlier in treatment lines and investigating its use in conjunction with conventional regimens, clinical trials are continuing to broaden the indications [3].

Additionally, the landscape is becoming more varied. Dual-targeted CAR T cells, such as BCMA/CD19 and CD19/CD20, are demonstrating promise in reducing antigen escape [4]. In order to combat the immunosuppressive tumour microenvironment, armoured CAR T cells that are designed to release cytokines or withstand inhibitory signals are beginning early phase trials [2]. Preclinical and early clinical research is advancing the CAR NK and CAR M platforms, which provide safer, "off the shelf" substitutes with possible benefits in solid tumours [3, 5].

Looking forward, the field is moving toward universal, scalable, and safer cell therapies. The newest developments include biomaterial-assisted delivery systems, logic-gated CARs, and immune cells derived from iPSCs. The ultimate objective is to

reduce cost, toxicity, and manufacturing complexity while expanding the revolutionary effects of CAR-based therapies beyond blood cancers to solid tumours.

Conclusion

With its remarkable success in treating haematological malignancies and its ability to transform immuno-oncology, CAR T cell therapy has ushered in a new era of personalised cancer treatment. Its journey is far from finished, though. The need for ongoing innovation is underscored by the constraints presented by solid tumours, toxicity, antigen escape, and manufacturing challenges. The therapeutic horizon is being expanded by novel approaches like dual targeted and armoured CAR T cells, integration with checkpoint inhibitors, and investigation of alternative immune platforms like CAR NK and CAR M.

With next-generation platforms and stem cell-derived products seeking to provide safer, more scalable, and more accessible therapies as the clinical landscape is changing quickly. The future of CAR-based immunotherapy rests in developing adaptable, long-lasting, and universal treatments that can address solid tumours as well as blood cancers, as research converges with developments in synthetic biology, gene
editing, and biomaterial delivery.

CAR T therapy, which turns the immune system into a living medication, is essentially a paradigm shift rather than merely a treatment. As it develops further, it has the potential to revolutionise cancer treatment and bring us one step closer to the long-awaited development of immunotherapies that are effective, long-lasting, and widely available.

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Manufacturing of Biofertilizer by Using Rhizobium

Surbhi Dhaka, Shivani Singh

Department of Biotechnology, Kunwar Satyavira College of engineering and management

Bijnor 246701

Abstract

One of the biggest issues in agriculture these days is the careless application of chemicals fertilizer. These substances have serious negative effects on both human and animal health in addition to their effects on the environment, therefore finding new alternatives or sustainable agricultural systems is essential. Biofertilizer is a bioformulation that contains living organisms. One such biofertilizer is Rhizobium, a member of the Rhizobiaceae family, which infects leguminous plant root nodules and converts atmospheric nitrogen to ammonia so that the plants can use it. The Rhizobium shelf-life studies are crucial for monitoring the quality of the biofertilizer formulation. The serial dilution and plating methods are the most appropriate, while there are other ways to determine the shelf life. By solubilizing soil, native plant growth-promoting rhizobacteria (PGPR) create microbial formulations known as biofertilizers, which either directly or indirectly stimulate plant growth. By optimizing Rhizobium application to increase production, lessen reliance on chemical fertilizer inputs to prevent nutritional deficiencies, and support ecosystem health, the findings should support sustainable agriculture practices.

Keywords: biofertilizer, bioformulation, Rhizobiaceae, leguminous plant, ecosystem.

Introduction

Given the current state of human population growth, it is predicted that by 2050, there will be 9 billion people on the planet, increasing the demand for food. However, the main causes include urbanization, biotic and abiotic stressors, limited quantity of productive land, and

unpredictable weather [1, 2]. Obstacles to supplying the ever rising demand for food [3]. Overuse of chemical fertilizers and pesticides has been done in the last ten years in an attempt to boost output. However, in actuality, just Plants only take a small portion of these nutrients, and the remainder is lost, polluting the environment [2]. Since the majority of the chemicals used are persistent, they cannot be broken down. Furthermore, the chemicals used degrade water bodies and cause disruption, as well as additional risks to public health in biogeochemical cycles. This demonstrated how eco-friendly and sustainable technologies are being developed to combat the overuse of synthetic fertilizers [4]. Plant production and soil health are significantly influenced by interactions among microorganisms, plants, and soil[5].Therefore, using beneficial microbiomes as biofertilizers can enhance soil fertility and plant growth in an environmentally responsible way[6,7]. Numerous bacteria in the plant microbiome are crucial to the establishment and growth of crops [7]. Soil improvement microbiological status by the use of biofertilizers can promote the natural soil microbiota, which influences nutrient accessibility and organic matter breakdown[8] Higher crop output may arise from biofertilizers' capacity to create a
more diverse population of soil microbes[9].

To check for plant growth, microbes extracted from the rhizosphere are tested encouraging colonization and having the capacity to function as a successful biofertilizer [2]. Additional characteristics that promote plant growth Utilizing bacteria as biofertilizers can improve nutrients. Organic matter's availability, breakdown, and participation in reduction of biotic and abiotic contaminants [4, 10]. The availability and uptake of nutrients from soil are enhanced by biofertilizers, which are applied to soil, seeds, or seedlings and include either live or latent microbial organisms[7].In order to extend the shelf life and facilitate the handling of microbial inoculants, biofertilizers are manufactured using appropriate carriers and applied in various forms[11]. Low cost, improved soil fertility and nutrient availability, increased resistance to biotic and abiotic stress, increased phytohormone synthesis, improved soil health, and reduced pollution are just a few advantages of biofertilizers[8]. Biofertilizers are marketed worldwide in two categories: those based on organic residues and those based on microorganisms. Green biofertilizers made of organic materials include animal dung, farmyard manure, processed sludge, and crop residues, but biofertilizers based on

microorganisms contain advantageous bacteria. Rhizobacteria that promote plant growth, phosphate solubilizing/mobilizing microbes, zinc solubilizing/mobilizing microbes, potassium solubilizing/mobilizing microbes, sulfur oxidizing microbes, and nitrogen-fixing microbes (free-living, symbiotic, and associative) are the different types of microorganism-based biofertilizers[2]. In order to quantify the yield benefits of biofertilizers, a meta-analysis of fenugreek seeds revealed that phosphate solubilizers and nitrogen fixers were quite successful. As inoculants in liquid media, biofertilizers that contain phosphate solubilizers or nitrogen fixers can be purchased in solid, liquid, or powdered form [12]. Liquid biofertilizers use Rhizobium, as inoculants. Using liquid biofertilizers has a number of additional benefits over solid ones, but the most significant is the addition of a sufficient quantity of nutrients in a liquid form [13, 14]. Cell protectants can occasionally be added to microbial biofertilizers to extend their shelf life. Furthermore, there are several issues with using solid-base inoculants that are uncommon with liquid inoculant formulations. Liquid biofertilizers have attributes like extended shelf life, improved survival, affordability, and ease of handling. In addition, liquid biofertilizers have additional benefits over carrier-based biofertilizers (CBFs). Unlike liquid biofertilizers, CBF are not as resistant to temperature changes accepting of such modifications. While liquid biofertilizers promote increased viability, CBF's poor moisture-retaining ability impacts organism viability over extended periods of time. Since bulk sterilization is not a very efficient way to prevent contamination, which can be easily controlled by suitable quality control methods and proper sterilization processes in the case of liquid-based biofertilizers, the likelihood of contamination is considerable in the case of CBF [15,16]. Using the viable count approach, Shravani et al. [13] conducted a study that compared the shelf life of liquid biofertilizers and CBF. When comparing carrier-based biofertilizers to liquid biofertilizers, their findings showed a subsequent decline in the microbial population accompanied by an increase in contamination. While CBF only demonstrated a consistent viable cell count for the first three months, liquid-based biofertilizers demonstrated a consistent viable cell count for at least five to six months. Furthermore, it was discovered that CBF had more noticeable pH variation and a gradual drop in moisture content. Therefore, on the parameters under study, liquid-based biofertilizers were found to be more effective than carrier-based fertilizers. The shelf life of liquid biofertilizer inoculants made with various cell protectants was assessed by another study. Colony-forming

units for liquid-based biofertilizers, such as Rhizobium, were measured weekly to estimate shelf life. Compared to other cell protectors, polyvinyl pyrrolidone produced the largest cell colonies, according to the study [14]. Similar to this, additional research chose the best carrier and liquid-based biofertilizers by conducting quality analyses on a number of characteristics [16]. To boost agricultural output, the Indian government has implemented a number of initiatives utilizing biofertilizers in addition to contemporary agrochemicals [17]. The majority of recent studies on biofertilizers have been on strain generation, determining the rhizosphere's method of action, and boosting biological nitrogen fixation. There isn't much research on inoculant manufacturing, though. Instead of employing liquid inoculants with a high microbial load, the bulk of Indian inoculant manufacturers continue to use the traditional Burton method [18]. Through extensive field research on inoculants and their impact on crop yield, thoughtful knowledge regarding the formulation of inoculant quality will grow microbiological.Inoculants are specific multifunctional microorganisms that, when used in agricultural operations, support improved plant development or biological control. The viability of the microbial inoculant is crucial for improving product quality. Improving microbial inoculant structure and activity assures biofertilizers are more reliable than chemical fertilizers for sustainable agriculture practices that promote soil health in the future[19]. Measurable protocols for producing and assessing inoculants have not been widely available for many crops. More research is needed to better understand the interplay between introduced microbial inoculants and native soil microbes. Thus, greater emphasis should be placed on raising public understanding of inoculant manufacturing.

RHIZOBIUM

Rhizobia are diazotrophic soil bacteria that fix nitrogen after settling inside legume root nodules (Fabaceae). Rhizobia come in a variety of genera, however they are all members of the Rhizobiales, a likely monophyletic group of proteobacteria. are soil bacteria distinguished by their special capacity to infect legume root hairs and cause the formation of efficient N2-fixing nodules on the roots. These living plants have a rod-like form and are only found in the vegetative stage. In contrast to numerous other soil microbes, rhizobia don't create .They are motile and aerobic spores. Rhizobia, which include Sinorhizobium, Azorhizobium,

Bradyrhizobium, Mesorhizobium, and Rhizobium species, form close symbiotic ties with legumes by chemotactically reacting to the flavonoid compounds that the bean host releases as signals. These plant substances cause rhizobia to express nodulation (nod) genes, which result in the production of lipo-chito signals from oligiosaccharides (LCO) that cause roots' mitotic cells to divide, resulting in the creation of nodules. Although the symbiosis between Rhizobium and legumes is a common example of mutualism, its evolutionary longevity is actually somewhat unexpected. Destroying the host plant that they are all dependent on. Each plant is infected by multiple unrelated strains, thus any one strain might divert resources from N2 fixation to its own reproduction without destroying the host plant that they are all dependent on. It turns out that by lowering the oxygen content, legume plants direct the evolution of rhizobia toward increased mutualism supply to nodules that fix less N2, which lowers the incidence of cheaters in the following generation. Numerous studies have been conducted on symbiotic N2-fixation, which has been used to boost crop yields. When plants die, the fixed N2 is freed, allowing other plants to use it and aiding in soil fertilization.

MATERIAL AND METHOD

The isolates were tested for contamination and to differentiate between Rhizobium and Agrobacterium using biochemical assays.

3.1 Glucose Peptone Agar medium

a) *Purpose*

This medium is used to test *Rhizobium* for glucose utilization and acid production. It's similar to lactose peptone agar, but glucose replaces lactose as the main carbon source.

Component	Amount (for 100 mL)
Peptone	1.0 g
Glucose	0.5 g
Agar	1.5 g

Distilled water	100 mL
(Optional indicator)	phenol red 0.0018 g or bromothymol blue 0.002 g

Table 1 Preparation for 100 mL GPA

- **Phenol red** – 0.018 g/L (medium turns yellow in acid, red in alkaline), or
- **Bromothymol blue** – 0.02 g/L (medium turns yellow in acid, blue in base)

b) Preparation Steps

1. **Dissolve** the peptone and glucose in ~80 mL distilled water.
2. **Add agar**, heat while stirring until completely melted.
3. Add the **indicator** if desired.
4. **Adjust pH** to **7.0 ± 0.2** using 0.1 N NaOH or HCl.
5. Make up the volume to **100 mL** with distilled water.
6. **Dispense** into tubes or flasks (e.g., 10 mL per tube).
7. **Autoclave** at **121°C for 15 minutes**.
8. **Cool and solidify** (plates or slants).

c) Usage for *Rhizobium* culture

- Inoculate the *Rhizobium* culture onto the medium.
- Incubate at **28–30°C for 3–5 days**.
- Observe:
  - **Good growth** → glucose utilized as energy source.
  - **Color change** (if indicator added):
    - Yellow → acid produced from glucose fermentation.
    - No color change → glucose not fermented (but may still be utilized oxidatively).

3.2 Lactose peptone agar

Lactose Peptone Agar (LPA) is a biochemical test medium used for studying carbohydrate (lactose) utilization and growth characteristics of *Rhizobium* species. Sometimes a pH indicator like **bromothymol blue** or **phenol red** is added to observe acid production, depending on the protocol.

a) Preparation for 100 mL

To make **100 mL** of Lactose Peptone Agar:

Component	Amount (for 100 mL)
Peptone	1.0 g
Lactose	0.5 g
Agar	1.5 g
Distilled water	100 mL

Table 2 Preparation of 100 mL of LPA

b) Preparation Procedure

1. **Weigh** the ingredients listed above.
2. **Dissolve** the peptone and lactose in about 80 mL of distilled water.
3. **Add agar** and heat gently (e.g., on a hot plate) while stirring until fully dissolved.
4. **Make up the volume** to 100 mL with distilled water.
5. **Adjust pH** to about **7.0 ± 0.2** using 0.1 N NaOH or HCl.
6. **Dispense** into test tubes or flasks as desired (e.g., 10 mL per tube).
7. **Sterilize** by autoclaving at **121 °C for 15 minutes**.
8. **Cool and solidify** (if in plates or tubes).

c) Use for *Rhizobium* cultures

- Inoculate *Rhizobium* cultures onto the medium.

- Incubate at **28–30 °C for 3–5 days**.
- Observe for **growth and lactose utilization** (acid production may change the color if indicator is present).

3.3 Growth on Yeast Extract Mannitol Agar (YEMA) slant :

When **Rhizobium** species are cultured on **Yeast Extract Mannitol Agar (YEMA)** slants, they show **characteristic colony morphology and growth patterns** that help in identification

a) Medium composition (YEMA)

- **Mannitol** – Carbon source
- **Yeast extract** – Nitrogen and growth factors
- **K_2HPO_4, $MgSO_4$, NaCl** – Minerals
- **Agar** – Solidifying agent

Sometimes, **Congo Red** is added (YEMA + Congo Red) for differentiation.

b) *Growth characteristics (without Congo Red)*

- **Growth rate:** Moderate to slow (visible after 2–4 days at 28–30 °C)
- **Colony appearance:**
  - Circular, convex, smooth, and glistening
  - White, creamy, or translucent
  - Entire margins (not irregular)
- **Texture:** Buttery or gummy consistency (due to extracellular polysaccharide)

3.3.1 *On YEMA + Congo Red*

a) **Rhizobium colonies:**
   - **Do not absorb Congo Red dye** → appear **white or pale pink**
   - Colonies remain **translucent**

b) **Agrobacterium (a contaminant) colonies:**

- **Absorb Congo Red** → appear **red or pinkish-orange**

c) *Biochemical confirmation*

Typical Rhizobium isolates are:

- **Gram-negative**, non-spore-forming rods
- **Catalase positive**
- **Oxidase positive**
- **Do not grow on glucose-peptone agar** (unlike Agrobacterium)

Summary Table

Characteristic	Observation
Medium	YEMA slant
Growth	Moderate, smooth, gummy
Colony color	White to creamy
Congo Red absorption	Negative (pale colonies)
Gram reaction	Negative rods
Incubation temp	28–30 °C
Time	2–4 days

Table 3 Summary

3.4 starch hydrolysis test:

It's also known as amylase test as it applies to **Rhizobium** species:

a) Purpose

In order to ascertain whether a bacterium generates the extracellular enzyme amylase, which hydrolyzes starch (a polysaccharide) into simpler sugars like glucose and maltose, the starch

hydrolysis test is utilized.

The metabolic profile of Rhizobium is characterized by this test, which is useful for identifying the species and setting it apart from other soil bacteria.

b) Principle

- Starch cannot be directly taken up by bacterial cells.
- Some bacteria secrete **amylase** to break down starch into smaller, soluble sugars that can be absorbed.
- When **iodine** is added to the medium, it reacts with starch to produce a **blue-black** color.
- If the organism has hydrolyzed starch, there will be a **clear zone** (halo) around the bacterial growth where starch is absent.

c) Materials

- Starch agar medium (nutrient agar + 0.2–0.5% soluble starch)
- Rhizobium culture (pure colony)
- Iodine solution (Gram's iodine: iodine + potassium iodide)
- Incubator (28–30°C for Rhizobium species)

d) Procedure

1. Prepare sterile **starch agar plates**.
2. Streak the **Rhizobium** isolate onto the plate.
3. Incubate at **28–30°C for 3–5 days** (Rhizobium grows slower than many other bacteria).
4. After incubation, flood the plate with **iodine solution**.
5. Observe the area around the bacterial growth.

e) Observation & Interpretation

Observation	Interpretation
Clear zone (halo) around growth	Positive for **starch hydrolysis** → amylase produced
No clear zone, entire plate blue-black	Negative for starch hydrolysis → no amylase production

Table 4 observation on Starch Hydrolysis

f) Expected Result for *Rhizobium*

Most **Rhizobium** species are **negative** for starch hydrolysis — they typically do **not** produce amylase.
This helps distinguish them from other soil bacteria like *Bacillus* species, which are usually positive.

g) Conclusion

If you observe **no clear zone** after adding iodine, your **Rhizobium** isolate is **starch hydrolysis negative**, meaning it does not secrete amylase.This is consistent with most members of the genus.

3.5 Hofer's Alkaline Medium Test:

a) Purpose

To test the ability of bacteria to **grow in an alkaline environment (pH ≈ 11)**. Rhizobium species **cannot tolerate or grow** in strongly alkaline conditions, while some related genera (like *Agrobacterium*) **can grow**.

b) Principle

- The test is based on the **sensitivity of Rhizobium to alkaline pH**.
- Hofer's medium is adjusted to a high pH (around **pH 11**) using **Na_2CO_3** or **NaOH**.

- If the organism grows in this medium, it is **alkali-tolerant** (not Rhizobium).
- Lack of growth indicates **Rhizobium**, which is sensitive to high alkalinity.

c) Composition of Hofer's Alkaline Medium (per liter)

Component	Quantity
Mannitol	10.0 g
Yeast extract	0.5 g
Dipotassium phosphate (K_2HPO_4)	0.5 g
Sodium chloride (NaCl)	0.1 g
Magnesium sulfate ($MgSO_4 \cdot 7H_2O$)	0.2 g
Agar	15.0 g
Distilled water	1000 mL

Table 5 Composition of Hofer's Alkaline Medium (per liter)

- Adjust **pH to 11.0** with **1 N NaOH** before autoclaving.
- Sterilize by autoclaving at 121 °C for 15 minutes.

d) Procedure

1. Prepare sterile Hofer's alkaline agar slants or plates.
2. Inoculate with a **pure Rhizobium culture** (from YEMA slant).
3. Incubate at **28–30 °C** for **3–5 days**.
4. Observe for visible growth.

e) Observation and Interpretation

Observation	Interpretation
No growth	Organism **cannot tolerate high pH → Rhizobium positive**
Good growth	Organism **tolerates alkaline pH → Not Rhizobium** (possibly *Agrobacterium* or other soil bacteria)

Table 6 Observation of Hofer's Alkalline medium test

3.6 Gram staining:

The following reagents were made for gram staining:
Iodine solution (Iodine-1 g, Potassium iodide-2 g, Ethyl alcohol-25 ml, Distilled water-400 ml); Iodinated alcohol (Iodine solution (b)-5 ml, Ethyl alcohol-95 ml); Crystal violet solution (Crystal violet-10 g, Ethyl alcohol 100 ml, Ammonium oxalate-4 g, Distilled water 400 ml);
Counterstain: 100 ml of distilled water, 10 ml of 2.5% safranin in ethyl alcohol. A loop full of a chosen bacterium was used to create a gram-stained smear, which was then spread out on a slide in a drop of water and let to dry in the open. After allowing the slide to cool and drying near the flame, it is stained using crystal violet solution in the manner described below: After rinsing with water for one minute and wiping away any excess water, the slide is flooded with iodine solution and decolored for another minute using iodinated alcohol. It is then rinsed in water for five minutes, drained, and counterstained with safranin. After the slide has been cleaned with water, drained, and allowed to air dry, it is examined while submerged in oil.

3.7 Growth in 2% sodium chloride concentration:

Each isolate was inoculated with slants of Yeast Extract Mannitol Agar (YEMA) medium containing 2% salt to test Rhizobium's adaptation at 2% NaCl concentration. After five days of incubation, growth was observed.

3.8 Gum Production:

Anderson's (1938) approach was used to study Rhizobium gum production. Sterilized flasks holding 100 ml of the Yeast Extract Mannitol (YEM) broth were incubated for 15 days on a rotating shaker after being inoculated in triplicate with 1 ml of each isolate's five-day-old culture broth at 121 0C for 15 to 20 minutes. Following incubation, the volume of culture media was lowered to 30 ml with the aid of boiling. The flasks for the gum's precipitation were filled with a three-volume mixture of ethanol and acetone, and

they were left undisturbed overnight to guarantee full precipitation. A record of each isolation was made. After the contents of the flasks were filtered, the amount of gum produced by each isolate was measured after the retentate, or gum, on the filter paper was dried in an oven set to 78°C for 24 hours.

3.9 Reduction of 2, 3, 5 triphenyl tetrazolium chloride (TTC) :

For this test, Herrigan et al.'s (1966) methodology was applied. TTC is a redox indicator that, when added to culture, shows if the isolates are capable of producing the dehydrogenase enzyme. After inoculating tubes with 5 ml of YEM broth with various isolates for 7 days, 1 ml of 2, 3, and 5 TTC (1% solution) was added, and the tubes were then incubated for an additional 30 minutes at 28 0C. The incubated tubes' pink appearance suggested that the TTC had decreased.

3.10 Action on Litmus milk :

Milk-based medium called litmus milk is used to differentiate between various bacterial species. Different kinds of bacteria can break down the lactose (milk sugar), casein (milk protein), and litmus (pH indicator) that are present in the medium. This test enables an accurate representation of bacterial kinds because milk is typically the first substrate utilized to maintain bacteria. In addition to indicating the pH type, litmus serves as an oxidation-reduction indicator. Whether the bacterium can ferment lactose, decrease litmus, produce clots, create gas, or initiate peptonization is determined by the test itself. In order to give the skim milk a grey-blue hue, bromocresol solution was added. For several days in a row, the milk was divided into test tubes and sterilized for 30 minutes. After that, the tubes were incubated at 28±2 0C after being infected twice with the appropriate culture. After 24 hours of inoculation, the milk's appearance and color change were specifically assessed.

3.11 Casein hydrolysis :

The purpose of the test is to ascertain whether an organism is capable of producing the

exoenzyme casesase. Certain bacteria create an exoenzyme called casease to break down casein. The big protein called casein is what gives milk its white hue. Milk agar, a complex medium comprising casien, peptone, and beef extract, is used for this test. A clearing zone will surround the bacterial development if the organism is capable of producing casein.

After being prepared and sterilized, skimmed milk agar (1000 ml of milk and 15–20 g of agar-agar) was transferred onto sterile petriplates. Short strokes of the culture loop were used to inoculate the middle of the plates, and they were then incubated for 48 hours at 37.0C. Following a 48-hour incubation period, the plates were examined against a black backdrop, and the existence or lack of visible halos surrounding the plate's center was noted.

3.12 Production of ammonia from peptone :

This test can be used to assess the production of ammonia from urea and the deamination of peptone by bacteria. A 1000 ml peptone broth was made with 1 g of peptone, 0.5 g of NaCl, 0.5 g of potassium nitrate, and 1000 ml of distilled water. Five milliliters of the broth were then poured into each test tube, and the tubes were sterilized. Test tubes were incubated for 48 hours at 37.0C following inoculation. One milliliter of Nessler's reagent was applied to the culture tube following incubation. Ammonia is present when the color changes from orange to brown.

3.13 Urease production :

Urease broth is a differential medium used to assess an organism's capacity to make urease, an exoenzyme that hydrolyzes urea to produce carbon dioxide and ammonia. Two pH buffers, urea, a trace amount of bacterial nutrients, and the pH indicator phenol red are all present in the broth. In an alkaline environment, phenol red turns fuchsia, and in an acidic environment, yellow. The media turns pink and an alkaline environment is established if the urea in the broth is broken down and ammonia is generated.

3.14 Triple sugar iron agar test:

The purpose of the test was to ascertain whether the isolates could use different sources of carbohydrates, such as lactose, glucose, sucrose, etc., as growth media. The purpose of the triple sugar iron test is to distinguish between the several groups that can ferment glucose and produce acid and hydrogen sulfide.

The ingredients for the Triple Sugar Iron Agar medium were as follows: 3 g/l of beef extract, 3 g/l of yeast extract, 15 g/l of peptone, 5 g/l of NaCl, 10 g/l of lactose, 10 g/l of sucrose, 1 g/l of dextrose, 0.2 g/l of ferrous sulphate, 0.3 g/l of sodium thiosulfate, 0.24 g/l of phenol red, 15 g/l, and agar until the pH was adjusted to 7.0 (Kligler, 1918; Hajna, 1945). Following inoculation and incubation, color was seen on the slant and butt[20].

4. RESULT AND DISCUSSION

Rhizobium is a beneficial soil bacterium that forms a **symbiotic association with leguminous plants** and plays a key role in **biological nitrogen fixation**. It protects plant roots from certain soil-borne pathogens and enriches the soil by converting **atmospheric nitrogen (which makes up about 78% of the atmosphere)** into forms that plants can readily absorb and utilize for their growth and development. Nitrogen is one of the most essential nutrients for plants, being a major component of chlorophyll, amino acids, and proteins.

In this study, we aimed to produce and evaluate an effective **biofertilizer** containing *Rhizobium* to enhance plant growth and soil fertility. The goal was to make a biofertilizer more efficient than existing ones by introducing *Rhizobium* as the active microbial component.

4.1 Experimental Setup

Initially, *Rhizobium* cultures were isolated, and a dense bacterial suspension was prepared. This suspension was thoroughly mixed with sterilized soil to form a **Rhizobium-enriched biofertilizer**. Tomato (*Solanum lycopersicum*) and chili (*Capsicum annuum*) plants were selected as test crops. Seeds were sown in pots containing the *Rhizobium*-treated soil and in control pots without the biofertilizer for comparison. All pots were watered regularly and kept in a location receiving adequate sunlight to ensure optimal growth conditions.

4.2 Observations

- **10th day:** Germination began, and small seedlings emerged in both treated and control pots. However, plants in the *Rhizobium*-treated soil appeared slightly healthier and greener.
- **18th day:** The average height of plants in the treated pot reached approximately **4 inches**, while those in the control pot were shorter and less vigorous.
- **31st day:** The treated plants continued to grow rapidly, reaching about **6 inches** in height. Leaves were broader and showed deeper green coloration, indicating better nitrogen availability.
- **36th day:** The plants in the *Rhizobium* biofertilizer pot reached a height of **8–9 inches**, showing significantly better growth and overall vigor compared to control plants, which displayed slower growth and lighter leaf color.

*4.3 **Result***

The experiment demonstrated that plants grown in *Rhizobium*-enriched soil exhibited **faster growth, stronger stems, and healthier leaves**. This confirms that *Rhizobium* biofertilizer effectively improves plant performance by supplying biologically fixed nitrogen, thereby reducing dependence on chemical fertilizers.

4.4 Discussion

The results indicate that *Rhizobium* inoculation enhances plant growth by improving nitrogen availability in the root zone. The symbiotic bacteria fix atmospheric nitrogen into ammonia, which plants can absorb and use for protein and chlorophyll synthesis. This leads to better photosynthetic activity, higher biomass production, and overall improved plant health. Additionally, *Rhizobium* can enhance soil structure and microbial diversity, promoting sustainable agriculture.

Although *Rhizobium* is primarily known for forming nodules in legumes, its indirect benefits to non-leguminous plants such as tomato and chili (through soil enrichment) are also

significant. This small-scale trial supports the idea that *Rhizobium*-based biofertilizers can be a cost-effective and eco-friendly alternative to synthetic fertilrizes.

5. FUTURE PROSPECTS

The present study on *Rhizobium*-based biofertilizer highlights its great potential for promoting sustainable agriculture and reducing dependence on chemical fertilizers. In the future, this work can be expanded by conducting large-scale field trials on different crops and soil types to confirm the effectiveness of *Rhizobium* under varied environmental conditions. Further research can focus on combining *Rhizobium* with other beneficial microorganisms such as *Azotobacter*, *Azospirillum*, and phosphate-solubilizing bacteria to create multi-strain biofertilizers that provide balanced nutrition and improve overall soil health. Genetic improvement of *Rhizobium* strains can also be explored to enhance their efficiency and tolerance to stress conditions such as salinity, drought, and acidity. Continuous application studies could assess the long-term effects of *Rhizobium* on soil fertility and microbial diversity. Moreover, commercial production and farmer awareness programs will be essential to promote the practical use of *Rhizobium* biofertilizers. Overall, *Rhizobium*-based biofertilizers hold great promise as an eco-friendly, cost-effective, and sustainable alternative for improving crop productivity and maintaining soil health in the future.

6. SUMMARY AND CONCLUSION

6.1 Summary Table

Parameter	Control	Rhizobium-treated
Nodule number	Few or none	Many, pink, healthy
Plant height	Lower	Higher
Biomass	Less	More
Yield	Lower	Higher

6.2 CONCLUSION

Overuse of chemical fertilizers to boost crop yields has resulted in major environmental risks as well as health harm. These chemical fertilizers also led to soil contamination, eutrophication, and pollutant leaching, which raised a number of health issues for people. Biofertilizers have nearly the same ability to increase output as chemical fertilizers and are less harmful, more affordable, and environmentally acceptable. Much progress has been made in agricultural research to improve biofertilizers as an alternative to chemical fertilizers. By boosting crop yield and soil fertility, biofertilizers help meet the demands of the food supply. Since it is organic, it doesn't harm the environment. Inadequate marketing, a lack of appropriate carriers for product formulation, a lack of storage facilities to prevent contamination of biofertilizers, and inconsistent biofertilizer efficacy are some of the current issues facing the application of microbial fertilizers. By raising farmer community understanding of microbial fertilizers and their advantages, these drawbacks can be addressed. Information from the many publications currently available shows that Rhizobium and cereals naturally associate, either rhizospherically or endophytically. phenomenon. Gaining more expertise in this field could help with the use of this technology. A thorough optimization process and a comparative analysis of the application's aftereffects are necessary for commercialization. Therefore, further study is required to understand how rhizobia or microorganisms that resemble rhizobia interact with wheat grains. Additionally, it is necessary to thoroughly evaluate whether the rhizobia fix N2 in conjunction with cereals in environments where these bacteria support plant growth, and if so, what percentage of the plant-N may be obtained by the BNF process. The natural capacity of rhizobia to suppress grains and promote their growth is still largely unknown to us. However, by strengthening its natural interaction with rhizobia, the innovative findings presented here contribute significantly to the technically difficult goal of enhancing wheat output by reducing its reliance on the chemical fertilizer-N.

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To prepare a natural perfume from rose petals using environmentally friendly method

Garima Chauhan, Insiya Zehra, Vaishali Sharma

Department of Biotechnology, Kunwar Satyavira College of Engineering & Management, Bijnor, Uttar Pradesh 246701, India

Abstract

This project aims to prepare a natural perfume from rose petals using environmentally friendly methods. Perfumesare mixtures of fragrant essential oils, alcohol, and water, used to provide a pleasant scent. In this study, rosepetals (Rosa species) were selected as the raw material due to their distinctive aroma and natural appeal. The essential oilwas extracted from fresh petals using the steam distillation method, which helps preserve the delicate fragrancecompounds. The obtained rose oil was then mixed with ethanol and distilled water in specific proportions toproducea stable and long-lasting perfume solution. The quality of the perfume was evaluated based on aroma intensity, persistence of fragrance, and overall acceptability. Factors such as temperature, freshness of petals, and concentration of oil were also analyzedfor theireffect on the final scent. The project demonstrates how natural perfumes can be produced without the use of synthetic chemicals, making them safer for human use and less harmful to the environment. Overall, the rosepetalperfume project highlights the combination of scientific extraction techniques and artistic formulation tocreateaneco-friendly, Naturally fragrant product.

Introduction

The use of perfumes goes back thousands of years. The Egyptians used plants, gums, and resins inreligiousrites. As the years went by, scented substances were

used to enhance body attractiveness and tomakehomes and public places more pleasant. Fragrances are considered normal components of our everydaylives. Many people feel the need to wear a fragrance in order to feel good: this is probably becausethereisa connection between scent and emotion as well as between scent and memory; moreover, studies have shownthat somefragrances can alter moods and even alleviate anxiety and stress. Perfumes can be defined as substances that emit and diffuse a pleasant and fragrant odor. Theyconsist ofmanmade mixtures of aromatic chemicals and essential oils. Until the nineteenth century perfumeswereusually composed of natural aromatic oil. Perfume is a mixture of fragrant essential oils or aromacompounds (fragrances), fixatives and solvents, usually in liquid form, used to give the humanbody, animals, food, objects, and living-spaces an agreeable scent. Perfumes can be defined as substancesthatemit and diffuse a pleasant and fragrant odor. They consist of manmade mixtures of aromatic chemicalsand essential oils. The 1939 Nobel Laureate for Chemistry, Leopold Ruzicka stated in 1945 that "right fromthe earliest days of scientific chemistry up to the present time, perfumes have substantially contributedtothe development of organic chemistry as regards methods, systematic classification, and theory." Modern perfumery began in the late 19th century with the commercial synthesis of aroma compoundssuchas vanillin or coumarin, which allowed for the composition of perfumes with smells previouslyunattainable solely from natural aromatics.

MATERIALS AND METHODS

Take 10 fresh roses and place the rose petals in a distill water which was in the beaker anboilingat the heating mental at 50 ° C temperature and seperate the rose oil and store for the 24 hours at roomtemperature in shade after 24 hours we mixed ethyl alcohol and gaxolide in the separated rose oil andkeepthe solution for 8 days at room temperature.

Rose:

Scientificname

: Rose

umsambas

Family:

Oleaceae

Fig.1.1

Chemicalconstituents:

Oseum sambas contains dotriacontanoic acid, do triacontanol, hesperdin,, and [+] roseoids A,B,C,D in its roots.

Leaves contain flavonoids such as rutin, quercetin and isoquercetin, flavonoids rhamnoglycosides as well as α-amyrin and β-sitosterol. A novel

plant cysteine-rich peptide family named rosetides was isolated from this plant.

Method

Take 9mlofrose oil &1ml of orange oil.

Add 39 ml ethyl alcohol. Add 1ml galaxolide to it.

Fill it in aerosol container for further us

Rose Oil extracts:

Method1: Maceration

→

Take 350 gm of *Rose umsambas* (rose flowers).

→Menstruumispo red in a vessel till the flowers recompletely dipped in it(water)

→Keep it for 8days in a glass vessel

1) **Steps**

Spray the fragrance twice in a down ward motion in frontofyou. Swiftly pass the blotter the fragrance'svapours cloud.

Quickly wave the blotter under your nose and inhale. Refer back to the card regularly to test its life cycle.

2) **SkinTest:**

- Steps

Spray the back of your hand twice while trespecting the correct spray distance. Leave to dry naturally & do not rub in fragrance.

Inhale the fragrance without letting it touch your nose. Refer back to your hand over time to see how it evolves.

EvalutionTest:

S.no.	Test	Observation
1.	pH	7
2.	Skintest	Noirritation
3.	Fragrance test	Pleasantaroma

RESULTS

The extracted perfume from rose petals showed a complex composition of fragrance compound, with geraniol and linalool as major constituent. The physical and organoleptic properties indicate a high-quality perfume suitable.

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Mathematical Modelling of an Engineering Problem: Heat Transfer in an Extended Surface (Fin)

Shubham Kumar[1], Rajan Singh[2], [3]B.K. Singh

, Applied Science Department, Kunwar Satyavira College of Engineering & Management, Bijnor (246701), India

, Department of Mathematics, IFTM University, Mooradabad (244001), India

[3], Department of Mathematics, IFTM University, Mooradabad (244001), India

Abstract

One of the best ways to improve heat transfer between a solid and the surrounding fluid is to use fins, or extended surfaces. They are widely utilized in air-cooled engines, condenser tubes, heat exchangers, radiators, and electronic component cooling, among other uses. This paper presents a comprehensive mathematical model of steady-state heat transmission via extended surfaces. A second-order differential equation describing the temperature distribution along the fin is developed using Newton's law of cooling and Fourier's rule of heat conduction. Key performance metrics fin efficiency and fin effectiveness is produced, along with analytical solutions for various boundary conditions. Additionally, a thorough numerical example shows how geometric and thermal parameters affect the result. Mathematical modelling plays an important role for solution of such a kind of engineering problems.

1. Introduction

Almost every engineering domain, from power plants and microelectronics to automotive and aerospace systems, need efficient heat transport. Low convective heat transfer coefficients frequently restrict the amount of heat that can be dissipated from surfaces, especially when gases are employed as cooling media. Engineers use expanded surfaces, or fins, to expand the effective area available for convection in

order to get around this restriction. A fin is a slender piece of material (usually metal) attached to a surface to enhance heat transfer by increasing the area exposed to the fluid. The performance of fins depends on their geometry, material properties, and environmental conditions. Understanding and predicting the temperature distribution, efficiency, and effectiveness of fins are crucial for their optimal design. This paper aims to develop a mathematical model describing heat transfer through an extended surface using fundamental principles of thermodynamics and differential equations.

2. Theoretical Background

2.1 Heat transfer occurs through three primary modes: conduction, convection, and radiation. In this study, we consider steady-state conduction along the fin and convection from the fin surface to the surrounding fluid.

2.2 Application of Fins

Fins are commonly found in:

- Engine cooling systems (e.g. cylindrical fins)
- Transformation and motor housings.
- Electronic heat sinks.
- Air conditioning and refrigeration units.
- Solar Panels and radiations

3. Mathematical Formulation

The following presumptions underlie the development of the fin model in steady-state conditions: uniform convective coefficient, constant thermal conductivity, one-dimensional heat conduction, and minimal radiation. An energy balance on a fin differential element is used to generate the governing equation.

3.1 Assumptions

1. Steady- state heat conduction.

2. One dimensional temperature variation along the fin length.

3. Constant thermal conductivity of the fin material.

4. Uniform convective heat transfer coefficient *h* .

5. Negligible radiation and internal heat generation.

6. The fin has uniform cross- sectional area *A* and perimeter *P*.

3.2 Energy Balance and Differential Equation

Consider a differential element of the fin of thickness *dx.*

The energy balance under steady- state conditions is:

Rate of heat conduction in at x = Rate of heat conduction out at x + Rate of heat lost by convection.

Mathematically,

$\frac{d}{dx}(kA\frac{dT}{dx})$ - hP (T-T_∞) = 0

Simplifying for constant *k* and *A:*

$$\frac{d^2T}{dx^2} - \frac{hP}{kA}(T - T_\infty) = 0$$

Let $\theta = T - T_\infty$

We obtain the governing equation

$$\frac{d^2\theta}{dx^2} - m^2\theta = 0$$

Where m = $\sqrt{\frac{hP}{kA}}$ is called the fin parameter.

The given differential equation is homogeneous linear differential equation of second order with constant coefficients. The equation can be put in the form $(D^2 - m^2)\theta = 0$

Solving the equation-

Complementary function (C.F.)

Auxiliary equation ($p^2 - m^2$) = 0

$$p = \pm m$$

Roots are real and distinct

C.F. = $C_1 e^{m_1 x} + C_2 e^{m_2 x}$

Particular Integral (P.I.)

P.I. = 0

Since the equation is homogeneous differential equation.

So, the complete solution of the given equation

$\theta(x) = \text{C.F.} = C_1 e^{m_1 x} + C_2 e^{m_2 x}$

The constants C_1 and C_2 depends on boundary conditions.

3.3 Boundary conditions and Fin configurations

Case I: Fin with Insulated Tip

Boundary conditions:

At x = 0, $\boldsymbol{\theta = \theta_b}$

At x = L, $\boldsymbol{\frac{d\theta}{dx} = 0}$

Solutions:

$$\theta(x) = \theta_b \frac{\cosh[m(L-x)]}{\cosh(mL)}$$

Heat transfer rate at the base:

$Q_b = k\,A\,m\,\theta_b\,tanh(mL)$

Case II: Fin with Convective Tip

Boundary conditions:

At x = 0, $\boldsymbol{\theta = \theta_b}$

At x = L, -kA $\boldsymbol{\frac{d\theta}{dx}}$ = $\mathbf{hA_t\theta_L}$

Solutions:

$$\theta(x) = \theta_b \frac{\cosh[m(L-x)] + \frac{h}{mk}\sinh[m(L-x)]}{\cosh(mL) + \frac{h}{mk}\sinh(mL)}$$

3.4 Fin Efficiency and Effectiveness

3.4.1 Fin Efficiency ($\boldsymbol{\mu}$)

$$\mu = \frac{Q_{actual}}{Q_{ideal}} = \frac{tanh(mL)}{mL}$$

3.4.2 Fin Effectiveness (ϵ)

$$\epsilon = \frac{Q_{Fin}}{hA_b(T_b - T_\infty)} = \sqrt{\frac{hP}{kA}} L\ tanh(mL)$$

4. Numerical Illustration

Given data:

Parameter	Symbol	Value
Thermal conductivity	K	200 W/m.K
Convective coefficient	H	40 W/m2.K
Perimeter	P	0.06 m
Cross-sectional area	A	$2.5\times\ 10^{-4}m^2$
Fin Length	L	0.1 m
Base temperature	T_b	373 k
Ambient temperature	T_∞	303k

Compute the Fin Parameter:

m = $\sqrt{\frac{hP}{kA}}$ = 1.86 m^{-1}

Fin Efficiency:

$$\mu = \frac{Q_{actual}}{Q_{ideal}} = \frac{tanh(mL)}{mL} = \frac{tanh(1.386)}{1.386} = 0.693$$

Heat dissipation:

$Q_{Fin} = \sqrt{hPkA}\ (T_b - T_\infty)\ tanh(mL)$

$$Q_{Fin} = 17.84W$$

5. Parametric Analysis

To study the influence of material and geometry:

Material	K(W/m.k)	M(1/m)	Efficiency (μ)	Heat Dissipation (W)
Aluminum	200	13.86	0.693	17.84
Copper	385	9.87	0.805	19.65
Steel	50	27.72	0.445	13.22

6. Observation:

(i) Higher thermal conductivity→ higher efficiency and heat transfer rate.

(ii) Longer fins yields diminishing returns as temperature gradient reduces towards the tip.

(iii) For small mL, fins are almost isothermal and efficiency is high for large mL, fins lose

Effectiveness.

7. Graphical Results

1. Temperature Distribution:

The temperature decays exponentially along the fin. Copper fins maintain a higher temperature along the length due to higher conductivity.

2. Effect of Fin Length:

Increasing L, Initially increases heat transfer but later saturated.

3. Effect of Convective Coefficient:

A higher *h* enhances heat loss but also increases m, reducing efficiency.

8. Discussion

The mathematical model accurately predicts the steady- state temperature distribution and performance of fins. The derived equations can be used for the optimizing the fin length, material, and cross- section for maximum heat transfer with minimal material

usage. For practical applications, the choice of material must balance thermal performance with cost weight considerations. Advanced techniques such as numerical techniques can be more useful for further advancement of the process.

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Enhanced Fuzzy Clustering via a Synergistic Combination of Intuitionistic Modified Fuzzy C-Means and Genetic Optimization

Niteesh Kumar

Department of Applied Science

Kunwar Satyavira College of Engineering and Management, Bijnor, Uttar Pradesh.

ABSTRACT

In this digital era, the exponential growth of data driven by the widespread use of the web, social media and various online platforms has made it increasingly challenging to extract meaningful insights from large and complex datasets. Although numerous clustering algorithms have been proposed for data mining, identifying a single method suitable for all types of datasets remains difficult. This study introduces a **hybrid fuzzy clustering algorithm** that integrates the *intuitionistic modified fuzzy c-means* technique with a *genetic algorithm*. The proposed method addresses the issue of high sensitivity to initial centroid selection by incorporating normalized crossover and mutation operators within the genetic algorithm. Additionally, it reduces the impact of noise through a newly developed metric and resolves uncertainty in membership value assignment using a single negation function. The effectiveness of the proposed algorithm is validated through experiments conducted on various real-world benchmark datasets, demonstrating superior clustering accuracy and robustness compared to existing methods.

Keywords: Crossover operator, Fuzzy clustering, Genetic algorithm, Intuitionistic fuzzy set, Objective function value.

1. Introduction

Clustering is a fundamental technique in big data analysis, machine learning, and data mining. Its primary objective is to categorize objects into distinct groups (clusters) such that the elements within the same cluster exhibit strong similarity, whereas

elements from different clusters show weak similarity [1]. Generally, no single clustering algorithm is universally suitable for all tasks, as each method possesses its own strengths and limitations. Consequently, several clustering approaches have been developed in the literature, including grid-based, hierarchical, partition-based, and density-based clustering methods. Even when applied to the same dataset, different algorithms or the same algorithm with varying parameters can yield diverse clustering results. Therefore, the selection of an appropriate clustering technique is critical to achieving desirable outcomes.

Among the various clustering algorithms, the Fuzzy C-Means (FCM) technique is widely recognized for its computational efficiency, simplicity, and ease of implementation. It is particularly effective for real-world datasets where cluster boundaries are not clearly defined [2]. However, despite its advantages, FCM suffers from certain drawbacks, such as susceptibility to local minima, sensitivity to noise, and dependence on the initial selection of cluster centroids. Numerous modifications have been proposed to overcome these limitations. Fundamentally, the FCM objective function relies on two key factors: the membership value and the Euclidean distance (ED). The ED metric, in particular, greatly influences clustering performance in noisy environments. To mitigate this issue, researchers have enhanced the FCM objective function over time by incorporating alternative distance measures tailored to specific problems.

Frigui and Krishnapuram [3] developed competitive agglomeration algorithms that integrate the benefits of hierarchical and partitional clustering by minimizing an appropriate objective function. Singh and Bansal [4] examined the influence of noise on clustering techniques, and Godwin and Ugwoke [5] investigated clustering algorithms capable of producing optimized cluster groups in healthcare datasets. Kumar et al. [6] proposed two novel FCM-based algorithms designed to minimize the impact of noise, while Kumar et al. [1] developed a hybrid method combining Particle

Swarm Optimization (PSO) with Improved FCM (IFCM) to address initialization and noise issues. Previous studies have demonstrated that the choice of distance metric can significantly mitigate noise effects in FCM; however, further improvement remains necessary. To address this, the present study introduces a new distance metric designed to minimize the influence of noise on FCM results.

One of the key advantages of FCM and its variants lies in their ability to assign a data point to multiple clusters rather than a single one. However, in practical datasets, inherent uncertainty often complicates the assignment of membership values. To handle this uncertainty, Atanassov [7] introduced the concept of Intuitionistic Fuzzy Sets (IFS) in 1986. IFS extend the traditional fuzzy set theory (FST) to better manage vagueness and uncertainty in data [8]. In recent years, the combination of IFS theory with FCM has gained popularity, leveraging not only membership values but also non-membership and hesitation degrees. Xu and Wu [9] further extended fuzzy clustering to the interval-valued intuitionistic fuzzy domain (IVIFS). Chaira [10] proposed an intuitionistic fuzzy clustering algorithm incorporating the concept of intuitionistic fuzzy entropy, applying it to medical data analysis. Kaur et al. [11] introduced a novel distance metric to design robust IFCM and kernel-based IFCM algorithms, enhancing their performance. Kumar and Harish [12] presented a modified IFCM variant for medical applications. Xian et al. [13] introduced a novel correlation coefficient function based on TIFLV and applied it in clustering algorithms. Zhou et al. [14] incorporated kernel functions as similarity measures and determined initial centroids using the state transition algorithm. Recently, Kaushal and Lohani [15] generalized IFCM through an adaptive fuzzification approach under an intuitionistic fuzzy framework.

Initially, FCM selects centroids randomly, which often leads to convergence at local minima. To overcome this, numerous meta-heuristic optimization techniques have been explored in the literature, including Genetic Algorithms (GA) [16], Particle

Swarm Optimization (PSO) [1], Simulated Annealing (SA) [17], and Quantum Genetic Algorithms (QGA) [18], among others. Over the past decades, GA has been particularly favored due to its flexibility and simplicity. Saha and Bandyopadhyay [19] proposed a fuzzy GA framework for dataset partitioning. Dong et al. [20] developed a hybrid GA-FCM model capable of automatically determining the number of clusters. Deep et al. [21] proposed a real-coded GA for mixed-integer optimization as an extension of the LXPM model. Hou et al. [22] further improved IFCM using weighted proximity measures such as aggregated weighted similarity and correlation alongside GA. Finally, Kuo et al. [23] proposed IFWCOM and GA-IFWCOM algorithms to enhance clustering accuracy in the presence of outliers. A hybrid fuzzy clustering technique combining intuitionistic modified fuzzy c-means and an improved genetic algorithm using Sugeno's negation function is proposed to reduce noise, resolve uncertainty, and overcome initialization sensitivity, achieving superior results on benchmark datasets [24].

Over the past several decades, numerous modifications have been introduced to overcome the limitations of the Fuzzy C-Means (FCM) algorithm. However, existing improved versions still exhibit certain shortcomings that affect the accuracy and robustness of FCM results. To address these challenges, the present study proposes a fuzzy clustering approach designed to improve clustering accuracy and optimize the objective function. The proposed method integrates the Intuitionistic Fuzzy C-Means (IFCM) algorithm with an Improved Genetic Algorithm (IGA) to develop a hybrid technique, termed the Improved Genetic Algorithm Intuitionistic Fuzzy C-Means (IGAIFCM) algorithm. In the proposed algorithm, FCM is modified through the introduction of a new distance metric that replaces the conventional Euclidean Distance (ED). This newly designed metric significantly reduces the influence of outliers and noise in the dataset. To handle the uncertainty associated with assigning membership values to data points, the Intuitionistic Fuzzy Set (IFS) theory is incorporated into the modified FCM, resulting in the IFCM technique. This approach

effectively accounts for hesitation and non-membership degrees in addition to traditional membership values. For assigning intuitionistic fuzzy membership values, Sugeno's negation function is utilized within the IFCM framework.

To overcome the sensitivity of IFCM to initial centroid selection, a Genetic Algorithm (GA) is employed to determine optimal initial cluster centers. Among various meta-heuristic optimization methods, GA is specifically chosen for its mutation and crossover operators, which prevent the algorithm from being trapped in local minima and enhance the exploration of the search space. The GA is further improved by introducing novel crossover and mutation operators, which increase its convergence rate and produce more stable centroids. The key contributions of the present study can be summarized as follows:

- A fuzzy clustering technique is developed through the integration of IFCM and IGA under an intuitionistic fuzzy environment.
- The improved genetic algorithm effectively addresses the initialization problem associated with IFCM.
- The IFCM technique employs Sugeno's negation function to incorporate hesitation degrees.
- The proposed algorithm demonstrates its effectiveness on real-world datasets, and its results are compared with existing clustering algorithms.

The remainder of this paper is organized as follows. Section 2 presents the preliminaries and related work. Section 3 describes the problem formulation and derivation of the proposed algorithm. Section 4 details the systematic steps of the proposed approach. Section 5 discusses the evaluation criteria, while Section 6 reports the performance of the proposed IGAIFCM algorithm on various datasets. Section 7 highlights the comparative analysis with existing methods to validate the effectiveness

of the proposed model. Finally, Section 8 concludes the paper with key findings and potential directions for future research.

2. Background information

Some basic concepts are given in this section, which is used in the proposed IGAIFCM technique.

2.1. Fuzzy c-means clustering technique

FCM is prominent clustering technique due to its straightforwardness. Dunn [25] used the concept of Ruspini [26] and gave the formulation of cluster, which was further improved by Bezdek [27] in 1985. The objective function of FCM with their minimizing conditions are represented as

$$J\left(u_i, C_k\right) = \sum_{i=1}^{n}\sum_{k=1}^{c} \mu_{ki}^{m} \left\|u_i - C_k\right\|^2 ; 1 < m < \infty \tag{1}$$

where, μ_{ki} is membership value, u_i is the i^{th} data point and C_k is k^{th} cluster centroid.

$\mu_{ki} = \dfrac{1}{\sum_{j=1}^{c}\left(\dfrac{\left\|u_i - C_k\right\|}{\left\|u_i - C_j\right\|}\right)^{2/(m-1)}}$ and $C_k = \dfrac{\sum_{i=1}^{n} \mu_{ki}^{m} u_i}{\sum_{i=1}^{n} \mu_{ki}^{m}}$ are necessary conditions which minimize Eq. (1).

Here, $\sum_{k=1}^{c} \mu_{ki} = 1$ and $\mu_{ki} \in [0,1]$.

2.2. Intuitionistic fuzzy set theory

Fundamental component of FST is only a membership value of a data point. But in real-life situations, there exists some uncertainty or hesitation about the membership value of a component. To resolve this issue, Atanassov [7] developed the notion of

intuitionistic FST. Intuitionistic fuzzy set (IFS) is formulated as

$$S = \left\{ \left\langle u, \mu_S(u), v_S(u) \right\rangle | u \in X \right\} \quad (2)$$

where $\mu_S(u)$ and $v_S(u)$ stands for membership and non-membership values s.t.

$$\mu_S(u) \in [0,1], v_S(u) \in [0,1] \& \; 0 \le \mu_S(u) + v_S(u) \le 1 \quad (3)$$

If the membership value of an element is not defined due to lack of knowledge [28], then hesitation comes into presence and is given by

$$\pi_S(u) = 1 - \mu_S(u) - v_S(u); 0 \le \pi_S(u) \le 1 \quad (4)$$

Due to hesitation, the membership values lies in the interval $\left[\mu_S(u), \mu_S(u) + \pi_S(u) \right]$.

2.3. Genetic algorithm

GA is a type of meta-heuristic optimizing technique, which was first given by John Holland in 1975 [29]. GA is an important search algorithm of natural genetics and natural selection which is based on the Darwin's principle. Even though a random collection of bizarre individuals can be the initial population but to form new generations the individuals will interact and breed. Stronger individuals will generate better offspring in the comparison of weaker individuals. As generations pass the weaker individuals will die out, this result in the population to get collectively stronger. The fittest survive is the central idea of natural selection. Genetic algorithm has major components: encoding, initializing population, selection, crossover & mutation [30].

3. Problem formulation

Some general notations and modified fuzzy c-means with their necessary conditions are discussed in the present section.

3.1. Notations

Numerous notations used throughout the present manuscript are:

c	number of clusters
m	fuzzy index (m> 1)
m_{ij}	$(ij)^{th}$ element
NX	normalized crossover
OFV	objective function value

3.2. Problem statement

Clustering is a widely used technique for analyzing the underlying structure and patterns within datasets. Among various clustering algorithms, the Fuzzy C-Means (FCM) method is one of the most popular due to its simplicity, efficiency, and ease of implementation. However, despite its advantages, the FCM algorithm suffers from several notable limitations, which can affect clustering performance. These issues include:

- High sensitivity to noise and outliers.
- Dependence on the initial selection of centroids.
- Susceptibility to convergence at local minima.
- Uncertainty in assigning appropriate membership values to data points.

To mitigate these drawbacks, the objective function of the FCM algorithm can be redesigned to minimize the impact of these factors and thereby improve clustering accuracy.

3.3. Distance function

Clustering techniques work on the principle of grouping objects. To group the objects, ED is most commonly used measure in fuzzy clustering technique. Due to noise in

data, the presence of ED easily influences the desired output of FCM. To overcome this disadvantage, a metric has been introduced in Eq. (5), which tolerates the noisy environment.

$$d\left(u_i, v_k\right) = 1 - \exp\left\{(-1)\frac{\left\|u_i - v_k\right\|^2}{2\sigma^2}\frac{\beta_i}{\theta_i}\right\} \tag{5}$$

Where $\beta_i = \frac{\left|\max m_{ij} - \min m_{ij}\right|}{2}$ and $\theta_i = \left|\sum_{j=1}^{d}\frac{m_{ij}}{d}\right|$ and d represent dimension. Eq. (5) is termed as metric, if it satisfies the following axioms [31]:

(I) $d(u_i, v_k) > 0$ iff $u_i \neq v_k$,

(II) $d(u_i, v_k) = 0$ iff $u_i = v_k$,

(III) $d(u_i, v_k) = d(v_k, u_i) \quad \forall u_i, v_k$

(IV) $d(u_i, w_r) + d(w_r, v_k) - d\left(u_i, v_k\right) \geq 0 \quad \forall w_r$

Theorem 1: Prove that Eq. (5) defines a metric.

Proof: Eq. (5) is as:

$$d\left(u_i, v_k\right) = 1 - \exp\left\{(-1)\frac{\left\|u_i - v_k\right\|^2}{2\sigma^2}\frac{\beta_i}{\theta_i}\right\}$$

Before satisfying axioms of metric, first show that $d(u_i, v_k)$ is always positive [1].

$$0 \leq \exp\left\{(-1)\frac{\left\|u_i - v_k\right\|^2}{2\sigma^2}\frac{\beta_i}{\theta_i}\right\} \leq 1 \qquad \forall u_i, v_k \in R$$

$$0 \leq 1 - \exp\left\{(-1)\frac{\left\|u_i - v_k\right\|^2}{2\sigma^2}\frac{\beta_i}{\theta_i}\right\} \leq 1 \qquad \forall u_i, v_k \in R$$

$$0 \leq d\left(u_i, v_k\right) \leq 1 \qquad \forall u_i, v_k \in R$$

Above result show that $d(u_i, v_k) \in [0,1]$. (I), (II) and (III) axioms are evidently satisfied by $d(u_i, v_k)$. Now, needs to satisfy last axiom.

$$d(u_i, w_j) + d(w_j, v_k) - d(u_i, v_k)$$

$$= 1 - \exp\left\{(-1)\frac{\|u_i - w_j\|^2}{2\sigma^2}\frac{\beta_i}{\theta_i}\right\} + 1 - \exp\left\{(-1)\frac{\|w_j - v_k\|^2}{2\sigma^2}\frac{\beta_j}{\theta_j}\right\} -$$

$$\left[1 - \exp\left\{(-1)\frac{\|u_i - v_k\|^2}{2\sigma^2}\frac{\beta_i}{\theta_i}\right\}\right] \quad \forall u_i, v_k, w_j \in R$$

$$\geq 1 - \exp\left\{(-1)\frac{\|u_i - w_j\|^2}{2\sigma^2}\frac{\beta_i}{\theta_i}\right\} - \exp\left\{(-1)\frac{\|w_j - v_k\|^2}{2\sigma^2}\frac{\beta_j}{\theta_j}\right\}$$

$$\left(1 - \exp\left\{(-1)\frac{\|u_i - w_j\|^2}{2\sigma^2}\frac{\beta_i}{\theta_i}\right\}\right) \quad \forall u_i, v_k, w_j \in R$$

$$= \left[1 - \exp\left\{(-1)\frac{\|u_i - w_j\|^2}{2\sigma^2}\frac{\beta_i}{\theta_i}\right\}\right] \times \left[1 - \exp\left\{(-1)\frac{\|w_j - v_k\|^2}{2\sigma^2}\frac{\beta_j}{\theta_j}\right\}\right] \quad \forall u_i, v_k, w_j \in R$$

$$= d(u_i, w_j) \times d(w_j, v_k) \geq 0$$

Hence, Eq. (5) is metric.

3.4. Improved genetic algorithm technique

The Fuzzy C-Means (FCM) algorithm is highly sensitive to initialization conditions, meaning that the selection of initial cluster centroids significantly influences its final clustering results. To address this limitation, the Genetic Algorithm (GA) is employed to determine the optimal initial centroids. The performance of GA largely depends on its crossover and mutation operators, which serve as the key components for exploration and exploitation in the search process [21]. To further enhance the performance of GA and reduce the chances of premature convergence, a Normalized Crossover (NX) operator is used based on the fundamental concept of the normal

distribution [24]. The probability density function used to generate the NX operator is expressed in Eq. (6).

$$h(u_k) = \frac{1}{\sigma^* \sqrt{2\pi}} \exp\left\{ -\frac{|u_k - \alpha|}{\sigma^* \sqrt{2\pi}} \right\}; -\infty < u_k < \infty \quad (6)$$

where, α and σ^* represents mean and variance of selected parent respectively. Now, a random number δ_k is constructed in the following ways:

$$G_k = 1 - \int_{\alpha}^{\delta_k} \frac{1}{\sigma^* \sqrt{2\pi}} \exp\left\{ -\frac{|u_k - \alpha|}{\sigma^* \sqrt{2\pi}} \right\} du_k$$

If $\alpha < u_k < \delta_k$, then, $|u_k - \alpha| = (u_k - \alpha)$

$$G_k = \exp\left\{ -\frac{(\delta_k - \alpha)}{\sigma^* \sqrt{2\pi}} \right\}$$

$$\delta_k = \alpha - \sigma^* \sqrt{2\pi} \log_e G_k; \quad \text{if } G_k > \frac{1}{2}.$$

Similarly, if $G_k \le \frac{1}{2}$, then

$$\delta_k = \alpha + \sigma^* \sqrt{2\pi} \log_e G_k$$

Thus,

$$\delta_k = \begin{cases} \alpha + \sigma^* \sqrt{2\pi} \log_e G_k; & \text{if } G_k \le 1/2 \\ \alpha - \sigma^* \sqrt{2\pi} \log_e G_k; & \text{if } G_k > 1/2 \end{cases} \quad (7)$$

By using the proposed algorithm, $u_{1j}^{(2)} = \left(u_{11}^{(2)}, u_{12}^{(2)}, ..., u_{1n}^{(2)}\right) \& u_{2j}^{(2)} = \left(u_{21}^{(2)}, u_{22}^{(2)}, ..., u_{2n}^{(2)}\right)$ be the two new generated off-spring based on the selected parents $u_{1j}^{(1)} = \left(u_{11}^{(1)}, u_{12}^{(1)}, ..., u_{1n}^{(1)}\right)$ $\& u_{2j}^{(1)} = \left(u_{21}^{(1)}, u_{22}^{(1)}, ..., u_{2n}^{(1)}\right)$. Then, the off-springs are generated by Eq. (8)

$$u_{1j}^{(2)} = u_{1j}^{(1)} + \delta_1 \left| u_{kj}^{(1)} - u_{2j}^{(1)} \right|$$
$$u_{2j}^{(2)} = u_{2j}^{(1)} + \delta_2 \left| u_{kj}^{(1)} - u_{1j}^{(1)} \right|$$

(8)

where, $u_{kj}^{(1)}$ represents the chromosome which has highest fitness value and $j = 1, 2,\ldots, d$. In IGA technique, Eq. (9) is used to calculate the mutated chromosomes.

$$^{*}u_{1j}^{(2)} = u_{1j}^{(2)} + \omega \left| u_{kj}^{(1)} - u_{1j}^{(2)} \right|$$
$$^{*}u_{2j}^{(2)} = u_{2j}^{(2)} + \omega \left| u_{kj}^{(1)} - u_{2j}^{(2)} \right|$$

(9)

where $\omega = \dfrac{iter_{\max} - iter_{current}}{iter_{\max}} \left(\omega_{\max} - \omega_{\min} \right) + \omega_{\min}$.

3.5. Intuitionistic fuzzy c-means

In real-world datasets, there exists some uncertainty. Due to this, uncertainty originates in assigning the membership value to an element in a desired cluster [8]. To handle such uncertainty, IFS is used with IFCM to obtain the hesitation value and non-membership value along membership value for data point representation. Sugeno's class of fuzzy complement generator is utilized to generate intuitionistic fuzzy membership.

3.5.1. Sugeno's negation function

In present article, intuitionistic fuzzy generator is evaluated by using Sugeno's negation function [32]. Fuzzy generator is represented as

$$N\left(\mu_{ki}\right) = g^{-1}\left(g\left(1\right) - g\left(\mu_{ki}\right)\right)$$

where, $g:[0,1] \to [0,1]$ and $g(\cdot)$ is strictly increasing.

Eq. (10) is used to represent Sugeno's class as:

$$g\left(\mu_{ki}\right)=\frac{1}{\xi_1}\log\left(1+\xi_1\mu_{ki}\right)$$

(10)

By using negation function, the non-membership values can be defined as

$$N\left(\mu_{ki}\right)=\frac{1-\mu_{ki}}{1+\xi_1\mu_{ki}};\xi_1>-1$$

So, the intuitionistic fuzzy membership value is obtained according to Eq. (11).

$$^{*}\mu_{ki}=\frac{\left(1+\xi_1\right)\mu_{ki}}{1+\xi_1\mu_{ki}};\xi_1>-1$$

(11)

3.6. Modified fuzzy c-means

The simplicity and ease of implementation make the Fuzzy C-Means (FCM) algorithm one of the most prominent fuzzy clustering techniques. To enhance the clustering performance and accuracy of FCM, the conventional Euclidean Distance (ED) in its objective function is replaced with the proposed distance metric. Consequently, the objective function of the Modified Fuzzy C-Means (MFCM) algorithm is formulated as presented in Eq. (12).

$$F(u_i,C_k)=\sum_{i=1}^{n}\sum_{k=1}^{c}\mu_{ki}^{m}\left[1-\exp\left\{(-1)\frac{\left\|u_i-C_k\right\|^2}{2\sigma^2}\frac{\beta_i}{\theta_i}\right\}\right]$$

(12)

The minimizing conditions of Eq. (12) are shown in Eqs. (13) and (14) as follows:

$$C_k=\frac{\sum_{i=1}^{n}\mu_{ki}^{m}\left[\exp\left\{(-1)\frac{\left\|u_i-C_k\right\|^2}{2\sigma^2}\frac{\beta_i}{\theta_i}\right\}\right]\frac{u_i\beta_i}{\theta_i}}{\sum_{i=1}^{n}\mu_{ki}^{m}\left[\exp\left\{(-1)\frac{\left\|u_i-C_k\right\|^2}{2\sigma^2}\frac{\beta_i}{\theta_i}\right\}\right]\frac{\beta_i}{\theta_i}}$$

(13)

$$\mu_{ts} = \frac{1}{\sum_{j=1}^{c} \left[\frac{1 - \exp\left\{ (-1) \frac{\|u_s - C_t\|^2}{2\sigma^2} \frac{\beta_s}{\theta_s} \right\}}{1 - \exp\left\{ (-1) \frac{\|u_s - C_j\|^2}{2\sigma^2} \frac{\beta_s}{\theta_s} \right\}} \right]^{1/(m-1)}} \quad (14)$$

3.7. Termination criteria for IGA technique

The selection of an appropriate termination criterion depends on the nature of the optimization problem. Although several termination rules exist, such as monitoring improvements in successive fitness values or limiting the number of iterations, the present work adopts a single, well-defined criterion to ensure computational efficiency and stability. In the proposed Improved Genetic Algorithm (IGA), the optimization process is terminated when the algorithm reaches a maximum of 100 iterations. This condition ensures a balance between convergence accuracy and computational time.

4. Description of the proposed model

The FCM algorithm faces several notable challenges, including its sensitivity to noise in datasets, dependence on initial centroids, and tendency to converge to local minima. To overcome these limitations, a hybrid fuzzy clustering algorithm is proposed by integrating the IFCM and IGA techniques. In the proposed approach, cluster centroids are represented as chromosomes, which are further optimized using the IGA to obtain more stable and reliable centroids. These optimized chromosomes are then utilized as the initial centroids for the IFCM algorithm to reduce initialization sensitivity and improve clustering accuracy. The proposed IGAIFCM algorithm is as follows:

<table>
<tr><th>IGAIFCM algorithm</th><th colspan="2">Formation of clusters through proposed algorithm</th></tr>
<tr><td>Step 1:</td><td colspan="2">Input $c, \omega_{\max}, \omega_{\min}$ and positive number $\in$ for termination of algorithms.</td></tr>
<tr><td>Step 2:</td><td colspan="2">Calculate θ_i and β_i by using $\beta_i = \frac{\left|\max m_{ij} - \min m_{ij}\right|}{2}$ & $\theta_i = \left|\sum_{j=1}^{d} \frac{m_{ij}}{d}\right|$</td></tr>
<tr><td>Step 3:</td><td colspan="2">By taking $t = 0$, obtained initial centroids
$C_k^0 = \sum_{i=1}^{s} \frac{u_i}{\mathrm{P}}$, where P represents no. of dp and $t = \frac{\text{Total no. of dp}}{c}$.</td></tr>
<tr><td>Step 4:</td><td colspan="2">Repeat step 3 till centers of all defined clusters are not determined. Use these centers as the initial centers to run IGA technique.</td></tr>
<tr><td rowspan="6">Step 5:</td><td colspan="2">Improved genetic algorithm technique:</td></tr>
<tr><td>Step 5.1:</td><td>Randomly select the encoding technique and choose the population size.</td></tr>
<tr><td>Step 5.2:</td><td>Randomly initialize the population:
The k clusters centroid encoded as an initial chromosome.</td></tr>
<tr><td>Step 5.3:</td><td>Calculate the fitness:
The fitness value of individual chromosome is determined according to $f_k = \frac{1}{J_k + \eta}$.</td></tr>
<tr><td>Step 5.4:</td><td>Selection:
Select those two chromosomes form the k chromosomes, which have least fitness values and assigned them as parent chromosomes.</td></tr>
<tr><td>Step 5.5:</td><td>Crossover: selected parental chromosomes undergo the crossover to produce two new offspring by:</td></tr>
</table>

		$u_{1j}^{(t+1)} = u_{1j}^{(t)} + \delta_1 \left\| u_{kj}^{(t)} - u_{2j}^{(t)} \right\|$ $u_{2j}^{(t+1)} = u_{2j}^{(t)} + \delta_2 \left\| u_{kj}^{(t)} - u_{1j}^{(t)} \right\|$
	Step 5.6:	Mutation: after crossover, the strings are required to mutate to manage the diversity in population. The mutation is evaluated by: $^{*}u_{1j}^{(t+1)} = u_{1j}^{(t+1)} + \omega \left\| u_{kj}^{(t)} - u_{1j}^{(t+1)} \right\|$ $^{*}u_{2j}^{(t+1)} = u_{2j}^{(t+1)} + \omega \left\| u_{kj}^{(t)} - u_{2j}^{(t+1)} \right\|$
	Step 5.7:	Termination criteria: Repeat the steps 5.3 to 5.7, until the termination criteria will not meet.
Step 6:	Intuitionistic fuzzy c-mean technique:	
	Step 6.1:	By taking $t = 0$, associates each data point with a membership value s.t. $\sum_{k=1}^{c} \mu_{ki}^{(t)} = 1$.
	Step 6.2:	Assign updated centroid as an initial centroid for IFCM, which are obtained in previous step.
	Step 6.3:	Calculate the intuitionistic fuzzy membership value by: $^{*}\mu_{ki}^{(t)} = \frac{(1+\xi_1)\mu_{ki}^{(t)}}{1+\xi_1 \mu_{ki}^{(t)}}; \xi_1 > -1$
	Step 6.4:	Update the clusters centroid by: $C_k^{(t+1)} = \frac{\sum_{i=1}^{n} {}^{*}\mu_{ki}^{m} \left[\exp\left\{ (-1) \frac{\left\| u_i - C_k^{(t)} \right\|^2}{2\sigma^2} \frac{\beta_i}{\theta_i} \right\} \right] \frac{u_i \beta_i}{\theta_i}}{\sum_{i=1}^{n} {}^{*}\mu_{ki}^{m} \left[\exp\left\{ (-1) \frac{\left\| u_i - C_k^{(t)} \right\|^2}{2\sigma^2} \frac{\beta_i}{\theta_i} \right\} \right] \frac{\beta_i}{\theta_i}}$
	Step	Calculate the fuzzy partition matrix according to the

	6.5:	formula: $$\mu_{ki}^{(t+1)} = \frac{1}{\sum_{j=1}^{c}\left[\frac{1-\exp\left\{(-1)\frac{\left\|u_i - C_k^{(t+1)}\right\|^2}{2\sigma^2}\frac{\beta_i}{\theta_i}\right\}}{1-\exp\left\{(-1)\frac{\left\|u_i - C_j^{(t+1)}\right\|^2}{2\sigma^2}\frac{\beta_i}{\theta_i}\right\}}\right]^{1/(m-1)}}$$
	Step 6.6:	Determine the OFV: $$F(u_i, C_k) = \sum_{i=1}^{n}\sum_{k=1}^{c}\mu_{ki}^{m}\left[1-\exp\left\{(-1)\frac{\left\|u_i - C_k\right\|^2}{2\sigma^2}\frac{\beta_i}{\theta_i}\right\}\right]$$
	Step 6.7:	Repeat the steps from 6.3 to 6.7, until the termination criteria will not meet.
Step 8:	End	

Flow chart represents the proposed IGAMFCM algorithm in Fig. 1.

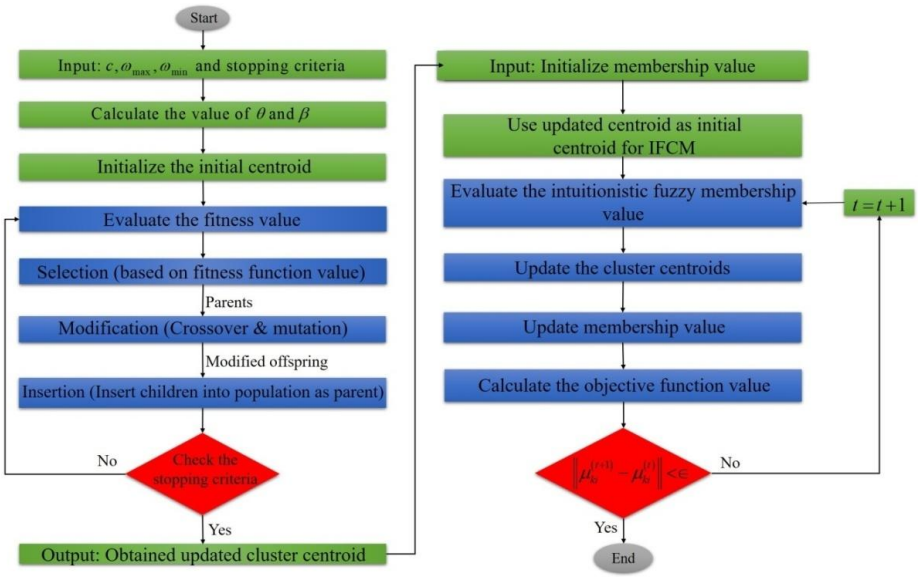

Fig. 1: Flow chart of the proposed IGAIFCM algorithm

5. Evaluation setups

The present section described some information about datasets, performance measure and parameter settings.

5.1. Data sets

Eleven real worlds [33] are considered in Table 1, to figure out the behavior of proposed algorithm.

Table 1 Information regarding data sets

Dataset	Number of instances	Number of clusters	Number of features
Balance scale	625	3	4
Breast cancer wiscosin (BCW)	569	2	20
Cancer	683	2	9
Contraceptive method choice (CMC)	1473	3	9
Glass	214	6	9
Haberman	306	2	3
Iris	150	3	4
Seed	210	3	7
Soybean	47	4	35
Tae	151	3	5
Wine	178	3	13

5.2. Performance measures

OFV, ICD and accuracy are used to measure the efficiency of proposed IGAIFCM algorithm with Sugneo's negation function. Upcoming section briefly discussed about ICD and accuracy.

5.2.1. Inter-cluster distance

Distance between the parent cluster centroid is termed as ICD [1] and it is formulated as in Eq. (15).

$$\mathrm{ICD} = \min\left\{ d_{ij}\left(C_i, C_j\right)\right\}$$

(15)

where $i = 1, 2, ..., k-1 \& \; j = i+1, i+2, ..., k.$

5.2.2. Accuracy

The ratio of number of correct labels to the total labels is termed as Accuracy [1]. Eq. (16) is used to obtained accuracy of proposed algorithm.

$$\text{Accuracy} = \frac{d}{n} \times 100 \tag{16}$$

where n is total labels and d is number of correct labels.

6. Performance evaluation

To demonstrate the effectiveness of the proposed algorithm, several experiments are conducted on both real-world and artificial datasets. In this section, the impact of noisy data on the proposed distance function is evaluated. Subsequently, five different datasets are processed using the IGAIFCM algorithm, and the results are compared with those obtained from existing clustering algorithms to highlight the advantages of the proposed approach.

6.1. Effect of noise

Noise easily influenced the results of FCM because it uses the ED. So, FCM is modified by using proposed metric instead of ED. Let $U = \{u_1, u_2, ..., u_n\}$ be a set of n data points defined on universe of discourse R^n. Eq. (17) is used to determine the minimizer's value, which is determined by differentiating $\sum_{i=1}^{n} \|u_i - C\|^2$ with respect to C.

$$C = \frac{\sum_{i=1}^{n} u_i}{n} \quad (17)$$

The artificial dataset {4.9, 6.2, 3.95, 5.3, 7, 4, 4.5, 5.9, 5, 4.7, 5.1} [34] is evaluated using Eq. (17) to determine the minimizer's value through the least squares method. For this dataset, the computed value of C is 5.1409. After introducing a noise value of 35 into the dataset, the minimizer's value shifts to 7.6292, indicating that the noisy point significantly influences the minimizer's result. To mitigate this effect, a new metric has been developed. The same dataset is further tested using the proposed metric, and the minimizer's value is calculated according to Eq. (13). Additionally, the impact of noise on other existing distance functions is examined, and the comparative results are presented in Table 2. As shown in Table 2, the proposed metric considerably reduces the influence of noise, demonstrating that the proposed algorithm is robust against outliers and noisy data.

Table 2 Study of effect of noisy data on different metrics

S. No.	Metric	Without noise	With noise
1	Euclidean metric [27]	5.1409	7.6292
2	Extended metric function [6]	5.0763	6.0277
3	New distance metric [35]	4.8651	7.4790
4	Exponential based function [36]	5.0896	6.1249
5	New metric [1]	5.0356	6.0024
6	Proposed metric	4.9846	5.2434

6.2. Implementation of proposed IGAIFCM technique

Example 1: In this example, the study of different metric has been carried out by implementing existing clustering techniques and comparing them with the IGAIFCM techniques, as shown in Table 3. From this table, it can be concluded that proposed techniques produce prosperous results in comparison of existing clustering techniques.

Table 3 Comparative study of different metrics on existing clustering techniques

ference	ta set	etric used	ustering techni que	Reference model			IGAIFCM		
				OFV	ICD	Acc. (%)	OFV	ICD	Acc. (%)
umar et al. [6]	lance scal e	lvanced metric	MFCM	6.2973	'863	.24	140.5697	1.9972	78
umar et al. [6]	lance scal e	tended metric	MFCM	8.3227	6183	.40	140.5697	1.9972	78
nxuan [37]	s	sine similarity	proved FCM (2)	8.6813	8816	.67	10.8554	1.8256	92
al et al. [38]	ass	freys-divergence	proved FCM (1)	8.9438	-----	.14	0.4539	1.1854	87.38
umar et al. [1]	CW	w metric	OIFCM	2.2954	.5883	.78	90.8552	20.5453	86.64

7. Comparison

Real-world datasets are utilized to perform the qualitative evaluation of the proposed algorithm. To assess its effectiveness in comparison with existing techniques, several performance measures are considered, namely the Objective Function Value (OFV), Intra-Cluster Distance (ICD), and accuracy. The comparative results are presented in Table 4. As observed from Table 4, the IGAIFCM technique achieves significantly better performance than other existing algorithms.

Table 4 Comparison table

S.No.	**References**	**Applied technique**	**Dataset**	**Reference model**			**IGAIMFCM_S**		
				OFV	**ICD**	**Acc. (%)**	**OFV**	**ICD**	**Acc. (%)**

1	Kennedy and Eberhart [39]	PSO	BCW	190.0230	1.1351	79.75	90.8552	20.5453	86.64
2	Bnadyopadhyay and Maulik [40]	KMVGA	Glass	35.4920	0.5126	86.45	0.4539	1.1854	87.38
3	Kanungo et al. [41]	K-means	Iris	78.9788	1.7972	88	10.8554	1.8256	92
4	Mehdizadeh et al. [42]	FPSO	Iris	60.5759	1.1289	90.67	10.8554	1.8256	92
5	Xiao et al. [43]	KMQGA	Glass	64.1060	0.4192	84.11	0.4539	1.1854	87.38
6	Manikandan and Selvarajan [44]	CS-PSO	Wine	97.1900	143.1262	------	25.5468	195.6524	75.84
7	Chen et al. [45]	HPSOFCM	Iris	96.6554	1.4526	90	10.8554	1.8256	92
8	Zhou et al. [14]	KIFCM	Seed	--------	2.6453	85	7.2987	3.5242	90.47
9	Liu et al. [46]	HRKM	Seed	570.9600	--------	81.34	7.2987	3.5242	90.47
10	Wu et al. [47]	AFCM	Iris	42.6873	--------	90.67	10.8554	1.8256	92
11	Wu et al. [47]	AFCM-SP	Iris	39.6817	0.9837	91.67	10.8554	1.8256	92
12	Parvathavarthini et al. [48]	IFPSO-IFCM	Haberman	6.9000	--------	61.27	5.9883	23.4863	78.73
13	Kumar et al. [49]	IABSFCM	Soybean	153.1980	--------	88.34	80.6528	4.3258	91.48
14	Sharma and Chhabra [50]	AHPSOM	Iris	96.6500	1.3827	90.67	10.8554	1.8256	92
15	Minxuam [37]	Improved FCM (2)	Iris	148.6813	1.3816	88.67	10.8554	1.8256	92

16	Seal et al. [38]	Improved FCM (1)	Glass	198.9438	--------	55.14	0.4539	1.1854	87.38
17	Kumar et al. [6]	AMFCM	Balance scale	166.2973	1.7863	74.24	140.5697	1.9972	78
18	Zhou et al. [14]	STA-KIFCM	Tae	--------	0.0110	95	29.1498	5.7624	97.35
19	Kumar et al. [1]	PSOIFCM	Seed	21.9716	3.5310	89.05	7.2987	3.5242	90.47
20	Hu et al. [51]	CWAFCM	Iris	20.6674	--------	90.67	10.8554	1.8256	92
Average				116.0922	11.5210	84.24	24.1432	14.0252	88.87

8. Conclusion

Fuzzy clustering is one of the most effective techniques for data analysis, among which the FCM algorithm is widely used due to its simplicity and efficiency. However, FCM still suffers from several limitations, such as high sensitivity to noise and initial centroids, as well as uncertainty in assigning membership values. To address these shortcomings, a hybrid fuzzy clustering technique integrating the IFCM and IGA under an intuitionistic fuzzy environment has been proposed. The main contributions of the proposed **IGAIFCM** technique using **Sugeno's negation function** are summarized as follows:

- A new metric is proposed and mathematically proven to be positive definite, symmetric, and compliant with the triangular inequality.
- The FCM is modified by incorporating the proposed metric to effectively handle noise in datasets.

- The improved genetic algorithm efficiently addresses the initialization issue of IFCM.
- The IGAIFCM technique employs Sugeno's negation function to calculate intuitionistic fuzzy membership values.
- The performance of the proposed IGAIFCM algorithm is validated using eleven benchmark real-world datasets, demonstrating superior results compared to existing techniques in the literature.

Hence, the proposed IGAIFCM technique provides an efficient and robust approach for forming accurate and reliable clusters. In future work, new methods will be explored for computing intuitionistic fuzzy memberships to handle uncertainty with greater precision.

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Green Electronics: Towards Sustainable and Eco-Friendly Devices

Pragati Garg

Department of Applied Science, Kunwar Satyavira College of Engineering and Management

Abstract

Electronics has become an inseparable part of our daily lives. From smartphones and computers to smart home appliances and industrial machines, electronic devices have made human life easier, faster, and more comfortable. However, behind these advancements lies a growing environmental problem. The manufacturing, usage, and disposal of electronic products produce harmful wastes and toxic materials, leading to serious pollution and resource depletion. The concept of Green Electronics has emerged as a promising solution to make electronic devices more sustainable, energy-efficient, and environment-friendly. This research paper explores the importance, principles, materials, technologies, and future directions of green electronics. It highlights how innovative approaches such as biodegradable materials, organic semiconductors, printed electronics, and renewable energy sources can help reduce e-waste and energy consumption. The paper also discusses the challenges faced in adopting these technologies and the possible strategies to overcome them. The aim is to create awareness among educators, researchers, and industries to adopt eco-friendly practices in electronic design and manufacturing. By promoting green electronics, we can move towards a cleaner environment, healthier ecosystems, and a sustainable technological future.

Keywords - Green Electronics, Sustainable Devices, Eco-friendly Materials, E-waste Management, Renewable Energy, Organic Semiconductors

1. Introduction

Electronics play a vital role in modern society. From communication and healthcare to transportation and education, every field depends on electronic systems. However, this rapid growth has also created a hidden environmental cost. Millions of tons of electronic waste, commonly called e-waste, are generated every year. Old mobile phones, computer parts, televisions, and batteries are often thrown away without proper recycling, leading to soil and water pollution.

Traditional electronic manufacturing uses materials like lead, mercury, cadmium, and brominated flame retardants. These substances are non-biodegradable and toxic to both humans and the environment. The production process also consumes large amounts of electricity and raw materials, contributing to global warming and resource depletion.

In this context, Green Electronics or Eco-friendly Electronics has gained significant attention. Green electronics aims to design, manufacture, and use electronic devices that minimize environmental harm. It promotes the use of recyclable, biodegradable, and low-toxic materials while ensuring energy efficiency throughout the product life cycle.

The goal of this paper is to provide a clear understanding of green electronics, its principles, and its applications. It also emphasizes the need for teachers, students, and industries to collaborate for a sustainable technological revolution.

2. Environmental Impact of Conventional Electronics

Traditional electronics have brought great convenience but also severe environmental challenges. Some of the main issues include:

2.1 E-waste Generation

Electronic waste is one of the fastest-growing types of waste in the world. According to the United Nations, more than 50 million tons of e-waste are produced every year globally, and only a small percentage is properly recycled. Most e-waste ends up in landfills or is burned, releasing toxic gases and heavy metals into the environment.

2.2 Toxic Materials

Conventional electronic components often contain harmful substances. For example: Lead in soldering can damage the nervous system. Mercury used in switches and lamps is poisonous to humans and animals. Cadmium in batteries and semiconductors contaminates soil and water.

2.3 Energy Consumption

Electronic devices consume a significant amount of energy during manufacturing and operation. The semiconductor fabrication process, in particular, requires ultra-pure water, chemicals, and electricity. This leads to a large carbon footprint.

2.4 Non-biodegradable Waste

Most traditional circuit boards and plastics do not decompose naturally. When dumped, they

remain in the environment for hundreds of years.

2.5 Global Challenge

Improper disposal practices are common in developing countries where electronic waste from richer nations is imported for manual recycling. This exposes workers to dangerous materials without proper safety measures.

Hence, there is a growing need for sustainable solutions in electronics — leading to the emergence of green electronics.

3. Principles of Green Electronics

Green electronics is not just about recycling old devices; it is about rethinking the entire process — from design to disposal. Its main principles are:

3.1 Eco-Design

Designing products with minimum environmental impact. This includes using fewer materials, avoiding toxic chemicals, and making products easy to disassemble for recycling.

3.2 Energy Efficiency

Devices should consume less power during operation and be designed for low standby energy loss. Energy-efficient circuits and renewable power sources should be encouraged.

3.3 Sustainable Materials

Replacing harmful materials with biodegradable or recyclable ones. For instance, using lead-free solder, organic polymers, and bio-based plastics.

3.4 Product Longevity

Encouraging products with longer lifespans reduces the frequency of replacements and, therefore, waste. Modular design helps users replace parts instead of discarding the whole device.

3.5 Waste Management

Proper collection, reuse, and recycling of e-waste ensure materials can re-enter the production cycle rather than harm the environment.

3.6 Education and Awareness

Promoting awareness among consumers, students, and manufacturers about eco-friendly practices plays a key role in achieving sustainability.

4. Green Materials and Technologies

Several new materials and technologies are being developed to support green electronics. Some of the most promising ones are described below.

4.1 Organic Semiconductors

Organic materials like carbon-based polymers and small molecules can conduct electricity and be used in devices such as organic light-emitting diodes (OLEDs) and organic solar cells. They are lightweight, flexible, and often non-toxic.

4.2 Biodegradable Substrates

Instead of using plastic or fiberglass circuit boards, researchers are exploring natural materials like paper, cellulose, or silk protein as substrates. These materials decompose safely after use.

4.3 Lead-Free Solder

Traditional solder uses tin and lead, which are toxic. Lead-free solders using tin, silver, and copper alloys are safer alternatives and are already used in many modern devices.

4.4 Printed Electronics

Printed electronics use conductive inks to print circuits directly onto flexible materials. This method reduces waste, lowers production cost, and consumes less energy.

4.5 Recyclable Plastics and Bioplastics

Conventional plastics used in electronics are non-degradable. Bioplastics made from renewable sources like corn starch or sugarcane can replace them, reducing dependence on petroleum-based materials.

4.6 Energy Harvesting Devices

Devices that can harvest energy from their surroundings (like solar cells, piezoelectric materials, or thermoelectric generators) reduce dependency on external power sources.

5. Case Studies and Real-World Examples

5.1 Biodegradable PCBs

Scientists have developed printed circuit boards made from plant-based materials that decompose naturally when discarded, reducing e-waste accumulation.

5.2 Organic LEDs (OLEDs)

OLED displays used in modern TVs and smartphones consume less energy and use organic compounds, making them more environmentally friendly than traditional LCDs.

5.3 Eco-Friendly Packaging

Some companies like HP and Dell use recycled paper and biodegradable packaging materials for their electronic products to minimize waste.

5.4 Solar-Powered Devices

Small solar-powered calculators, garden lamps, and chargers are simple examples of how renewable energy is used in electronics.

5.5 Green Data Centers

Many IT companies are now shifting towards "green data centers" that use renewable power, energy-efficient cooling systems, and recyclable components to reduce carbon footprints.

6. Role of Renewable Energy in Green Electronics

Green electronics also focuses on how devices are powered. Renewable energy sources such as solar, wind, and geothermal power can reduce dependency on fossil fuels.

6.1 Solar Energy

Solar panels convert sunlight into electricity and can be used to power homes, streetlights, and even portable gadgets. Integrating solar cells into electronic devices is becoming a new trend.

6.2 Energy Storage

Advancements in green batteries, such as lithium-iron-phosphate or sodium-ion batteries, are safer and more sustainable compared to traditional ones.

6.3 Smart Grids

Electronic devices connected to smart grids can monitor and optimize power usage, ensuring efficient distribution and minimal wastage.

7. Challenges in Green Electronics

- Despite its potential, green electronics still faces several challenges:
- Cost Factor: Eco-friendly materials and production methods are often more expensive.
- Performance Limitations: Some biodegradable materials have lower durability or conductivity compared to traditional materials.
- Lack of Awareness: Many consumers and industries are unaware of the importance of sustainable electronics.
- Recycling Infrastructure: In developing countries, proper recycling systems are still lacking.

- Standardization Issues: There are no universal standards or certifications for green electronic products yet.
- To overcome these barriers, government policies, industry collaborations, and public awareness campaigns are essential.

8. Future Prospects

- The future of green electronics is very promising. Some upcoming trends include:
- Nanotechnology: Use of Nanomaterials for energy-efficient and recyclable devices.
- AI-Driven Design: Artificial Intelligence can help optimize designs for minimal energy use.
- Circular Economy: Encouraging reuse and repair instead of disposal.
- Smart Materials: Development of self-healing or self-recycling materials.
- Education and Research: Introducing green electronics topics in school and college curricula to build awareness among students.
- As technology advances, more affordable and efficient green solutions will become mainstream, helping humanity achieve both technological and environmental goals.

9. Conclusion

Green electronics is not just a scientific innovation; it is a social responsibility. As the world becomes more dependent on electronic devices, it is crucial to ensure that progress does not come at the cost of nature. Adopting sustainable practices in design, manufacturing, and disposal can significantly reduce pollution, save energy, and protect future generations.

Teachers, students, researchers, and industries must work together to promote awareness and action. Simple steps like recycling e-waste, using energy-efficient devices, and supporting eco-friendly companies can make a huge difference.

By integrating environmental consciousness into technology, we can move towards a world where innovation and sustainability go hand in hand. The concept of green electronics truly reflects the idea that “technology should not harm nature but heal it.”

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Green Chemistry – Principles, Applications, and Future Perspectives

Harsh Rajput

Department of Applied Science

Kunwar Satyavira College of Engineering and Management, Bijnor.

Abstract

Green chemistry, also known as sustainable chemistry, represents an innovative approach to chemical research and engineering that aims to reduce or eliminate the use and generation of hazardous ssubstances.This paper explores the foundational principles of green chemistry, its role in fostering environmental sustainability, and the practical applications across diverse industries. It also examines case studies, recent advances, challenges, and future directions for integrating green chemistry in both academic research and industrial practices. One of the core ideas behind green chemistry is prevention. Rather than dealing with pollution after it has been created, green chemistry aims to design chemical products and processes that are inherently non-toxic and waste-free. This includes using safer solvents, minimizing energy use, and selecting raw materials that are renewable or biodegradable.

KEYWORDS: Green chemistry, Twelve Principles of Green chemistry, Applications, Case studies, Future directions.

1. Introduction

The environmental impact of traditional chemical processes has led to significant concerns over pollution, resource depletion, and ecological damage. Green chemistry emerged in the 1990s as a proactive approach to design safer chemicals and processes from the outset. Unlike environmental chemistry, which focuses on

managing pollutants post-release, green chemistry aims to prevent pollution at its source. This paper examines the fundamental concepts, guiding principles, and significance of green chemistry in promoting sustainable development. Green chemistry,also known as sustain able chemistry ,is a modern and innovative approach to chemical science that emphasizes the design of products and processes that minimize or eliminate the use and generation of hazardous substances. Unlike traditional chemistry, which often focuses solely on the function and efficiency of chemical reactions, green chemistry prioritizes environmental protection, human health, and sustainability alongside performance and productivity.

The concept of green chemistry emerged in the 1990s in response to growing concerns over pollution, chemical waste, and the environmental damage caused by industrial and laboratory processes. The U.S. Environmental Protection Agency (EPA) and scientists like Paul Anastas and John Warner played a major role in defining the framework of green chemistry through the introduction of the 12 Principles of Green Chemistry. These principles guide chemists in designing safer chemicals, using renewable feedstock, improving energy efficiency, and reducing waste at the source.

Green chemistry is not limited to a single sector; its applications span across pharmaceuticals, agriculture, energy, textiles, plastics, and even cosmetics. For example, in the pharmaceutical industry, green chemistry helps reduce the use of toxic solvents and increases yield through efficient reactions. In agriculture, it promotes the use of bio-based pesticides and fertilizers. In the energy sector, green chemistry contributesto the development of biofuels and cleaner battery technologies.

As global environmental challenges intensify—such as climate change, pollution, and resource depletion—green chemistry offers a proactive solution that integrates

scientific innovation with environmental responsibility. It represents a shift toward a more sustainable way of thinking about chemistry, where the goal is not only to create effective products but also to ensure they are safe for both people and the planet. In summary, green chemistry is a vital field for building a sustainable future. By rethinking how chemicals are designed and used, it provides an essential path toward reducing environmental harm while supporting technological and economic growth.

2. The Twelve Principles of Green Chemistry

Paul Anastas and John Warner developed twelve guiding principles of green chemistry that serve as the framework for environmentally responsible chemical design. These include:

- **Prevention** – Avoid waste rather than treating or cleaning it up.
- **Atom Economy** – Maximize incorporation of all materials into the final product.
- **Less Hazardous Chemical Syntheses**
- **Designing Safer Chemicals**
- **Safer Solvents and Auxiliaries**
- **Design for Energy Efficiency**
- **Use of Renewable Feedstocks**
- **Reduce Derivatives**
- **Catalysis** – Prefer catalytic reagents over stoichiometric ones.
- **Design for Degradation**
- **Real-time Analysis for Pollution Prevention**
- **Inherently Safer Chemistry for Accident Prevention**

3. **Importance of Green Chemistry:** Green chemistry, also known as sustain able chemistry ,is a vital field that focuses on designing products and processes that minimize the use and generation of hazardous substances. Its importance lies in its proactive approach to preventing pollution and promoting sustainability rather than treating problems after they occur.

Green chemistry addresses several critical challenges:

- **Environmental Protection**: Minimizes toxic waste and emissions.
- **Public Health**: Reduces human exposure to carcinogens and endocrine disruptors.
- **Economic Benefits**: Enhances efficiency and reduces cost of waste disposal.
- **Regulatory Compliance**: Supports industries in meeting environmental regulations.

4. **Applications in Industry**

Pharmaceuticals: Green chemistry has revolutionized the pharmaceutical industry by promoting safer, more sustainable, and cost- effective methods for drug development and manufacturing. Its principles are applied throughout the drug life cycle—from synthesis to disposal—ensuring minimal environmental impact and improved health outcomes.

- Development of greener synthetic pathways (e.g., Pfizer's green synthesis of sertraline).
- Use of biocatalysts and enzyme catalysis.

Agriculture: Green chemistry plays a crucial role in making agricultural practices more sustainable, safe, and environmentally friendly. By applying the principles of green chemistry, farmers, scientists, and agrochemical companies can reduce pollution, protect ecosystems, and improve food safety, all while maintaining or enhancing crop yields.

- Green pesticides and biodegradable agrochemicals.
- Reduced reliance on harmful solvents.

Textile and Dyeing: Green chemistry helps reduce the use and creation of toxic substances, which makes it especially valuable in toxicology — the study of harmful effects of chemicals on living organisms .and The dyeing and textile industry is a major polluter, knownfor heavy water use, toxic dyes, and waste. Green chemistry offers more sustainable alternatives.

- Waterless dyeing using supercritical CO_2.
- Biodegradable dyes.

Energy Sector: Green chemistry plays a vital role in making energy production and consumption more sustainable, efficient, and less harmful to the environment. It helps reduce the use of fossil fuels, lowers greenhouse gas emissions, and promotes cleaner technologies for a greener future.

- Biofuels and sustainable hydrogen production.
- Use of green solvents in battery production.

Plastics and Polymers: The traditional production and disposal of plastics cause significant environmental pollution, including microplastic

contamination and greenhouse gas emissions. Green chemistry offers innovative, sustainable solutions for creating and managing plastics and polymers in eco-friendly ways.

- Bioplastics from renewable resources.
- Degradable and compostable materials.

5. Case Studies

Warner-Babcock Institute

The Warner-Babcock Institute for Green Chemistry exemplifies innovation by creating environmentally friendly formulations for consumer products.Their work includes the development of hairdyes free from toxic compounds like ammonia and PPD, as well as anti-agingcreams with enhanced skin compatibility and flame retardants that are non-toxic, biodegradable, and free of halogens. These advancements demonstrate how green chemistry can align product performance with environmental safety.

Dow Chemical

Dow Chemical revolutionized the production of propylene oxide—a key industrial chemical—by developing a cleaner process that uses hydrogen peroxide instead of traditional chlorinated solvents. This HPPO (Hydrogen Peroxide to Propylene Oxide) process significantly reduces wastewater, eliminates co-products, and enhances atom economy. It is safer, more energy-efficient, and aligned with multiple green chemistry principles, including the prevention of waste and the use of safer solvents.

Merck

Merck's redesign of the catalytic process for synthesizing Sitagliptin (the active ingredient in the diabetes drug Januvia®) is a landmark in pharmaceutical green chemistry. By replacing a high-pressure rhodium-catalyzed step with an enzymatic biocatalyst, Merck reducedhazardous waste, increased yield, and eliminated the need for heavy metals. This effort won the Presidential Green Chemistry Challenge Award in 2010.

Pfizer

Pfizer applied green chemistry in the synthesis of Sertraline (Zoloft®), an antidepressant. They modified the synthetic route to include a crystallization-induced diastereomer transformation (CIDT**)**, which reduced the number of purification steps and improved overall yield. This change cut the use of solvents and reagents, minimized waste, and reduced production time, showcasing the economic and environmental benefits of process intensification.

Anastas-PoliakoffCO_2SolventSystem

Researchers John Warner and Paul Anastas, in collaboration with Martyn Poliakoff, developed supercritical carbon dioxide as an alternative solvent system. Used in applications such as dry cleaning and polymer production, supercritical CO_2 replaces volatile organic solvents, reducing toxic emissions and flammability hazards. This breakthrough not only reduces environmental harm but also improves safety and recyclability.

BASF – Water-Based Automotive Coatings

BASF introduced waterborne automotive coatings to replace traditional solvent-based paints, which are major sources of volatile organic compound (VOC) emissions. These coatings maintain high durability and color quality while significantly reducing VOC release. This innovation aligns with green chemistry's principle of designing safer chemicals and processes.

DuPont – Bio-based Polymer (Sorona®)

DuPont developed Sorona®**,** a bio-based polymer derived partially from corn glucose instead of petroleum. Used in textiles and carpets, Sorona® has a lower carbon footprint and improved properties like softness and stain resistance. The process illustrates the shift toward renewable feedstocks and energy-efficient production.

6. **ChallengesandLimitations:** While green chemistry of fer ssustain able solution store due environmental impact, it also faces several challenges and limitations that hinder its widespread adoption.

One major challenge is economic feasibility. Green processes and materials, especially in early stages, can be more expensive than conventional alternatives. Industries may hesitate to invest in new technologies due to high initial costs and uncertain returns. Additionally**s,** technical limitation exist, such as the lack of efficient green alternatives for some industrial chemicals, reactions, or solvents.

Limited awareness and training among chemists and engineers also slow progress. Many professionals are not fully educated in green chemistry principles, leading to resistance in changing long-established methods. Furthermore, regulatory gaps and lack of strong policies in some regions reduce incentives for adopting greener

practices.

There are also performance concerns; some bio-based or green materials may not match the durability, strength, or stability of traditional products. Finally, scalability remains a hurdle—what works in a lab may not always be feasible at an industrial level.

Despite these challenges, continuous innovation, better education, and supportive regulations can help overcome these barriers and makegreen chemistry a practical and essential part of modern science and industry.

- **Economic Barriers**: Initial R&D costs and transition challenges.

- **Knowledge Gaps**: Limited access to greener alternatives for all chemical processes.
- **Policy and Incentives**: Need for stronger governmental support.
- **Scalability**: Laboratory-scale methods don't always translate to industry-scale production.

7. **Future Directions:** Green chemistry is poised to play an increasingly vital role in shaping a sustainable future. As global environmental concerns grow and industries seek cleaner technologies, the future of green chemistry will focus on innovation, efficiency, and large-scale impact.

One major direction is the development of renewable feedstocks**.** Future green chemistry will rely heavily on biomass, agricultural waste, algae, and other bio-based sources to replace fossil fuels in the production of chemicals, plastics, and fuels. These alternatives not only reduce carbon emissions but also promote circular

resource use.

Catalysis will continue to evolve, with a focus on enzyme-based and metal-free catalysts that operate under mild conditions. These catalysts will enable cleaner, energy-efficient reactions with high selectivity and minimal waste. Artificial intelligence and machine learning will alsobe integrated to design safer molecules and optimize synthetic routes.

Another key area is green solvents and solvent-free systems, such as supercritical CO_2, water-based processes, and ionic liquids, which minimize toxicity and waste. Continuous flow chemistry and microwave-assisted reactions will improve energy efficiency and scalability.

The future also includes smart materials and green nanotechnology, allowing the creation of biodegradable, self-healing, and functional materials with minimal environmental impact. Green chemistry in education will become essential, ensuring future scientists are equippedwith the knowledge to prioritize sustainability from the start.

Lastly**,** policy support and global collaboration will be critical. Governments, industries, and academic institutions must work together to set standards, fund research, and promote environmentally responsible practices.

In summary, the future of green chemistry lies in innovation, interdisciplinary collaboration, and sustainability-driven design. By advancing greener alternatives across all sectors—pharmaceuticals, agriculture, energy, and manufacturing—green chemistry will be central to achieving a cleaner, healthier, and more sustainable world.

- **Green Chemistry Education**: Integrating into curricula globally.
- **AI and Machine Learning**: Designing safer molecules computationally.
- **Circular Economy Models**: Encouraging reuse, recycling, and lifecycle thinking.
- **Green Nanotechnology**: Developing sustainable nanoscale materials.

8. Conclusion

Green chemistry is not merely a scientific discipline but a transformative philosophy that influences how chemicals are designed, synthesized, and utilized. While challenges persist, the potential for long-term environmental and economic benefits makes green chemistry an indispensable tool in achieving global sustainability goals. Through innovation, interdisciplinary collaboration, and education, the widespread adoption of green chemistry practices can shape a cleaner and safer future. Green chemistry represents a transformative approach to the way chemicals and chemical processes are designed, produced, and used.It is not just a scientific discipline but a comprehensive philosophy aimed at reducing or eliminating the use and generation of hazardous substances. In an era where environmental degradation, climate change, and resource depletion pose serious threats to human health and global ecosystems, green chemistry stands out as a key solution for achieving sustainability across industries.

At its core, green chemistry is preventive rather than corrective. Traditional chemistry often focuses on treating the harmful effects ofchemical use after they have occurred—such as pollution control or waste treatment—whereas green chemistry seeks to prevent these problems at the source. This is done by rethinking chemical synthesis to maximize atom economy, minimize toxic inputs, and reduce energy consumption. By designing processes that are cleaner, safer, and more efficient,

green chemistry contributes to a future that is both environmentally responsible and economically viable.

One of the most important contributions of green chemistry is in reducing pollution. Whether in agriculture, pharmaceuticals, plastics, or energy production, green chemistry offer salter natives thatarelessharmful to the environment and human health.Biodegradable plastics,non- toxic dyes, renewable biofuels, and safer pharmaceuticals are all outcomes of applying green chemistry principles. These innovations not only lessen environmental impact but also lead to safer products and healthier lives.

Furthermore, green chemistry contributes significantly to economic benefits. It reduces costs associated with waste treatment, energy consumption, and raw material sourcing. By improving process efficiency and encouraging the use of renewable feedstocks, industries can achieve better profit margins while aligning with environmental regulations and social responsibility goals.

However, despite its many benefits, the full potential of green chemistry is still being realized. Challenges such as high initial costs, limited awareness, technical constraints, and lack of policy support have slowed its widespread adoption. Nevertheless, ongoing research, innovation, and education are steadily overcoming these barriers. Increased collaboration among governments, industries, and academic institutions is further accelerating the development of sustainable technologies.

Education and training are particularly critical for the future of green chemistry.Equipping future scientists, engineers, and policy makers with the knowledge and skills to implement green principles are essential for long-term progress. Curricula at all levels must integrate sustainability and green chemistry concepts to inspire a new generation of environmentally conscious professionals.

In conclusion, green chemistry is more than just a scientific advancement—it is a necessary shift toward a sustainable and responsible future.It promotes innovation without compromise: creating new materials and processes that are safe, efficient, and environmentally benign. As the world continues to grapple with the consequences of industrial pollution and climate change, green chemistry provides a hopeful path forward—one that balances the needs of society, the economy, and the environment. By embracing green chemistry, we invest not only in scientific progress but also in the well-being of future generations and the health of our planet.

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Adaptive Inventory Control with Hybrid Game Theory Models for International Supply Chains

Neha Bharadwaj

Dr. Riddhi Garg

Head of Mathematics Department, IFTM University

Abstract

This study for custom proposing hybrid game-the oretic model that adapts to supply chain disruptions (e.g., natural disasters, political instability), focusing on international inventory management. Inventory control is critical component for supply chain managemen twhich influence operational cost, service level sandal profitability. Traditional inventory management objects such as Economic Order Quantity (EOQ), Just-in- Time (JIT) often fail for account the strategic interactions and interdependencies that arise in modern multi-actor supply chains. This research paper explores the optimization inventory control through the application of game theory, a mathematical framework that models competitive and cooperative inter actions between rational decision-makers. The study focuses on two primary game-theoretic models: the Nash Equilibrium for non-cooperative scenarios and the Stackel berg game for hierarchical leader- follower relationships. Adaptive game theory is also investigated to an analyze cost-sharing agreements that enhance overall supply chain efficiency. Using a two-echelon supply chain simulation involving manufacturers and retailers, the research evaluates the impact of game theory on inventory decisions under varying levels of demand uncertainty and market dynamics.

Introduction

Inventory control is essential aspect of encompassing strategies, supply chain

management and process which involve in regulating stock level of meet customer demand when minimize cost associate with holding, ordering, and stock outs. In today's competitive and interconnected business environment, achieving efficient inventory control is critical for maintaining operational efficiency, ensuring Customer satisfaction and maximizing profit ability. However, traditional inventory models often operate under the assumption of independent decision-making, overlooking the complex interactions and interdependencies which characterize multi-actor supply chains. The globalization of markets and advancements in technology has added layers of complexity to inventory management. Supply chains now frequently involve multi stakeholder who include retailer, manufacturer, distributor and third-party logistics provide reach with distinct objective and constraints. These actors often engage with strategic interaction where decisions of any party influences and influenced by the action of others. This inter dependence creates challenges such as conflicting demand uncertainty, objectives and risk misalignment which lead in efficiencies in inventory control if it not properly addressed. Game theory a mathematics framework which done by model strategic interaction between rational decision-makers which offer powerful tool for tackling all these challenges. With analyzing scenario here participant's decisions are interdependent. Game theory enables development of inventory policy whose account for both cooperative behavior and competitive among supply chain actors. Let an example a retailer decision on order quantity may depend on manufacturer pricing strategy on other hand a supplier's inventory policy can be influenced on demand forecasts from multiple retailers. Game theory provides insights into such scenarios, enabling stakeholders to optimize their decisions and achieve better outcomes for the supply chain whole. On current search investigate application for game theory to inventory control with focus on two primary models first one Nash Equilibrium for non-cooperative setting and second one is Stackel berg game for hierarchical leader-

follower relationships. In non-cooperative scenarios stakeholders act independently to maximize their individual utility often resulting in suboptimal outcomes for the supply chain. The Nash Equilibrium offer way to predict stable strategies for such competitive environments. Conversely Stackel berg model addresses hierarchical relationships such as these between manufacturers and retailers, where one party (the leader) makes a decision that influences the subsequent actions of the other party (the follower).This study explores cooperative game theory which examines scenario where stakeholders collaborate with minimize costs and share risks. On other hand Cooperative strategies such as cost-sharing agreements and joint replenishment policy have show promise for enhancing supply chain efficiency particularly for Environments characterized by high demand uncertainty and influence market conditions. This research paper aims bridge for gap between traditional inventory control models and the strategic complexities for modern supply chains. In incorporating game-theory principle into inventory management. This research provides a comprehensive framework which optimizes stock levels improve coordination and aligning objectives of diverse supply chain actors. The findings have significant implications for which business seeking for enhances supply chain resilience and competitive in dynamic market. Remainder of current paper is organize as follows which are view of relevant literature on inventory control and game theory detailed explanation of methodology and models use an analys is the result through simulations and case study a discussion of practical implications, future research direction.

Literature Review

Integration of game theory with inventory control has garnered significant attention like supply chain become increasingly complex and interconnects. Here literature review examines the evolution of inventory controls method and application of game theory in supply chain management and gaps in existing research which necessitate

further exploration**.**

Traditional Inventory Control Models

Inventory control has traditionally been guided for models such as the Economic Order Quantity (EOQ) model and Newsvendor model and Just-in-Time (JIT) systems. Such models primarily focus for minimizing holding ordering and shortage cost by optimize replenishment policy. For instance EOQ give straightforward formula which determines optimal order quantity which minimizes total costs in static demand environment. Similarly Newsvendor model address inventory decision under demand uncertainty balance cost of excess inventory against cost of stock out. JIT emphasizes lean inventory management for aligning production schedule with real-time demand for reduces waste and holding costs. While in these model has proven effective for isolated scenario and they often assume that decision is made independently now neglecting interdependencies among supply chain actor. This limitation has led to inefficiency for multi-actor environments where actions of one stakeholder invariably affect outcomes of other. Example a retailer decision for overstock may result in excessive production by manufacturer leading for increased cost across supply chain. Recognize all these shortcomings researcher has sought to incorporate strategic interaction into inventory control framework.

Game Theory in Supply Chain Management

Game theory introduces by von Neumann and Morgenstern in the 1940 provide robustframeworkformodellingandanalysingstrategicinteractionbetweenrational decision-maker. In this application supply chain management have expand significance over past two decade address challenge such as demand uncertainty pricing strategy and inventory replenishment.

Non-Cooperative Game Theory Models

Non-cooperative game theory model many focus on competitive scenario where every actor seeks maximize his utility independently. Nash equilibrium key concept in non-cooperative game represent a state where no player can improve their payoff for unilaterally changing their strategy. In the inventory control Nash equilibrium has been used to model situations where retailer competes for market share with optimize order quantity and pricing strategy.

Cooperative Game Theory Models

The Cooperative game theory emphasize collaboration between stakeholders for achieve mutually beneficial outcomes. In context of inventory control cooperative models often focus cost-sharing agreement joint replenishment policy and collaborative fore casting. Research of Lengand Parlar (2005) highlights potential of cooperative strategy to reduce supply chain cost by aligning objectives sharing risks among actors.

Hybrid Approaches

Currently study has explored hybrid model which combine element for cooperative and non-cooperative game theory which address limitations of individual approaches. For instance researcher has proposed multi-stage game that incorporates cooperative strategies in the initial stages of inventory planning follow by competitive strategy during execution. This approach allows supply chain actor to align their objective while maintaining flexibility to adapt for market dynamics.

Research Gaps and Opportunities

Despite growing body of research with game-theoretic inventory models several gaps remain. Many existing study assume staticdem and pattern, perfect information, which are rarely observe in real-world scenario. Here is a need for models that account of dynamic demand, asymmetric information, and impact of external factor like as market trends and disruption. Additionally integration of advanced technologies like artificial intelligence and block chain with game-theory models present a exciting avenue for future research.

Conclusion

Literature highlight potential of game theory to revolutionize inventory control of address strategic inters dependence among supply chain actors. Non-cooperative, cooperative and Stackel berg models every offer unique insight into inventory decision-making while hybrid approach provides a balanced framework of optimizes supply chain performance. Further research needs overcome practical implementation challenge and enhance the applicability of game-theoretic models in dynamic real-world environments

Methodology

This section outlines framework use investigate optimization of inventory control use game theory. Methodology integrates mathematical modelling, simulation and analysis the evaluate strategic interaction between supply chain stakeholders.

Framework and Assumptions

The study base on following assumptions:

1. Supply Chain Structure: Two-echelon supply chain with onemanufacturer and

multiple retailers.

2. Rationality: All players arerationaldecision-makerseekingthemaximize their individual payoffs (profit, utility).
3. Demand Distribution: Customer demand follows probabilistic distribution with known parameter.
4. Information Sharing: Level of information sharing depend on gamemodel (limited for non-cooperative games extensive in cooperative games).
5. Costs: Costcomponentsincludeorderingcostsholding costsandshortage costs for retailers, production and inventory costs for manufacturer.

6. Strategies: Retailer decide order quantity,while manufacturer determine wholesale pricing and production level.

Game-Theoretic Models

Two game-theoretic models are utilized to examine inventory control strategies:

Nash Equilibrium Model (Non-CooperativeGame):

This model retailers operateind ependently each optimize their order quantity based on demand forecast and cost structure.

Cooperative Game Model:

Stakeholder collaborate minimize total supply chain cost through joint replenishment and cost-sharing agreement.

Mathematical Formulation

Non-Cooperative Game (NashEquilibrium)
Each retailer is seeks to maximize their pay
off Ui: Ui=Ri (Qi) −Ci (Qi)

Where:

Ri (Qi) is the revenue function based on orderquantity.

Ci(Qi) represents costs,including ordering,holding,and short age costs. The Nash equilibrium is achieved when:

$\partial Ui/\partial Qi=0$for alli.

Cooperative Game (Cost-SharingModel) The total cost TC

$$TC=\sum i=1n(Ci(Qi)+Cm(Qm)),\ TC=i=1\sum n(Ci(Qi)+Cm(Qm)),$$

Where Cm(Qm) is the manufacturer cost. The Shapley value is applied to allocate costs:

$$\phi i=\sum[[|S|!(|N|-|S|-1)!]/|N|!][v(S\cup\{i\})-v(S)].$$

SimulationDesign

Models are test educing with simulation involving supply chain for fast-moving consumer goods (FMCG):

Demand Data: Historical demand data is used fort simulate demand variability.
ScenarioAnalysis:
Non-cooperative: Retailer optimize independently.
Cooperative: Retailer and manufacturer collaborate on cost-sharing.
Stackelberg: Manufacturer sets prices and retailers respond withorder quantity.

The simulation evaluate performance metric such as

- Total costs (ordering, holding).

- Service levels (percentage of demand).
- Profit ability of individual player and overall

supply chain. Performance Metrics

Key performance indicator (KPI) use for comparison includes:

1. Tota lCost Reduction: Ability of each model for minimize overall supply chain cost.
2. Equity: Fair distribution of cost profit among stakeholder (measure use Shapley value).
3. Efficiency: Improvement for service level and inventory turn over rate.
4.

AnalyticalTools

Mathematical model and simulation is implementing. Graphs, diagram are generate for visualize inventory level, cost distribution and strategic interaction. Result is validate by comparing simulate outcome with theoretical prediction.

Results and Analysis

This section presents the outcomes of applying game-theoretic models to the inventory control problem in a two-echelon supply chain. The results are analyzed across three scenarios: the Nash equilibrium model (non-cooperative), the cooperative game model, and the Stackelberg game model (leader-follower). Performance metrics such as total supply chain costs, individual payoffs, service levels, and cost-sharing efficiency are examined, with graphs and diagrams illustrating the findings.

Results from the Nash Equilibrium Model (Non-Cooperative Scenario): The non- cooperative

scenarior retailer operates independently for optimize their order quantity, considering their respective demand forecast cost sand actions of competing retailer

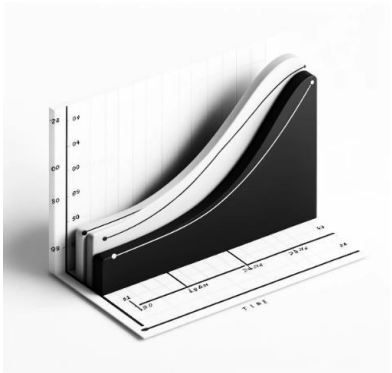
KeyFindings:

Inventory Levels: Retailer inventory decision under Nash equilibrium led to suboptimal outcome for supply chain. Each retailer focus minimizing it own cost resulting in instances of overstock for one retailer and stockouts for another.

Total Costs: Total supply chain cost was higher compare cooperative and Stackelberg model due to inefficient caused by lack of coordination.

Service Level: Variability inservice level was observe with retailer achieving 90% service level while another dropped with 70% indicating uneven demand fulfilments.

Graph1:Retailer Inventory Levels Under Nash Equilibrium



Results from the Cooperative GameModel

The cooperative scenario retailer and manufacturer collaborate to minimize total supply chain cost and share savings equitably using the Shapley value.

KeyFindings:

Cost Reduction: Cooperative strategy led 25% reduction for totalsupplychaincost compare the Nash equilibrium model.

ServiceLevel: Service level improves significantly with retailer achieving over 95% demand fulfilments.

Cost-Sharing: The Shapley value ensured fair distribution for joint savings.

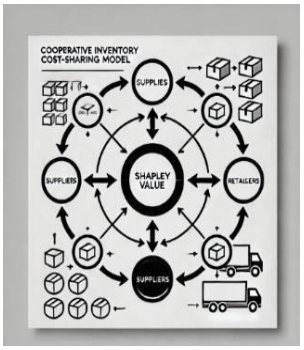


Diagram1: Cooperative InventoryCost-SharingModel

Analysis:

Cooperative game model demonstrate potential for significant cost saving and improve service level through collaboration. However success of these model depend son trust transparency and effective mechanism forcost allocation. Shapley value was instrumental achieving fairness encouraging stakeholder the participate in cooperative framework.

Table1: Performance Metrics across Models

Metric	NashEquilibrium	CooperativeGame

TotalSupplyChainCost	High	Lowest
ServiceLevels	Uneven (70–90%)	High(>95%)
ManufacturerProfit	Moderate	High
RetailerProfit	Moderate	High
Feasibility	High	Moderate

InsightsandPracticalImplications

Strategic Decision-Making:

Non-cooperative models are suitable for competitive environment.
Cooperative model work when trusta nd transparency facilitates collaboration.

Operational Efficiency:

Collaborate drive significant cost saving which improve service level but practical challenge such as information sharing and trust must be address.Leader follower dynamic provide a middle ground achieving coordination without require full cooperation.

ConclusionofResults

Results demonstrate applying game-theoretic model for inventory control provide valuableinsightintostrategicdecision-making.Whenevercooperativestrategyyield most efficient outcome. Non-cooperative models though less efficient remain relevant for competitive scenarios. They find has significant implication for design inventory policy which balance individual and collective goal.

Discussion

Finding this research highlight transformative potential of game theory for optimizing inventory control with supplychain. Which address strategic interaction game-theoretic model offer nuance insight into decision-making process with their impact on overall supply chain performance. Each model explored—Nash equilibrium, cooperative games.

The Nash equilibrium model exemplifies challenges of non-cooperative behaviour where independent decision-making often lead to inefficiency like as higher total cost and uneven service level. These outcome underscore importance of coordination among supply chain actor as individual optimization frequently come at expense of overall efficiencies.Conversely cooperative game modeldemonstratesignificantcostsavingandservice level improvement achieve through collaboration. With leverage cost sharing mechanism like Shapley value can equitably distribute benefit fostering trust and long-term partnership.

FutureScope

Futurere search shall explore hybrid model integrate element of competition and co-operate with address complexities of modern supply chain.In-corporative real-time data and AI-driven decision-making and block chain for transparent collaboration can enhance applicability of game the oretic approaches.This advancement will help business adapt the dynamic demand ,mitigate risks and improve overall supplychain

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Linear Programming Methods for Transportation Problems

Anmol Kumar Rajput, Subham Rajput, Nitish Kumar

Department of Applied Science, Kunwar Satyavira College of Engineering and Management, Bijnor

Abstract

Transportation problems are a specific class of Linear Programming Problems (LPP) that focus on determining the most efficient way to distribute goods from multiple sources to multipledestinationsatminimumcost.Thisthesisinvestigatestheformulationandsolution of transportation problems using linear programming methods such as the Northwest Corner Rule, Least Cost Method, Vogel's Approximation Method, and the Modified Distribution (MODI) Method. The study emphasizes the optimization of resources, computational efficiency, and practical applications in logistics and supply chain management. Computational results demonstrate the effectiveness of the MODI method in achieving optimal solutions faster than traditional methods.

Keywords: Linear Programming, Transportation Problem, Optimization, MODI Method, Cost Minimization.

1. IntroductionBackground of the Study

Efficient allocation of limited resources is a key concern in operations research. The transportation problem is a fundamental model in linear programming that helps minimize the cost of distributing a product from several suppliers to various consumers. It finds wide applications in business, logistics, and industrial systems.

Problem Statement

Given multiple origins with known supply capacities and multiple destinations with specific demands, the challenge is to determine an optimal distribution plan that minimizes the total transportation cost while meeting supply and demand constraints.

Objectives

1. To formulate transportation problems as Linear Programming Problems.
2. To compare the efficiency of various methods for finding initial feasible and optimal solutions.
3. To analyze cost efficiency and computational effectiveness using sample data.

Scope and Applications

Transportation problems have applications in

1. Supply chain optimization
2. Resource allocation
3. Logistics management
4. Airline scheduling and shipping networks

2. Literature Review

This chapter summarizes the contributions of past researchers on transportation problems linear programming problem.

1. Hitchcock (1941) first presented the transportation problem as a special type of linear programming problem.
2. Danzig (1947) introduced the Simplex Method, forming the foundation for

modernoptimization.

3. Vogel (1958) proposed the Vogel's Approximation Method (VAM) to improve initial feasible solutions.Recent studies highlight th ecomputational superiority of the MODI (Modified Distribution) method for obtaining optimal solutions efficiently.

The literature reveals that transportation problems remain an active research area due to their real-world relevance and potential integration with computer algorithms and machine learning optimization.

3. Methodology

Mathematical Formulation

Let m=number of sources, n=number of destinations,cij=cost of transport in gone unit from source i to destination j, ai = supply at source i, bj = demand at destination j, and

xij = quantity transported from source i to destination j.

Objective Function MinimizeZ=ΣΣcij*xij Subject to constraints:

Σxij=aifo

ralli

Σxij=bjfo

rallj xij ≥

0

Methods Used

1. Northwest Corner Rule (NWC) – Simple, provides an initial feasible solution.

2. Least Cost Method (LCM) – Chooses the lowest cost cell first.

3. Vogel's Approximation Method (VAM)–

Balancespenaltycostsforbetterinitial solutions.

4. MODI Method – Used to test and improve the solution until it becomes optimal.

Tools and Software

MS Excel Solver and Python (PuLP) for computation; manual calculation for small data sets.

Data Analysis and Result

Example problem:

Source/Destination data table:

S1:D1=2,D2=3,D3=1,

Supply=20

S2:D1=5,D2=4,D3=8,

Supply=30

S3:D1=5,D2=6,D3=8,

Supply=25 Demands:

D1=10, D2=35,

D3=30

Total costs obtained:

NorthwestCornerMethod=355

Least Cost Method = 315

Vogel'sApproximationMethod=300

MODI Method (Optimal) = 295

Interpretation: The MODI method gives the minimum transportation cost and is computationally efficient.

Discussion and Conclusion

Findings

The MODI method consistently provides the optimal or near-optimal solution. VAM gives better initial feasible solutions than NWC or LCM. Linear programming effectively reduces total transportation cost.

Conclusion

Linear Programming provides a systematic and efficient approach to solving transportation problems. The integration of modern computational tools enhances accuracy and efficiency, making these methods indispensable in logistics and supply chain management.

Future Work

Future work can focus on the application of fuzzy linear programming for uncertain costs,

Integration with AI-based optimization, and automation using Python and cloud computing.

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Predictive Role of Parenting Styles and Peer Dynamicsin Adolescents Decision-Making Ability

Manpreet Kaur & Amandeep Kaur

Khalsa College of Education, Amritsar, Punjab, India.

Abstract

Decision may be big or small, important or less important; it significantly influencesone's life and need to be carefully carried out.Along with other skills that an adolescent requires to develop, the skill of good decision-making is very essential, because many important and essential decisions taken at this stage of life, lasts for a long time and have a significant impact on their future. But now the question arises, whom to depend on or seek help, while making a decision? Especially, in the case of adolescents, they are not mature enough to make decisions independently. Inside the home and outside it, are the two social circles to which adolescents come across. Parents are the first social circle with whom every child interacts and come across, but as they enter adolescence, they start being more social outside the home. They are highly impressed by the peers of the same group. Hence, researcher has identified that parenting styles and peer pressure are two major factors that plays a significant role in making particular decision of their life among adolescent students. So, this study is an attempt to explore parenting styles and peer pressure as predictors of decision-making ability of adolescent students.For this purpose, a sample of 200 senior secondary school student including 100 boys and 100 girls were taken. Decisions Making Scale (Mincemoyer, Perkins & Munyua, 2001), Parenting Style Scale (Gafoor & Kurukkam, 2014) and Peer Pressure Scale-Revised (Singh & Saini, 2016) were administered on the sample. The results of correlation analyses revealed Parenting

styles (namely parental responsiveness and parental control) peer pressure and decision-making ability of adolescent students are significantly correlated with each other. Regression analysis shows parenting styles (namely father's responsiveness, mother's responsiveness, parental esponsiveness, father's control, mother's control and parental control) positively and significantly predict decision-making ability among adolescent students. Peer Pressure is significantly and negatively predicting Decision-Making Ability of Adolescent students.

Keywords: **Decision-Making Ability, Parental Responsiveness, Parental Control, Peer Pressure**

INTRODUCTION

Adolescence is a developmental stage between childhood and adulthood. This stage encompasses many social, emotional, intellectual and physical changes that create both opportunities and challenges for an adolescent, his/her family, friends, peers, society, etc. The experiences gained by an individual during the adolescent period results in personality shaping and are seen in the latter periods of life. Therefore, proffering opportunities to develop social competence and sense of responsibility towards self, parents, friends and other members of the society is very essential for them. Providing opportunities and experiences to an adolescent to cope with the real-life challenges, and solve problems related to their daily life, will bring many essential and important cognitive developments in them.

During adolescence period many cognitive abilities like decision-making, judgment, planning, thinking, etc.ares till developing.Decision-making ability is one of them important cognitive ability that is required in everyday life. Decision may be big or small, important or less important;it significantly influences one's life and need to be carefully carried out. Along with other skills that an adolescent requires to develop,

the skill of good decision-making is very essential, because many important and essential decisions taken at this stage of life,lasts for along time and have a significant impact on their future. A good decision-making ability can change the life of an individual constructively; on the other hand a wrong decision can destroy the life completely.

But now the question arises, whom to depend on or seek help, while making a decision? Especially, in the case of adolescents, they are not mature enough to make decisions independently. They often rely on others for their decisions. They seek help from their parents, teachers, peers and friends to make decision, like choosing a career, course, particular game as co-curricular activities etc. This sometimes results in good decisions while sometimes results in bad decisions, depending on the source they are relying to seek guidance to make decision. They are often seen taking wrong, impulsive and immature decisions. Even they rely mostly on their peers or friends while making decisions, in order to get their conformity or feeling easy to share their feeling, emotions and actions with peers as compared to their parents. This sometimes increase the rate of making a wrong decision, as their peers are also not mature or experienced enough for making decisions as compared to their parents. Hence, decision-making ability plays a very important role in the daily lives of the adolescents. Parent and peer are the factors that deeply influence the decision-making ability of the adolescents.

Decision-Making Ability

Decisions are made to solve problems, tackling the situation, handling crises and resolving conflicts that are inevitable. Decision-making ability is at the core ofplanning. The concept of decision-making involves defining the problem, finding, comparing and choosing a course of action. It is a process or activity of choosing an appropriate course of action from several alternatives' courses. The term "Decision-Making" has been defined as a process of judging various available options and

narrowing down choices to a situation one. The process of choosing or adopting a certain course of action, particularly when there is ambiguity, is known as decision making. A single decision can sometime make a large difference because your future is determined by your choices. For example, suppose you have just completed the tenth grade and must decide which stream to pursue. This might be a difficult option for students because some may still be confused about what they want to do at that age.If

You make wrong choice; it may make their career suffer. This makes developing decision making ability in adolescent students more important. Decision-making ability is a cognitive ability that develops with age. Making a choice among variousalternatives while making a decision, depends on an individual's knowledge, experience, sensitivity to rewards, and even feedback of the previously made decision. Having a great ability to make good decision at a particular time is depends upon a very important factor and that is how parents raise their children to cope-up with particular environment. This is nothing but the parenting style consisting the strategies, methodologies used by the parents while rearing their child.

Parenting Styles

Parenting styles refer to the manner in which parents raise their children. This can refer to the parent's level of expectations, performance, demands, attentiveness to rules, etc., as well as the style of discipline that the parent's utilize to enforce their expectations. Parenting styles is supporting healthy growth and development, because the way parents interact with their child and how parents' discipline has a lifelong impact. Parents are the individuals who beget an offspring. The parent's role is not only to give birth to a child but it's their duty to provide them with a congenial environment that may bring maximum development in them. The development may be physical, intellectual, emotional, social, spiritual or psychological.

The participation of the parents in their child's everyday life by listening to them, being the part of their activities, helping them in studies, regular interaction with teachers and friends, sitting together for food, planning some outings, listening to the problems and even guiding them about what is right and wrong, leaves a long lasting positive/negative influence on their child.

Adolescence is a transitional phase between childhood and adulthood. Adolescents are not small children and are not mature enough also to handle their responsibilities as adults can. They are not clear about their roles in society. They under go many hormonal changes which bring changes in their physical appearances and also in mental health. These lead to many psychological traumas causing impulsivity and mood swings in them.They are in a hunt for self-identity and are often confused about their role in the society. They are in a continuous search for their identity which will ultimately lead to self-development, self-exploration, and understanding their internal and external world. Parents play a very vital role in this search by providing them with proper love, attention, support, care, affection, and understanding. This parental responsiveness will help their children in achieving higher levels of self-understanding, cognition, and self-esteem leading to self-satisfaction, goodintrapersonal and interpersonal relationships.

Hence, parents play a very essential part in helping their teenage children in searching for their own-self by providing them with proper love, attention, support,care, affection and understanding their problems. Along with this responsiveness, parent's behavioral control on children is also very essential to develop their understanding of their self, achieving higher levels of cognition and self-esteem leading to self-satisfaction, good intrapersonal and interpersonal relationships. These developments will help an adolescent to understand his/her capabilities, responsibilities and potentials, distinguish between right and wrong and enable him/her to make the right decisions in everyday life. An adolescent will be able to understand his/her own potentials ,strengths ,and short comings with parent's support,which will enable

him/her in handling the various problematic situations with confidence, by acting wisely in solving the problem in the best possible way by taking appropriate decisions. Parent's behavioral control, demand for perfection and maturity, helps them to manage their emotions effectively and work properly following the rules and regulations of the society. This collectively results in developing high self-esteem, good mental health, autonomy, work efficiency, life satisfaction, good interpersonal relationships, mastery over the environment, understanding the meaning of life, and optimum personal growth.

Along with the parenting style there is another factor will influence the decision-making ability of an adolescent and that is peer pressure. In adolescence stage, children are more comfortable to share his/her emotions with their peer. Peer refers to the group of individuals of the similar age and when a person's attitude, behavior or activities are changed under the influence of peers it is called as peer pressure

Peer Pressure

Peer refers to the group of individuals of the similar age and pressure is the force exerted by something or someone, combining the two terms, peer pressure is the forceof a person or group of people of the same age group on another person which makes a person to behave, think and act in a different manner. When a person's attitude, behavior or activities are changed under the influence of peers it is called peer pressure. An adolescent on an average spends 11/12 hours with their peers or friends (at school, reference coaching or at play), 8 hours at sleep and only around 4 hours with their parents or guardians at home. So, they spend the maximum of his/her active hours with peers. As a result, they always try to fit themselves in the peer group by changing their behavior, attitude, activities and even sometimes morals. To be a part of the group or to get peer conformity, they indulge in dangerous activities which not only destroy their present and future even cause harm to their parents and guardians.

Sometimes, they lose their identity to get fit in the particular peer group. Increasing

support from their friends and peers make them feel independent, happy and confident, but slowly this increasing support makes them come under the pressure or control of their peer group. They start behaving in an impulsive and risky manner. They start acting in the way which makes them fit in the group. For achieving thisconformity, they may take wrong decision and start indulging in some ill legal activities like stealing, drug abuse and destroying others properties.

The peer pressure can also bring positive impacts, by bringing positive changes in an individual for example by the making a child motivated to complete their home work or development of new skills by observing their peers.Peers also sit together jointly on some projects and with the collaborative discussion comes new and good ideas. Such an influence of all peers is not equal. The range of peer pressure depends on the nature of the connection, the stronger the relationship is, and i.e. closeness with the peer or friend, more is the pressure. Also, the presence and absence of a peer or a friend also effects an adolescent's risk-taking behavior and decision-making power. They take more risks in the presence of peers as compared to in their absence.

From the above discussion, it can be concluded that decision making ability in adolescents highly depends on their peers.They make decisions to ge tconformity in the peer group without examining the pros and cons of their actions. They always want to get fit and have acceptance from their peers, so they decide to take actions or behave in the manner, in which they have never tried or thought at their own. But some adolescents are seen making independent decisions at their own,their decisions are least affected by their peers. Good, study-oriented and good moral valued peers help one to make good and right decisions. While bad and anti-social peers, encourage one to make wrong and faulty decisions. Hence, decision making ability among adolescents is very closely related to peer pressure. Parents are the first social circle with whom every child interacts and comes across, but as they enter adolescence, they start being more social outside the home. They are highly impressed by the teenagers of the same age. They start changing their attitude, behaviors and

actions following the peer group in which they want conformity. For such acceptance, they indulge in some dangerous activities and start hiding things from their parents.They start getting separated from their parents and try to come closer to their peers. Hence, during the stage of adolescence, two important factors that can affect the making of adolescents are parenting styles andpeer pressure. This study is an attempt to explore and study parenting style and peer pressure as predictors of decision-making ability of adolescent students.

REVIEW OF RELATED LITERATURE

There are number of research studies that find the direct as well as indirect connection between decision-making ability, parenting styles and peer pressure. Dogan and Kazak (2010) conducted a study to investigate the relationship between adolescents decision-making skills and their parental attitude. The study was conducted on 99 female and 53 male adolescents. Decision-making skills comprised of self-esteem, complacency, vigilance, panic and cop-out; parental attitude consisted of democratic, protective and authoritarian attitude. The results of the correlational analysis revealed that self-esteem decision making skill was positively and significantly correlated to parental protectiveness; complacency was positively correlated to protective and authoritarian parental attitude; vigilance was positively correlated to democratic and negatively to authoritarian parental attitude; panic decision-making was negatively correlated to democratic and positively to protective and authoritarian parental attitude, and cop-out was positively correlated to authoritarian parental attitude. Gender differences were seen in panic decision making style, whereas girls represented more panic decision making in comparison to boys. It was concluded that decision making skills in adolescents are very closely related to their parental attitudes and parents play a very vital role in developing these skills in their children.

Albert and Steinberg (2011) found the factors influencing the decision-making and judgement making ability during adolescence. Firstly, their decisions are affected by

their responsiveness towards some rewardor to avoid punishments.Such adolescents choose those options that help them to achieve the desired reward or avoid certain punishment. Secondly, adolescents make more faulty and risky decisions in thepresence of their peers in comparison to their absence. The study also found significant age-related influences on decisions. With an increase in age, the decision-making abilities of an individual increases and the adolescents make sound and rational decisions in the latter stages of life. Their self-regulating competence also enhanceswith age and this slowly declines their risk-taking tendencies.

Shakya, Christakis, and Fowler (2012) indicated that the parental responsiveness significantly affects the adolescent's susceptibility to peer pressure. The adolescents receiving more parental responsiveness had high level of self-esteem, self-monitoring and self-construal. Such adolescents were less prone to peer pressure. They have more capacity to think rationally and make more rational decisions. This rational and logical thinking help adolescents to stop substance and drug abuse and get rid of peer pressure. This brings positive psychological outcomes and behaviors in adolescents.

Karabanova and Poskrebysheva (2013) conducted a study, to correlate the development of value system and autonomy of decision making in adolescents with the parent-child relationship The results reveal that the parents who are motivating and allow their adolescent children to make independent decisions, are able to develop good value system and good decision-making skills in their children.Hence,development of value system and good decision-making skills is directly proportional to good and optimistic parent child relationship.

Reddy and Sekar (2018) conducted a review with an objective to study the influence of perceived parenting style on the decision making of adolescents.The results of the study showed that perceived parenting style has a significant influence on their child'sdecision-makingability.Supportive parents give more chances of independent and autonomous decision making and problem solving to their children. These

chances of independent decision making raise theirt hinking skills and cognitive functions.It makes them more confident decision maker throughout their lives. Hence, parenting style and decision making are very closely and significantly related to each other.

From all the above review it can be concluded that parenting style and peer pressure are directly or indirectly related to the decision-making ability of adolescent students. Therefore, all the variables are linked in one way or the other, but the researcher rarely find the study in which parenting style and peer pressure act as predictors for decision making ability of adolescent students. Hence, the present study is aimed to explore parenting style, peer pressure as predictors of decision-making ability of adolescent students.

Hypotheses of the Study

The following hypotheses were derived:

1. Parenting styles (namely parental responsiveness and parental control), peer pressure and decision-making ability of adolescent students are significantly correlated with each other.
2. Parenting styles (namely parental responsiveness and parental control) will not significantly predict decision making ability of adolescent students whichfurther implies:

a) Parental responsiveness will not significantly predict decision making ability of adolescent students.

b) Father's responsiveness will not significantly predict decision making ability of adolescent students.

c) Mother'sresponsiveness will not significantly predict decision making ability of adolescent students.

d) Parental control will not significantly predict decision making ability of adolescent students.
e) Father's control will not significantly predict decision making ability of adolescent students.
f) Mother's control will not significantly predict decision making ability of adolescent students.
g) Peer pressure will not significantly predict decision making ability of adolescent students.

METHOD AND PROCEDURE

Method of The Study

The present study was come under the domain of descriptive research which has undoubtedly been the most popular, wisely and used method in education.

Sample of the Study

Sample in a research study is one of the most important sources through which researcher can reach the conclusion. In the particular case, a sample of 200 senior secondary school student including 100 boys and 100 girls were selected from schools of Amritsar. Psychological Measured Used

Following tools were used to collect the data for present study:

1. Decisions Making Scale (Mincemoyer, Perkins & Munyua, 2001).
2. Parenting Style Scale (Gafoor & Kurukkam, 2014).
3. Peer Pressure Scale-Revised (Singh & Saini, 2016).

RESULTS AND DISCUSSION

In order to find the correlation between the variables parentings tyles, peer pressure and decision-making ability among adolescent students, Pearson product-moment correlation coefficient(r) analysis was used and the following inferences were drawn.

Table Showing the Coefficient of Correlation between Decision-Making Ability, Parenting Styles and Peer -Pressure.

Correlations							
	Parental Responsiveness	Father's Responsiveness	Mother's Responsiveness	Parental Control	Father's Control	Mother's Control	Peer Pressure
Decision making ability	.54**	.53**	.49**	.45**	.47**	.40**	-.28**

p<0.01 level

Table shows that the coefficient of correlation of decision-making ability with parental responsiveness was .54, which is significant at 0.01 level, this signifies that there exist a moderate and positive significant correlation between decision making ability and parental responsiveness whereas the coefficient of correlation of decision making ability with father responsiveness was calculated .53, which is again significant at 0.01 level, this indicated that there exist a moderate and positive significant correlation between the variables. Also, the correlation coefficient of decision-making ability with mother responsiveness

was .49, i.e. significant at 0.01 levels, this indicate there exist a weak and positive significant correlation between decision making ability and mother responsiveness.

Similarly, the coefficient of correlation of decision making ability with parental control was found to be .45, which is significant at 0.01 level, this signifies that there exist a weak and positive significant correlation between decision making ability and parental control where as the correlation coefficient of decision making ability with father control was calculated .47, which is significant at 0.01 level , this indicates there exist a weak and positive significant correlation between decision making ability and father control. The correlation coefficient of decision-making ability with mother responsiveness was calculated .40, which is significant at 0.01 levels, which further indicates that there exists a positive significant correlation between the variables.

Also, the coefficient of correlation of decision-making ability and peer pressure was found to be -.28, which is significant at 0.01 level, this further implies that there exist a weak and negative significant correlation between decision making ability and peer pressure.

Hence, the hypothesis, “Parenting styles (namely parental responsiveness and parental control) peer pressure and decision-making ability of adolescent students are significantly correlated with each other.” is accepted. Parenting styles and peer pressure will influence the decision-making ability of adolescent students whereas parenting styles will positively influence the decision-making ability i.e. more the parents are involved with their adolescent, better are the decision-making ability of them and peer pressure will negatively influence the decision-making ability of adolescent students.i.e. more will be the peer pressure, worse will be the decision of adolescent students. After calculating the

coefficient of correlation of dependent variable and independent variables, regression analysis is often used because it allows us to understand the nature of the relationship between variables more deeply. While correlation indicates the strength and direction of the relationship between two variables, regression analysis helps us predict the value of one variable based on the value of another variable and provides insights into the magnitude of the effect and potential confounding factors. Essentially, regression analysis helps us uncover more patterns and make more accurate predictions compared to just looking at correlation coefficients.

Regression Analysis

Regression analysis was used to infercausal relationships between the predictors and criterion variable.In the present study; the variables under investigation were parenting styles, peer pressure and decision-making ability. So, the significance was found between the variables by applying regression analysis.

Table Showing the Regression Analysis of Decision-Making Ability and Parental Responsiveness, Father Responsiveness, Mother Responsiveness, Parental Control, Father Control, Mother Control, Peer Pressure

Variables	**Decision-Making Ability**						
Parental Responsiveness	**b**	**SE**	**B**	**t- value**	**R**	**R-square**	**F**
	.24	7.67	.54	9.21	.54	.30	84.97

Father Responsiveness	.45	7.77	.53	8.82	.53	.28	77.87
Mother Responsiveness	.40	7.99	.49	7.93	.49	.24	66.99
Parental Control	.20	8.15	.45	7.25	.45	.20	52.69
Father Control	.37	8.09	.47	7.50	.47	.22	56.33
Mother Control	.33	8.39	.40	6.22	.40	.16	38.74
Peer Pressure	.21	8.78	.28	-4.23	-.28	.08	17.96

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Above table reveals that Parental Responsiveness is significantly predicting Decision-Making Ability of Adolescent students as it is explaining 30% of variance in decision-making ability of adolescent students ($F = 84.97$, $p<0.01$). Further analysis of unstandardized beta coefficient is showing +ve relationship between parental responsiveness and decision-making ability. Moreover ($b = .24$, $t = 9.21$, $p<0.01$),which indicates one unit change in parental responsiveness will bring 0.247 unitschange in decision making ability.

Father Responsiveness is significantly predicting Decision-Making Ability of Adolescent students as it is explaining 28% of variance in decision making ability of adolescent students ($F = 77.87$, $p<0.01$). Further analysis of unstandardized beta coefficient is showing +ve relationship between father responsiveness and decision-making ability. Moreover ($b = .45$, $t = 8.82$, $p<0.01$), which indicates one unit change in father responsiveness will bring 0.453 units change in decision making ability.

Mother Responsiveness is significantly predicting Decision Making Ability of Adolescent students as it is explaining 24% of variance in decision making ability of adolescent students ($F = 66.99$, $p<0.01$). Further analysis of unstandardized beta

coefficient is showing +ve relationship between mother responsiveness and decision-making ability. Moreover (b = .40, t = 7.93, p<0.01), which indicates one unit change in mother responsiveness will bring 0.408 units change in decision making ability.

Hence, the hypothesis, 2 (a), 2 (b) and 2 (c) stands disapproved. We can say that the parents who love, understand, care and provide a supportive environment at home, their children develop good decision-making abilities in them. This is because, the adolescents with supportive parents can discuss their issues and problems, freely and openly with their parents.This open discussion helps them to fully understand the issue, search alternatives and make the correct choice among the alternatives. Hence, they are more likely to make sound, logical and rational decisions in their everyday life. Specifically, Father's responsiveness enables an adolescent to seek help and support of their father before making a decision. Father's practical knowledge and experience help an adolescent to make a correct decision. As Mother's are the most loving and caring creatures of God. Their love and support make an individual internally strengthened. Sharing tensions and stresses with mother makes an adolescent psychologically healthy and allows him/her to think clearly and rationally before reaching at a decision.Furthermore, Parental control is significantly predicting Decision-Making Ability of Adolescent students as it is explaining 20% of variance in decision-making ability of adolescent students (F=52.69,p<0.01).Further analys is of unstandardized beta coefficient is showing +ve relationship between parental control and decision- making ability. Moreover (b = .20, t = 7.25, p<0.01), which indicates one unit change in parental control will bring 0.201 units change in decision making ability. Father control is significantly predicting Decision-Making Ability of Adolescent students as it is explaining 22% of variance in decision-making ability of adolescent students (F =56.33, p<0.01). Mother Control is explaining 16% of variance in decision- making ability of adolescent students (F = 38.74, p<0.01).

Hence, the hypothesis, 2 (d), 2 (e) and 2 (f) stands disapproved. Parental control is the

parent's behavioural control over their child, they make them their children do right deeds and follow the norms of the society. They have a continuous check on their children and demands perfection from them. Adolescents reared by such a parenting style follow the rules and regulations of the society and avoid illegal and risk-taking behaviours in their everyday lives. Such adolescents think logically and rationallybefore reaching at a decision. Thus, parental control develops decision making abilities in adolescents. Specifically, Father's demand for perfection and obedience makes an adolescent disciplined and well behaved. Such adolescents often think twice before reaching at a choice, they generally make decisions that are ethically correct and may not create disobedience to their father and society. Mothers are very close to their children. They always try to fulfill the appropriate demands of their children. They help their adolescent children to distinguish between right and wrong; and help them to follow a right path to achieve the desired objectives of life. This inclines one to make good decisions in everyday life.

A glance at the table reveals that Peer Pressure is significantly predicting Decision-Making Ability of Adolescent students as it is explaining 8% of variance in decision-making ability of adolescent students ($F = 17.96$, $p<0.01$). Further analysis of unstandardized beta coefficient is showing -ve relationship between peer pressure and decision-making ability. Therefore, the hypothesis "Peer pressure will not significantly predict decision making ability of adolescent students" stands disapproved. Negative and significant correlation, shows that with the increase in peer pressure the decision- making ability of adolescent decreases and vice versa. The scale used for the present research work calculates the value of negative peer pressure among adolescents.The negative peer pressure restricts one's capacity to think about the negative consequences of the acts that they are performing. It inclines an individual to do such activities to get peer acceptance and it can be interpreted from the results that, the adolescents who possess more peer pressure on them, lacks in independent decision-making ability. Therefore, the adolescents who are highly

influenced by their peers and work under their pressure and are more likely to make irrational and illogically decisions in their everyday life which in the long run may make them repent for it.

CONCLUSION

On the basis of the current study, we can say that the positive and significant correlation of decision-making with father's responsiveness, mother's responsiveness, parental responsiveness, father's control, mother's control and parental control shows that with the increasing support, warmth and understanding from the side of both parents, increases the decision-making ability of adolescents. Along with love and support; strictness and regular check of both the parents on their adolescent child is essential to enhance their decision-making ability. Hence, parental responsiveness and parental control both, play a very essential and vital role in enabling adolescents tomake good and healthy decisions in their everyday lives. The adolescents who are highly influenced by their peers and work under their pressure and are more likely to make irrational and illogically decisions in their everyday life which in the long runmay make them repent for it.

IMPLICATIONS

The present study examined parenting styles and peer pressure as predictors of decision-making ability of adolescent students. As strengthening decision-making ability among adolescents is essential. For this, teachers can integrate decision-making exercises into the curriculum, while parents can encourage open communication and critical thinking at home. School administration can offer workshops or counseling services focused on decision making ability.Parents are

advised to provide such a home environment that their adolescents may share and discuss their problems and issues regarding which a decision has to be made, freely with them. This will guide them to follow a right path in a right direction and eventually leading to make good, mature and meaning decisions in their life. The study shows that adolescents should not blindly follow their peers to get their conformity. They must look for the pros and cons of a decision before executing it. Because a good decision makes a life while a faulty and wrong decision can destroy the life of an individual.

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Smart Inventory Management through Digital Transformation: A Path to Sustainable Business Practices

Manisha Prajapati*, Ashendra Kumar Saxena

Teerthanker Mahaveer College of Engineering, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh (244001), India

Abstract

In the era of Industry 4.0, businesses are rapidly adopting digital technologies to enhance efficiency, transparency, and sustainability. Among various operational areas, inventory management holds a critical role in optimizing resources, minimizing waste, and achieving sustainable growth. Traditional inventory systems often lead to overstocking, inefficiency, and excessive resource utilization. By enabling real-time data tracking, predictive forecasting, and automation, the integration of digital transformation tools like Artificial Intelligence (AI), the Internet of Things (IoT), Big Data Analytics, and Blockchain has transformed this job. Smart inventory management promotes eco-efficient practices by aligning supply with demand, reducing energy consumption, and supporting circular-economy models. By leveraging digital innovation, organizations can reduce carbon footprints, optimize logistics, and foster responsible business operations. Furthermore, digitalized inventory systems enhance decision-making and transparency, helping companies balance economic goals with environmental commitments. This study examines the role of digital transformation in reshaping inventory management towards sustainable business practices. It highlights how technological integration can strengthen supply-chain resilience, drive cost efficiency, and contribute to global sustainability objectives. The findings underscore that smart inventory systems are not only a technological upgrade but a strategic shift toward sustainable and future-ready business management.

Keywords:

Smart Inventory Management, Digital Transformation, Sustainable Supply Chain, Industry 4.0, Green Business Practices, Circular Economy, Resource Optimization

1. Introduction

1.1. Motivation and Relevance to Mathematical Sciences

Organisations are under increasing pressure to maximise resources, cut expenses, and run sustainably in the rapidly changing business environment of today. Since ineffective inventory systems can result in overstocking, stockouts, unnecessary waste, and increased carbon emissions, inventory management is essential to accomplishing these goals.The growing complexity of global supply chains demands mathematical rigor to model, predict, and optimize inventory levels effectively. A Decision-makers can integrate economic, operational, and environmental goals into inventory plans by using methods like mixed-integer optimisation, linear and nonlinear programming, and metaheuristic algorithms. In addition to improving operational effectiveness, the integration of applied mathematics with inventory management supports evidence-based decision-making in sustainable business practices.

1.2 Research Gap and Objectives

Despite advances in digital technologies and smart inventory systems, most organizations still rely on traditional approaches that inadequately capture environmental and sustainability considerations. Specifically, there is limited research on integrating real-time digital monitoring tools (IoT, AI, Blockchain) with mathematical models that quantify environmental impacts, such as carbon emissions or resource consumption. This paper aims to fill this gap by exploring how digital transformation enables mathematically optimized inventory systems that align economic efficiency with sustainable objectives. The main objectives are:

1. To examine the role of smart inventory systems in reducing waste and energy consumption.

2. To investigate optimization techniques that incorporate environmental constraints in inventory planning.

3. To demonstrate how digital tools enhance transparency, predictive accuracy, and decision-making in supply chains.

1.3. Scope and Structure of the Paper

This paper explores the intersection of digital transformation, mathematical optimization, and sustainable business practices in inventory management. It presents a comprehensive review of smart inventory systems, examines optimization techniques under uncertainty, and discusses digital enablers such as AI, IoT, and Blockchain. Additionally, the paper highlights sustainability-oriented strategies, including green supply chains, circular inventory models, and life cycle assessment (LCA) approaches. Finally, it addresses practical challenges, provides recommendations, and identifies opportunities for further research in applied mathematics and sustainable operations.

2. Fundamentals of Smart Inventory Management

2.1. Essential Ideas in Inventory Theory

A crucial component of supply chain operations, inventory management guarantees that the appropriate number of items are available at the appropriate time and place. Conventional inventory theory is based on ideas like: • Economic Order Quantity (EOQ): The ideal order size that reduces the whole cost of inventory, including ordering and holding expenses.

• **Reorder Point (ROP):** To prevent stockouts, a new order should be placed at this inventory level.

• **Safety Stock:** Extra inventory kept on hand to protect against supply or demand fluctuations.

• **Lead Time:** The amount of time that passes between placing and receiving an order.

2.2. Smart Inventory Systems in the Digital Era

Smart inventory management leverages digital technologies to transform traditional inventory processes into adaptive, real-time, and data-driven systems(López et al., 2013). Key features of smart inventory systems include:

- **Real-Time Monitoring:** IoT-enabled sensors track stock levels, storage conditions, and movement of goods continuously.
- **Predictive Forecasting:** AI and machine learning algorithms predict demand trends, reducing overstocking and stockouts.
- **Automation:** Automated replenishment systems place orders based on predictive insights, minimizing human intervention.
- **Integration with Supply Chain:** Digital platforms synchronize inventory across suppliers, distributors, and retailers, enabling agile and responsive operations.
- **Transparency and Traceability:** Blockchain ensures secure, immutable records of inventory movement, supporting compliance and ethical sourcing.

By integrating these technologies, businesses can maintain optimal inventory levels, reduce waste, and align inventory practices with sustainability goals(Pattnaik et al., 2021).

2.3. Traditional vs. Smart Inventory Models

Smart inventory management is thus not merely a technological upgrade but a strategic transformation that improves efficiency, sustainability, and resilience across the supply chain (Iqbal, 2025).

Table 1: Difference between smart and traditional inventory models.

Feature	**Traditional Inventory Models**	**Smart Inventory Models**
Data Collection	Periodic/manual updates	Real-time via IoT and sensors
Forecasting	Historical average or simple trends	AI/ML-based predictive analytics
Decision Making	Manual, experience-based	Automated, data-driven, and optimized
Flexibility	Low, slow to adapt	High, adaptive to demand fluctuations

Sustainability Focus	Limited	Embedded (energy usage, waste reduction, carbon footprint)
Integration	Limited across supply chain	Fully integrated across suppliers, logistics, and retailers

3. Optimization Techniques for Smart Inventory Management

Inventory optimization ensures that businesses maintain optimal stock levels while minimizing costs and environmental impact. Modern smart inventory systems use advanced mathematical and computational techniques to address complex challenges such as uncertain demand, supply disruptions, and sustainability constraints(Salas-Navarro et al., 2022).

3.1. Linear and Nonlinear Programming

- **Linear Programming (LP)** is widely used for optimizing inventory costs when relationships between variables are linear. LP can minimize total costs (ordering, holding, and shortage costs) while satisfying constraints such as storage capacity or budget limits(Nemtajela&Mbohwa, 2016).
- **Nonlinear Programming (NLP)** handles more complex scenarios where cost, demand, or emission functions are nonlinear. For example, energy consumption in storage facilities may increase nonlinearly with stock levels, and NLP allows integrating such environmental factors into inventory decisions(Kaushik, 2025).
- **Mixed-Integer Linear Programming (MILP)**

MILP combines linear programming with integer constraints, making it suitable for decisions like batch ordering, discrete shipment sizes, or facility selection. MILP is particularly useful for:

1. Optimizing multi-echelon supply chains.
2. Incorporating both continuous variables (inventory levels) and discrete decisions (order quantities, shipment schedules).
3. Integrating sustainability objectives, such as minimizing carbon emissions alongside costs.

3.2. Metaheuristic Approaches (e.g., Genetic Algorithms, PSO)

Metaheuristic algorithms provide approximate solutions for highly complex, large-scale, or non-convex optimization problems. Common techniques include:

- **Genetic Algorithms (GA):** Simulate evolution to find optimal inventory configurations.
- **Particle Swarm Optimization (PSO):** Models inventory decision-making as a collaborative search problem.

- **Ant Colony Optimization (ACO):** Useful for optimizing multi-echelon inventory networks and logistics routes.

These approaches are flexible and capable of handling multiple objectives, uncertainties, and non-linear constraints(Mahajan et al., 2024).

4. Digital Transformation Enablers in Inventory Systems

The rapid advancement of digital technologies has revolutionized inventory management by enhancing visibility, accuracy, and decision-making efficiency. Digital transformation enablers such as IoT, Big Data, AI, Blockchain, and Digital Twins provide innovative solutions for optimizing inventory operations while aligning with sustainability and transparency goals. These technologies collectively transform traditional inventory systems into intelligent, data-driven networks capable of real-time responsiveness(Chauhan et al., 2022).

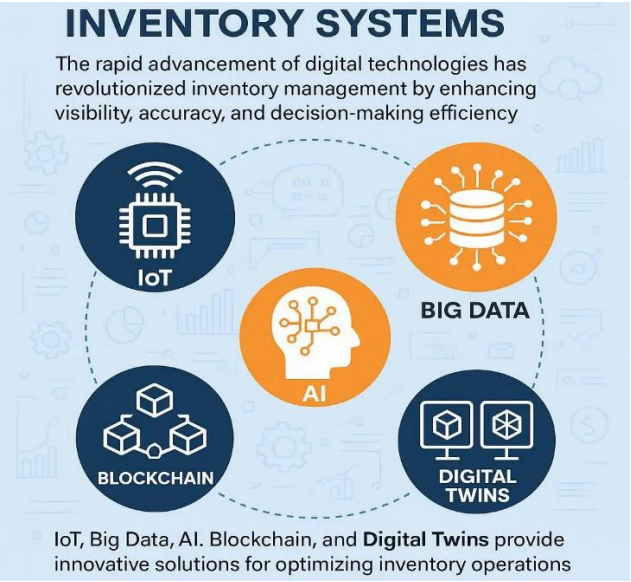

Fig 1: Technology Enablers for Smart Inventory Systems

4.1. Role of IoT in Real-Time Inventory Monitoring

Through connected sensors and RFID devices, the Internet of Things (IoT) makes it possible to track and manage inventory in real time. IoT systems automatically capture data related to stock levels, temperature, humidity, and product movement, reducing manual errors and delays. In food supply chains, IoT plays a crucial role in maintaining product quality and traceability by continuously monitoring environmental conditions during storage and transport. This real-time visibility allows businesses to make prompt decisions, prevent stockouts or overstocking, and enhance overall operational efficiency (Zhang & Aslan, 2021).

4.2. Big Data and Predictive Analytics

To find patterns and trends, big data analytics analyses massive amounts of structured and unstructured data from numerous sources, including distributors, suppliers, and consumers. This data is used by predictive analytics to eliminate waste, optimise inventory levels, and predict future demand. Businesses can make data-driven decisions that lower uncertainty and raise service standards by combining information from sales records, weather trends, and customer behaviour. In sustainable inventory management, Big Data supports better resource utilization and helps minimize environmental impact through optimized replenishment cycles(Chen et al., 2022).

4.3. AI and Machine Learning for Optimisation and Forecasting

Algorithms for machine learning (ML) and artificial intelligence (AI) improve forecasting accuracy and automate intricate decision-making procedures in inventory systems. AI-powered models are able to predict demand variations, modify order quantities, and dynamically optimise replenishment schedules by learning from past data.These tools also enable adaptive inventory control, reducing human intervention while improving precision(Boute& Udenio, 2022). In

sustainable operations, AI helps minimize waste and energy use by aligning production and supply with actual consumption patterns.

4.4. Blockchain for Secure Inventory Tracking

Transparency, traceability, and security in inventory transactions are guaranteed by blockchain technology.Each product movement is recorded as an immutable digital entry, creating a trustworthy and verifiable chain of custody from producer to consumer. In food supply chains, blockchain helps authenticate product origin, verify sustainability certifications, and detect inefficiencies or fraud(Ray et al., 2019). This decentralized approach enhances collaboration among stakeholders, builds consumer trust, and supports compliance with environmental and safety regulations.

4.5. Digital Twins and Simulation Modelling

To mimic and examine real-world activities, digital twins build virtual versions of actual inventory systems. Combined with **simulation modelling**, this technology allows managers to test "what-if" scenarios, optimize warehouse layouts, and evaluate the impact of different inventory policies before implementation. In sustainable inventory management, digital twins help identify inefficiencies, reduce resource consumption, and improve system resilience(Wang, 2022). By integrating real-time data, organizations can continuously refine operations, ensuring both efficiency and environmental responsibility.

5. Sustainable Business Practices and Inventory Management

The goal of sustainable business practices is to strike a balance between social and environmental responsibility and economic success. Sustainability in inventory management is centred on cutting carbon emissions, minimising waste, and

encouraging resource efficiency all the way through the supply chain. Integrating sustainability into inventory systems requires the adoption of green technologies, eco-friendly policies, and data-driven decision-making tools(Lazar et al., 2021). The following subsections outline key approaches and models that support sustainable inventory operations.

Fig 2: **Sustainable Business Practices**

5.1. Mathematical Models Incorporating Environmental Costs

Traditional inventory models primarily focus on minimizing total costs related to ordering, holding, and shortage. However, modern approaches now incorporate **environmental costs** such as carbon emissions, energy consumption, and waste disposal. These models use optimization techniques to balance economic objectives with ecological performance. By including environmental parameters in the cost function, firms can identify optimal inventory policies that minimize both financial expenses and environmental damage (Gautam & Khanna, 2018). For example, mathematical programming models can be designed to reduce the total cost per unit while maintaining sustainability constraints.

5.2. Green Supply Chain and Circular Inventory Models

Green Supply Chain Management (GSCM) emphasizes reducing environmental impact through eco-friendly material sourcing, energy-efficient logistics, and waste minimization. In this context, **Circular Inventory Models** focus on reusing, recycling, and remanufacturing products to extend their lifecycle and reduce raw material dependency. These models encourage closed-loop systems where returned or defective items are reintegrated into the production cycle(Novitasari& Agustia, 2021). Such sustainable inventory frameworks not only reduce waste but also enhance profitability and brand reputation by promoting responsible consumption.

5.3. Carbon Emission Constraints in Inventory Optimization

With increasing global emphasis on climate change mitigation, incorporating **carbon emission constraints** into inventory models has become essential. These models consider emission limits generated from production, transportation, and storage activities. By including emission caps or carbon pricing mechanisms, companies can determine eco-efficient inventory policies that comply with environmental regulations(Verma & Mishra, 2024). Carbon-aware inventory optimization ensures that cost reduction does not compromise sustainability goals, thereby promoting greener supply chain practices and contributing to international sustainability targets like the **UN Sustainable Development Goals (SDGs)**.

5.4. Life Cycle Assessment (LCA) Techniques in Inventory Decisions

Life Cycle Assessment (LCA) offers a methodical way to assess how products affect the environment at every stage of their life cycle, from the extraction of raw materials to disposal. Organisations can make well-informed decisions on sourcing, production volumes, and storage techniques by incorporating life cycle assessment (LCA) into inventory management. It makes it possible for managers to pinpoint high-impact phases, maximise the use of resources, and implement more environmentally friendly options**.** LCA-based inventory decisions ensure that sustainability is embedded across

all supply chain levels, fostering long-term ecological and economic balance(Lu et al., 2017).

6. Challenges and Recommendations

The successful adoption of sustainable and technologically advanced inventory management systems is hampered by a few issues, notwithstanding the increased focus on sustainability and digital transformation. One significant obstacle is the high upfront costs of implementing technologies like blockchain, IoT, and AI. Due to their limited financial and technical resources, small and medium-sized businesses frequently face difficulties. Furthermore, inconsistent implementation and measurement result from the absence of standardised frameworks for incorporating environmental concerns into inventory optimisation. The availability and quality of data is another important concern since inadequate or erroneous data can result in ineffective forecasting and decision-making.**b** Governments and policymakers can support this transition through **green incentives, tax benefits, and carbon credit mechanisms**. Collaboration between academia, industry, and policymakers is crucial to create adaptable and scalable green inventory models that align economic efficiency with environmental responsibility.

7. Conclusion and Prospects for the Future

7.1. Conclusion

This study highlights the critical intersection of mathematical modelling, digital transformation, and sustainability within inventory management. By integrating technologies such as IoT, AI, and blockchain with environmental models that account for carbon emissions and resource consumption, organizations can achieve both operational efficiency and ecological balance. The proposed frameworks emphasize that economic performance and environmental stewardship are not mutually exclusive

but can be mutually reinforcing when guided by data-driven insights and sustainable principles. Ultimately, green inventory management contributes to achieving the **UN Sustainable Development Goals (SDGs)** related to responsible production (SDG 12) and climate action (SDG 13), paving the way for long-term resilience and competitiveness.

7.2. Opportunities for Further Research in Applied Mathematics

Future research in applied mathematics offers promising opportunities to enhance sustainable inventory systems. Scholars can explore **multi-objective optimization models** that simultaneously address cost, emission reduction, and service-level trade-offs under uncertainty. Advanced techniques such as **stochastic programming, fuzzy logic, and reinforcement learning** can further improve the accuracy of inventory forecasting in complex supply networks. There is also scope for developing **dynamic, real-time decision-support systems** that integrate live data from IoT devices and predictive analytics. Furthermore, the **integration of game theory and behaviouralmodelling** could provide insights into collaborative decision-making among supply chain partners. Expanding research in these mathematical domains will strengthen the theoretical foundation and practical applicability of sustainable inventory management in a digitally connected world.

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APPLICATION OF STATISTICAL TECHNIQUES IN ECOLOGICAL SYSTEM

Anshul Dubey[1], Rajan Singh[2], B.K. Singh[3], Nidhi Tiwari[4]

[1,2,3,4]*Department of Mathematics, IFTM University, Moradabad, 244001, India.*

ABSTRACT:

The study of ecology has become quantitative and mathematical during the last few decades because of the general awareness of the importance of the relationships between the environment and biomass of a population, interactions between populations and within populations and the effect of population on the environment. Different kinds of pollution (e.g. water, air, noise, etc.) have appeared as a real threat to the existence of human population, the most intelligent species on the planet earth. To measure the effect of these hazards to the animal and plant populations and on the overall ecological balance, the knowledge of different statistical methods and techniques have become indispensable. In the first part of this paper elementary concept of descriptive statistics, probability distribution, regression and correlations, the chi-square distribution, etc. will be reviewed briefly, while in the second part we shall dwell on the specialized topic, namely species-abundance relations, and the measurement of species-diversity.

KEYWORDS: Environment, biomass of a population, species-abundance relations, Mean and Variance, Standard Deviation, Histogram.

INTRODUCTION:

Statistics may be considered as the science and technique of collecting, analysing, and making inferences from data and these references are stated as probabilities. The term probability is used in the sense of relative frequency of multiple or repeated events in the long run. The data under consideration of statistical analysis consists of two kinds

of variables: (a) continuous and (b) discrete. Mean and Variance are the best-known statistical measures of a series of observations. Suppose the following numbers of individuals have been observed in a series of 10 quadrats.

16, 11, 19, 28, 34, 62, 18, 20, 10, 12

The average number of individuals in a quadrat, the mean, is the sum of all observations divided by the number of observations

$$\bar{x}\,(mean) = \frac{1}{n}\sum_{i=1}^{n} x_i$$

So, $\bar{x} = (16 + 11 + 19 + 28 + 34 + 62 + 18 + 20 + 10 + 12)/10 = 23$ individuals. To have an insight it is desirable to estimate the average deviation of each observed value from the mean$\bar{x}$. The average deviation measured from the mean is always zero because the sum of the deviation is zero. To eliminate this problem the squared deviation is usually calculated, i.e. $\sum_i (x_i - \bar{x})^2$. The average of the squared deviations is called variance σ^2 where σ is called standard deviation.In the present example $\sigma^2 = 220$, and$\sigma = \pm 14.83$.

Let us now consider the weights of 2,089 individuals in a population shown table 1. The observations are divided into 10-kilogram groupings. A total of 216 individuals was found to weight between 70 and 80 kilograms. The number of observations occurring in each group is plotted against groups. This is known as frequency distribution. If the length of each class interval becomes smaller the discontinuities of the distribution become smaller, considering the weight groups to be infinitesimally small, the distribution would resemble the dotted curve. It is a bell-shaped curve known as the normal curve. This curve represents a function known as the normal probability density function. The density function is continuous; the distribution approaches the normal curve in shape in the limit as the class interval becomes smaller.

Table: 1

Weight Group	No. of Observation	Fraction of Total
30-40	13	0.03714
40-50	30	0.06666
50-60	68	0.12364
60-70	135	0.20769
70-80	216	0.288
80-90	300	0.35294
90-100	345	0.36316
100-110	338	0.32190
110-120	275	0.23913
120-130	190	0.152
130-140	101	0.07481
140-150	48	0.03310
150-160	20	0.01290
160-170	7	0.00424
170-180	3	0.00171
	N=2089	

The Gaussian or normal curve is described by the equation

$$y = \frac{1}{\sigma\sqrt{2\pi}}\exp[-\frac{(x-\mu)^2}{2\sigma^2}]$$

In this equation y represents the relative frequency of some variable quantity x. The values for transcendental numbers π and e are constant. This equation has two important parameters μ, the arithmetic means and σ, the standard deviation which may be taken as the measure of the spread of the data about the mean. The curve is completely determined if the values of the parameters μ and σ are known, since π and e are constants. Knowledge of the following important properties of normal curve are important:

(a) It is a bell-shaped, symmetrical curve. The median and mode coincide with mean μ.

(b) It is initially convex upward but soon becomes concave. There is a point of transition from convex to concave called a point of inflexion. The distance of this point horizontally from the mean is equal to the standard deviation σ.

The area under the curve is unity i.e. the sum of all the probability (possible relative frequencies) represents certainly.

Thus, the area lying under the curve between the values x_1 and x_2 shown as the shaded are represents a fraction of the total are proportion to its probability. In other words, this fraction represents the probability of obtaining a sample value lying between x_1 and x_2. As the curve is symmetrical, half the area under the curve lies above the mean while half lies below it.

The shaded area lying under the curve between the inflexion points. This area constitutes about 2/3 of the total area or more-approximately 67%.

The equation of the normal curve can be expressed in the form

$$y = \frac{1}{\sqrt{2\pi}} e^{-z}$$

Where $Z = \frac{x-\mu}{\sigma}$

This is known as the normal curve in the standard form. Here mean is zero and standard deviation is unity. The discussion so far being made assumes that the estimates of the mean and standard deviations are accurately known because of the large sample sizes.Suppose we select a value of Z for which the excluded or shaded area constitutes 5% of the total area. This means that 95% of the estimates, $\bar{x}$ will lie no further from μ than Zs/$\sqrt{n}$. This value will be found to be 1.96. In other words

$$\mu - 1.96\frac{s}{\sqrt{n}} < \bar{x} < \mu + 1.96\frac{s}{\sqrt{n}}$$

As an example, let us consider the mean weight of all men in the world to be 130lb and s.d. to be 8 lb. then 95% of the time the mean weights of samples of 100 women

selected at random would fall within the limits described by the equation

$$\bar{x} = 130 \pm 1.96(\frac{8}{\sqrt{100}})$$

In biological and ecological studies 95% probability is conventionally accepted as adequate assurance. If the limits are extended from 1.96s to 2s on either side of the mean to 3s, the probability goes up from 95% up to 99%. Thus, for a 50% increase in range around the mean, we gain a mere 4% increased assurance. Conventionally, we speak of the limits around the mean $\pm 1.96s/\sqrt{n}$, as the 95% confidence limits or fiducial limits. Sometimes, in real situations, it is not always feasible to have a large sample. With small samples we cannot do this safely without making some allowance for the unreliability of s as a measure of σ. We do this in the following way by multiplying Z by a factor g while setting fiducial limits of the mean, namely

$$\mu = \bar{x} \pm (gZ)s$$

Where g depends on the sample size n as g(n)$>$ 1 when n is small, but approaches the value 1 as n increases. The correction factor gZ was solved first by W.S. Gesset (1908) under the pseudonym 'Student'. The various values of g(n) for different sample size n were tabulated under the heading 't' and use of this corrected value is known as Student`s t-test. For example, *n*=30 or more, t and Z are practically equal. Therefore

$\mu = \bar{x} \pm ts$, where $n < 30$

RECOMMENDATION: We shall now consider some of the distributions which have been proposed to fit the observed species-abundance frequency distribution. The logarithmic distribution will be discussed first, because it is useful in providing empirical fit to observed species-abundance relationship.

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APPLICATIONS OF ARTIFICIAL INTELLIGENCE AND MACHINE LEARNING IN MODERN PHARMACEUTICAL SCIENCES: A COMPREHENSIVE REVIEW

Mohd Ali Nawaz Rain[1*], Mohammad Akhlak[2], Aijazul Haq[3]

[1*,2]*Department of pharmacy, Krishna Pharmacy College, Bijnor, 246701*

[3]*Department of Mechanical Engineering, Kunwar SatyaVira College of Engineering and Management, Bijnor, 246701*

ABSTRACT

Artificial Intelligence (AI) and Machine Learning (ML) have transformed pharmaceutical sciences by changing nearly every step in the path of drug discovery, development, formulation, clinical trials, and safety monitoring, known as pharmacovigilance. AI represents computer algorithms that can reproduce human cognitive tasks, such as reasoning, learning, and solving problems, while ML enables them to learn from data and improve performance without programmers explicitly coding an algorithm. The adoption of AI and ML tools into the pharmaceutical pipeline will increase the pace of scouting for new drug candidates, increase efficiency in formulation development, and increase production efficiency through predictive modeling and automation. AI and machine learning tools facilitate analysis of complex biological and chemical data through neural networks, deep learning, and data mining to introduce innovative products at lower cost and with on time measures, development times, and efficiency increases. AI and the use of bigger quality management systems, process analytical technologies (PAT) and Industry 4.0 will enable more precise manufacturing and engineering with lower batch-to-batch variability increasing the right-first-time rates. In pharmacovigilance, AI can detect adverse events and enable safety monitoring in real-time employing

sources of clinical data, electronic health records, various social media streams, and focused data sets. As personalized medicine rises, and predictive analytics persists, more treatment for individual patient populations will be tailored. Despite the advances presented with AI and ML, challenges remain, particularly with standardizing data, interpretability, regulatory acceptance, and ethical considerations. Nonetheless, AI and ML generally represent important tools toward achieving efficiency.

Keywords: Artificial Intelligence (AI), Machine Learning (ML), Pharmaceutical Sciences, Development formulation, Drug Discovery, Pharmacovigilance, Personalized Medicine, Quality Management Systems.

INTODUCTION

The ability of a computer or robotic system to process information and produce outcomes that are similar to how a human may think when learning, making decisions, and solving issues is known as Artificial Intelligence (AI).Within the discipline of computer science, Artificial Intelligence (AI) focuses on using symbolic programming to solve problems. It has evolved into a science of problem solving with wide-ranging applications in engineering, business, and medicine. To meet the needs of doctors and society in the twenty-first century, the current medication development process needs to be drastically altered. To work more efficiently and greatly improve the success of early drug development, the pharmaceutical sector in particular has a genuine chance to alter the way it conducts research and development. Machine learning and artificial intelligence have created this opportunity [1-3].

AI is a quickly evolving technology that has many applications in both daily life and business. Recently, the pharmaceutical industry has discovered creative

and novel ways to use this powerful technology to help address some of the most urgent problems facing the sector right now. Artificial intelligence in the pharmaceutical sector refers to the application of automated algorithms to tasks that would typically need human intelligence. Over the past five years, the use of artificial intelligence in the biotech and pharmaceutical industries has completely changed how researchers develop new drugs, manage illnesses, and much more [4].

The main objective of this artificial intelligence is to identify real-world information processing problems and offer a theoretical justification for solving them. In mathematics, a description of this kind, called a method, is related to a theorem. Algorithms are developed and utilized in artificial intelligence research to analyze, comprehend, and learn from data. Several statistics and machine learning disciplines Artificial intelligence encompass learning, pattern recognition, grouping, and similarity-based approaches [5].

Artificial Intelligence (AI) has gradually flooded many clinical journals, such as the ones brand new photo processing and scientific physics. AI refers to pc algorithms which can mimic functions which might be feature contemporary human intelligence, which include trouble fixing or trendy. The latest fulfillment state-of-the-art AI has been made feasible thanks to super growths brand new each computational power and facts availability. The aim trendy this evaluates is to provide the primary technological pillars cutting-edge AI, together with the device modern-day strategies and their software to scientific imaging [6-7].

ML is the simple paradigm that includes more than one approach based totally domain names and several algorithms to apprehend the sample inside the records. Each automation-based totally approach makes use state models DL and ML however holds the difference [8]. This model is the well-set up mathematical model that indicates underlying styles available in the statistics

and information and applies to study strategies for predicting future information several models are used to educate a unmarried dataset to keep away from brute force sensitivity and optimize specifically by means of understanding the perspective in various version architecture [9].

Big data and AI-driven analytics have changed the pharmaceutical industry's innovation paradigm. Machine learning, according to Nagy et al. can encourage innovation, optimize productivity, and produce stellar outcomes across every phase of the value chain. Sets breakthroughs and facilitates the development of new business models may significantly enhance the value proposition of the medical business. Managers of drugs are searching for methods to apply machine learning as well as artificial intelligence in biotechnology with health industries. Artificial intelligence (AI) holds great potential for adjusting the commercial operations picture of the pharmaceutical industry. There are concerns that larger businesses are applying the software applications that are there now, which is setting up the digital future of this market. Recognized drug firms work with healthcare professionals in artificial intelligence (AI) to apply AI to general drug discovery, development, and research [10].

RECENT TRENDS

Pharmaceutical Automation in Research and Development

Artificial intelligence is a very recent notion. Even though laboratory automation technologies have existed since the 1990s, large-scale bio-repositories, automated clinical and analytical testing, combinatorial chemistry, high throughput screening, and other related activities have only recently begun to take off in these laboratories. Thanks to advancements in robotics and other technologies, a fully automated library is now a reality. A robust automated

information system is necessary due to the high speed and volume of samples being handled, which results in an enormous flow of measurements and data. LIMS (laboratory information management system) is one example [11].The automated infrastructure for the pharmaceutical sector is operational. The roles that have already been automated will continue in their position thanks to the little advancements brought about by the regular cycle of technological progress.

PAT (Process Analytical Technology)

Another cutting-edge trend is PAT. This is essential in supporting pharmaceutical businesses' efforts to enhance their production processes through innovation and a continuous improvement mind-set. Patients save money as a result of increased product yields, better usage, and less waste. PAT encompasses more than simply hardware. To comprehend crucial process parameters, it has to be able to communicate with and collect information from numerous sensors and analysers. It also has to carry out intricate multivariable computations and modelling [12].

INDUSTRIAL APPLICATIONS OF ARTIFICIAL INTELLIGENCES OR MACHINE LEARNING IN THE PHARMACEUTICAL SECTOR

Can Artificial Intelligence replace humans in the pharma sector?

AI can replace 35 % of UK jobs in next 10–20 years", given by a survey conducted by Deloitte in collaboration with Oxford Martin Institute. There are also some studies saying that automation is not possible because of cost of adopting automation technologies is so high and regulatory considerations in the pharma sector. The pharmaceutical industry cannot be fully automated. AI

can just assist humans but cannot replace them.[13]AI presents a transformative opportunity in the discovery of drugs and the formulation and testing of pharma dosage forms. Pharmaceutical automation technology plays a major role in the real-time monitoring of critical quality attributes and performance attributes of raw materials and in-process materials to design, analyze, and control manufacturing. AI algorithms are available to design the process of manufacturing that leads to the final product complying with predetermined specifications. AI is an innovative technology to carry out tedious tasks in the pharmaceutical industry. The main limitation of the use of AI in pharma is that data given by AI is a black box phenomenon, it is not known how the conclusion reached by AI technology. AI cannot be a model of the human brain. There is a false notion that automation leads to unemployment and a reduction of human intervention in the pharma industry. Skilled data scientist's software workers and AI experts are required to handle all these technological activities and these AI are not perfect and accurate. To check their reliability human intervention is mandatory mainly in healthcare sectors.AI training datasets are never static. These datasets will be updated as technology advances, and using the updated dataset, AI will also be finetuned. For all of these, human intervention is required [14-15].

AI/ ML in Drug Formulation

Pharmaceutical sciences have seen various formulations, for example solid dispersions, extrudates, pellets, nanoparticles, and liposomes, arise in addition to standard dosage forms. The name "formulation techniques" is given to these techniques because they empower the development of formulations or incorporate functionality into common dosage forms such as tablets. AI applications in formulation techniques are even more worthwhile to investigate

in order to create next-generation drug products with desired efficacy and health outcomes because these methods can successfully address a variety of API issues, such as low solubility, stability, bioavailability, and production capability [16].Drug formulation requires projections regarding appropriate delivery vehicle and the best excipient properties to obtain desired drug form. ML models have been reported to accelerate this process of formulation with a high degree of accuracy by making extensive modeling of complex variables possible with ANNs. Neural networks have been widely employed to assist pre-formulation studies.For Example: To monitor in vitro release kinetics of a drug from microparticles using data on microparticle formulations from literature [17].

AI/ML in Quality Assurance and Quality Control

AI model in quality assurance that is useful in the software development sector such as Regression testing. AI transforms the quality process in drug manufacturing by enhancing inspection accuracy process speed is increased with the help of the data and images available during the manufacturing process and hence any deviations in product quality are identified [18].

AI in quality assurance and quality control shows better quality of the developed product, less scrap, cost savings, and higher profits for pharmaceutical companies. Digitization in pharma quality control laboratories ensures better quality and compliance by reducing manual errors and fluctuations and solving problems faster and more effectively. The digitization of quality control laboratories has led to an improvement in product quality by reducing the amount of manual documentation work and optimizing planning and scheduling to improve the use of personnel, equipment, and materials [19-20]

AI/ ML in Pharmacovigilance

Artificial intelligence's (AI) application in pharmacovigilance (PV) has

expanded significantly, promising to improve the speed and accuracy of adverse event detection. The transition has been driven by the increasing complexities in drug development and post-market surveillance, including the unprecedented volume of data, complexity of drug–drug interactions, and patient variability [21-23]The application of AI in PV began in the early 2000s with the introduction of data mining algorithms for signal detection in spontaneous reporting systems (SRS). Pioneering approaches such as the Bayesian Confidence Propagation Neural Network (BCPNN) method and the Multi-item Gamma Poisson Shrinker (MGPS) laid the groundwork for more sophisticated AI applications in PV. Since then, AI has been applied to various aspects of PV, including automated case processing, signal detection, and real-world evidence analysis [24-26].

RESEARCH AVENUES OF AI/ML IN PHARMACY

AI/ML in QSAR/QSPR

Quantitative structure-activity/property relationship (QSAR/QSPR) models aim to determine the relationship between molecular structures and properties such as biological activity, chemicals, pharmaceuticals, toxicants, and environmental pollutants. Molecular properties descriptors, including quantitative or qualitative attributes of compound molecules are extracted to statistically represent the chemical structure properties. Molecular properties have mathematical correlation with biological activity and chemical properties. These relationships can be emerged traditionally using data mining or machine learning algorithms. QSAR has been applied in drug design, ADME/T modeling, chemical, food, agricultural and environmental sciences.[27]

Applying QSAR in drug design has brought several benefits such as saving time and cost, logical prediction of biological activity or chemical properties, and making use of previously collected data. More significantly, QSAR has proved to be efficient in the prediction of values for ADMET properties including absorption, distribution, metabolism, excretion and toxicity. Various analytical tools from statistics and machine learning are used in QSAR analysis, including predictive modeling (both classification and regression), visualization, exploratory data analysis and cluster analysis. These studies rely on the principle that similar compounds tend to have similar properties. Machine learning strategies as powerful tools have been applied in QSAR modeling, in which chemical information from large compound databases is mined to design compounds with desired biological properties. The robustness of a learning based QSAR modeling is evaluated according to its productiveness. Machine learning strategies for QSAR modeling faces challenges such as unstructured data, various molecular dimensions, and various structures and compositions [28-29] which affect the model's performance.

AI/ML in Clinical Trial Design

Clinical trials aim to verify the safety and effectiveness of a drug in human subjects for a specific medical condition. These trials demand a significant investment of around 6 to 7 years and substantial financial resources. Regrettably, the pharmaceutical industry experiences a daunting reality where only one out of every ten compounds that advance to these trials ultimately achieves the necessary approvals. This staggering attrition represents a substantial setback and financial burden for the industry. Approximately one-third of the duration of a trial is consumed by the process of enrolling patients.

The effectiveness of a clinical trial's outcome hinges significantly on the ability to successfully recruit appropriate participants. In instances where patient recruitment falls short, it results in a substantial 86% of clinical trial failures. AI could help recruit patients with a specific medical condition during different phases of clinical trials such as phase II and III. By utilizing patient-specific genome AI can aid in the identification of a specific population affected by the disease. This approach enables the primary prediction of viable targets for drugs within the selected patient group, enhancing the precision and efficiency of clinical trials. The reasons behind these failures often stem from factors such as improper patient screening, insufficient adherence to technical prerequisites, and inadequate infrastructure. Nevertheless, the considerable wealth of digital medical data presents an opportunity to mitigate these failures through the integration of AI. In essence, leveraging AI could lead to a reduction in these setbacks by enhancing patient selection, meeting technical criteria, and improving overall trial infra structure.

The use of various facets of AI, including ML and other methods, can contribute significantly to the preclinical identification of molecules and the anticipation of potential lead compounds even before commencing clinical trials. This approach aids in the early identification of lead molecules that have a higher likelihood of successfully navigating clinical trials, taking into account the specific patient population that has been targeted for the study.Patient attrition, responsible for causing 30% of clinical trial failures, imposes extra recruitment needs and results in wasted resources. This challenge can be mitigated through vigilant patient monitoring and support in adhering to the trial protocol. To address this, Ai Cure developed mobile software that effectively tracked medication adherence among schizophrenia patients in a clinical trial like phase II. Implementing this technology increased patient adherence by 25%, ensuring clinical trial success [30].

CONCLUSION AND FUTURE ASPECTS

Artificial Intelligence (AI) and Machine Learning (ML) have recently emerged as transformative technologies that are transforming Pharmaceutical Science. Integration of AI/ML technologies across multiple operational areas, including drug discovery, formulation design, quality control, clinical trials, and pharmacovigilance, has contributed to more rapid, accurate, and cost-effective outcomes. AI systems bring efficiencies by analyzing large data sets, identifying molecular targets, predicting drug-drug interactions, and optimizing production processes, all of which serve to enhance pharmaceutical innovation. AI does not, and cannot, take the place of human intelligence, but rather augments human judgement and enhances the precision of decision-making while also delivering efficiency through the automation of repetitive tasks. Remaining challenges include an unmet appetite for rigorous adherence to data privacy, regulatory stay authorities, the interpretability of AI and models, and skilled professionals to embrace the management of AI systems and ongoing validation of performance-driven implementation**.**

In the next decade or two, AI/ML will likely continue to drive a paradigm shift toward precision and personalized medicine, matching drug development and treatment plans to unique genetic and physiological profiles of individuals. Application of deep learning and predictive modeling will improve in silico drug screening and reduce the incidence of failed clinical trials while simultaneously enabling real-time monitoring of therapeutic effects. The coupling of AI with newer technologies such as the Internet of Things (IoT), blockchain, quantum computing, for example, and big data analytics - will pave the way for exciting opportunities for secure and transparent.

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An Extension of the Beta Function Using the Logarithmic Kernel and the One Parameter Mittag-Leffler Function.

Md Afzal and Ashendra Kumar Saxena

Research Scholar, Department of Mathematics, College of Engineering, Teerthanker Mahaveer University, Moradabad, India
Email: connect2afzal@gmail.com
Professor, Department of Mathematics, College of Engineering,
Teerthanker Mahaveer University, Moradabad, India.
Email: drashendra.computers@tmu.ac.in

Abstract:

This paper introduces a logarithmic Mittag–Leffler (LML) extension of the classical Beta function by incorporating a natural logarithmic kernel into the one-parameter Mittag–Leffler framework. The proposed formulation yields a compact analytic structure that preserves essential characteristics of the Euler Beta function, including symmetry, recurrence, and integral representations. Several closed-form expansions and Mellin transform formulas are derived, linking the new function with fractional-type integral operators and extended hypergeometric functions. Generating functions for the logarithmic Mittag–Leffler hypergeometric and confluent hypergeometric cases are also established, demonstrating that the extended model retains the generating-function symmetry of the classical theory. Furthermore, a corresponding statistical distribution, the LML Beta distribution is formulated, showing that the new function can serve as a normalizing constant in probability models. In all limiting cases, the function reduces smoothly to known Beta and Mittag–Leffler type extensions, ensuring analytical consistency and unification with existing results.

Keywords: Extended Beta function; Mittag–Leffler function; logarithmic kernel; fractional calculus; Mellin transform; generating functions; extended hypergeometric and confluent hypergeometric functions; statistical distribution.

Mathematics Subject Classification (2020): 33B20; 33C05; 33E12; 33 C60; 44A20; 26A33.

Introduction

The Euler Beta function is one of the fundamental special functions in analysis, with important links to the Gamma and hypergeometric functions. Its applications span

diverse areas such as probability, statistics, and mathematical physics. Because of its central role, many authors have explored possible generalizations by modifying the kernel of the classical Euler integral.

Among the earlier contributions, Chaudhry and Zubair introduced a p-extended form of the Beta function by incorporating an exponential kernel [4, 5]. Later, Shadab, Jabee, and Choi [6], followed by Goyal, Momani, Agarwal, and Rassias [7], proposed new extensions based on Mittag-Leffler functions.
Further developments have included multi-parameter Mittag-Leffler generalizations [8, 9], approaches based on fractional differential operators (Srivastava and Choi, 2012) [3] , and adaptations in statistical contexts (Khan and Husain, 2022; Ghayasuddin et al., 2020) [10, 11].
Despite these efforts, most existing formulations treat the exponential and MittagLeffler kernels independently. The present article addresses this gap by introducing a logarithmic Mittag-Leffler extension of the Beta function. The proposed definition provides a unified setting that naturally contains several earlier models as special cases, while retaining analytical tractability.
Contributions. The main contributions of this paper can be summarized as follows:
We define a new extension of the Beta function in which a logarithmic kernel is coupled with the one parameter Mittag-Leffler function, thereby presenting a novel framework that incorporates logarithmic modulation alongside fractional memory effects through the Mittag-Leffler structure.
The extended function preserves fundamental features of the classical Beta function, including symmetry, recurrence, and integral representations.
Several well-known extensions are obtained as limiting cases. In particular, the construction reduces to the Shadab-Jabee-Choi Mittag-Leffler form when $\delta = 0$, and to the Chaudhry-Zubair p-extended Beta function when $\delta = 0$ and $\alpha = 1$.
We establish Mellin transforms, series expansions, and generating functions associated with the new Beta function, which broaden its applicability to generalized hypergeometric functions and probability distributions.

Preliminaries

In this section, we present a concise overview of certain classical functions that form the analytical framework for subsequent developments. These include the Gamma, Beta, Mittag–Leffler, and Hypergeometric functions, all of which are interrelated through integral or series representations. The definitions provided here follow standard references such as Andrews, Askey, and Roy [1], and Gorenflo et al. [2], Srivastava and Choi [3] .

Gamma Function

The Gamma function generalizes the factorial operation to the complex domain and is expressed as,

$$\Gamma(\mu) = \int_0^\infty v^{\mu-1} e^{-v}\, dv, \qquad \Re\ (\mu)\ >\ 0.$$

(1)

It satisfies the recurrence relation $\Gamma\ (\mu+1) = \mu\Gamma\ (\mu)$ and the reflection formula $\Gamma(\mu)\Gamma(1-\mu) = \frac{\pi}{\sin(\pi\mu)}$, which are fundamental to many functional identities in analysis and special function theory.

Beta Function

The Euler Beta function, also known as the Eulerian integral of the first kind, is defined by

$$B(p_1, p_2) = \int_0^1 u^{p_1-1}(1-u)^{p_2-1}\, du, \qquad \Re(p_1),\Re(p_2)\ >\ 0.$$

(2)

A fundamental connection between the Gamma and Beta functions is established through,

$$B(p_1, p_2) = \frac{\Gamma(p_1)\,\Gamma(p_2)}{\Gamma(p_1+p_2)},$$

(3)

Which shows that the Beta function can be viewed as a normalized product of two Gamma functions. This relationship serves as the foundation for many generalizations and extensions in mathematical physics and fractional calculus [3, 1].

Mittag–Leffler Functions

The Mittag-Leffler function family extends the exponential function and is a central tool in the study of fractional differential and integral equations [2, 14, 15]. The most basic form, the one-parameter Mittag-Leffler function, is defined by,

$$E_\alpha(z) = \sum_{m=0}^{\infty} \frac{z^m}{\Gamma(\alpha m+1)}, \qquad \Re(\alpha) > 0 \qquad (4)$$

It reduces to the exponential function when $\alpha = 1$. The two parameter Mittag–Leffler function generalizes this form:

$$E_{\alpha,\beta}(z) = \sum_{m=0}^{\infty} \frac{z^m}{\Gamma(\alpha m+\beta)}, \qquad \Re(\alpha), \Re(\beta) > 0$$

(5)

A still broader generalization by Prabhakar [13] introduces an additional parameter γ,

$$E_{\alpha,\beta}^{\gamma}(z)=\sum_{m=0}^{\infty}\frac{(\gamma)_m z^m}{m!\Gamma(\alpha m+\beta)},\qquad \Re(\alpha),\Re(\beta),\Re(\gamma)>0\,,$$

(6)

Where $(\gamma)_m$ denotes the Pochhammer symbol,

$$(\gamma)_m=\frac{\Gamma(\gamma+m)}{\Gamma(\gamma)}=\gamma(\gamma+1)(\gamma+2)\cdots(\gamma+m-1),\qquad (\gamma)_0=1.$$

These functions form the analytic core of many fractional and extended integral representations, including the generalized Beta functions discussed in this paper.

Hypergeometric Functions

The hypergeometric series plays a unifying role among special functions and is written as

$${}_2F_1(a,b;c;z)=\sum_{n=0}^{\infty}\frac{(a)_n(b)_n}{(c)_n\,n!}\,z^n,\ |z|<1,$$

(7)

In particular, by employing integral representations involving Beta-type kernels, one can derive relations such as,

$${}_2F_1(a,b;c;z)=\frac{\Gamma(c)}{\Gamma(b)\Gamma(c-b)}\int_0^1 t^{b-1}(1-t)^{c-b-1}(1-zt)^{-a}\,dt,\quad \Re(c)>\Re(b)>0 \quad (8)$$

This representation serves as the conceptual basis for constructing extended hypergeometric functions via modified Beta integrals, as developed in later sections of this work ([4-7]).

Previous Work and Motivation

The Beta function is a classical special function with a wide range of applications in analysis, probability, statistics, and mathematical physics (see Andrews, Askey, and Roy [1], Srivastava and Choi [3]). To broaden its applicability, several authors have proposed extensions by modifying the kernel of the Euler Beta integral.

The extensions began with Chaudhry and Zubair who introduced a p-extended form

of the Beta function by incorporating an exponential kernel [4]:

$$B_p(x,y)=\int_0^1 t^{x-1}(1-t)^{y-1}e^{-\frac{p}{t(1-t)}}dt, \qquad \Re(x),\Re(y)>0,\ p\geq 0.$$

(9)

Later generalized this approach by considering a more complex exponential structure [5]:

$$B_{p,\nu}(x,y)=\int_0^1 t^{x-1}(1-t)^{y-1}\exp\left(-\frac{p}{t^{\nu}(1-t)^{\nu}}\right)dt, \qquad \Re(x),\Re(y)>0,\ p>0,\nu>0.$$

(10)

Building upon these exponential extensions, Shadab, Jabee and Choi proposed a Mittag-Leffler based extension by replacing the exponential kernel with the one parameter Mittag-Leffler function [6]:

$$B_p^{\alpha}(x,y)=\int_0^1 t^{x-1}(1-t)^{y-1}E_{\alpha}\left(-\frac{p}{t(1-t)}\right)dt, \qquad \Re(x),\Re(y)>0,\alpha>0,\ p>0.$$

(11)

Further developments included Khan et al. who introduced an extension with separate parameters in the Mittag-Leffler kernel [8]:

$$B_{p,q}^{\lambda}(x,y)=\int_0^1 t^{x-1}(1-t)^{y-1}E_{\lambda}\left(-\frac{p}{t}-\frac{q}{1-t}\right)dt, \qquad \Re(x),\Re(y)>0,\ p,q\geq 0,\lambda>0.$$

(12)

Again, Goyal, Momani, Agarwal and Rassias extended Shadab work by employing the two-parameter Mittag-Leffler (Wiman) function [7]:

$$B_{(\rho_1,\rho_2)}^{(r)}(x,y)=\int_0^1 t^{x-1}(1-t)^{y-1}E_{\rho_1,\rho_2}\left(-\frac{r}{t(1-t)}\right)dt, \qquad \Re(x),\Re(y)>0,\rho_1,\rho_2>0,r\geq 0.$$

(13)

Most recently, Khan and Husain proposed a generalized Mittag-Leffler Beta function with additional power parameters [10]:

$$B_{p,\mu,\nu}^{\alpha,\beta}(x,y)=\int_0^1 t^{x-1}(1-t)^{y-1}E_{\alpha,\beta}\left(-\frac{p}{t^{\mu}(1-t)^{\nu}}\right)dt,$$

(14)

Which converges for $\Re(x)$, $\Re(y) > 0$, $\alpha,\beta > 0$, $\mu,\nu > 0$, and $p \geq 0$.

Other notable contributions include the work of Ghayasuddin et al. [11] on statistical applications of extended Beta functions, Khan and Khan [9] on a new Mittag–Leffler–type extension, Ata [12] on Beta-type generalizations in fractional calculus, and Srivastava, Parmar, and Chopra [3] on extended fractional operators with related integral formulas and generating functions.

Motivation. Although the above generalizations are valuable, most rely on exponential kernels or involve multi-parameter Mittag-Leffler functions such as the Prabhakar form [13], which often increases algebraic complexity. The motivation of this work is to construct a compact one-parameter extension of the Beta function by employing a natural logarithmic kernel together with the one-parameter Mittag-Leffler function. This approach reduces complexity while preserving symmetry, integral representations, and other key properties of the classical Beta function.

Definition of the Natural Log Mittag-Leffler Beta Function

In this section we define Logarithmic Mittag-Leffler (LML) extension of beta function

$$\mathrm{B}_{\alpha}^{(\log)}(p_1, p_2; \delta, p) = \int_0^1 u^{p_1-1}(1-u)^{p_2-1} \ln\left(e + \delta u(1-u)\right) E_{\alpha}\left(-\tfrac{p}{u(1-u)}\right) du. \tag{15}$$

For $\alpha > 0$, $\delta \geq 0$, $p \geq 0$, and $\Re(p_1), \Re(p_2) > 0$,

The integrand is well behaved for $\delta \geq 0$ (since $\ln(e+\delta\, u(1-u)) \geq 1$ and remains bounded on (0,1)), and the Mittag-Leffler kernel E_{α} maintains established properties for $\alpha > 0$, $p \geq 0$.

Connections to Known Beta Extensions. The proposed definition admits the following limiting cases:

(i) For $\delta = 0$,

$$\mathcal{B}_{\alpha}^{(\log)}(p_1, p_2; 0, p) = \int_0^1 u^{p_1-1}(1-u)^{p_2-1} E_{\alpha}\left(-\tfrac{p}{u(1-u)}\right) du,$$

Which coincides with the Mittag-Leffler extension of Shadab-Jabee-Choi [6].

(ii) Setting $\delta = 0$ and $\alpha = 1$ (so $E_1(z) = e^z$) gives

Chaudhry–Zubair p-extended Beta [4]: $\int_0^1 u^{p_1-1}(1-u)^{p_2-1}\exp\left(-\frac{p}{u(1-u)}\right)du.$

(iii) Further, for δ = 0 and p = 0 (so $E_\alpha(0) = 1$) ,

$$B_\alpha^{(\log)}(p_1, p_2; 0, 0) = B(p_1, p_2),$$

Where $B(p_1,p_2)$ is the classical Beta function.

(iv) For the special case when δ = 0 and considering the two parameter Mittag-Leffler function $E_{\rho1,\rho2}$ instead of E_α, we recover the Wiman-type extension proposed by Goyal, Momani, Agarwal, and Rassias [7]:

$$B_{(\rho_1,\rho_2)}^{(r)}(x, y) = \int_0^1 t^{x-1}(1-t)^{y-1} E_{\rho_1,\rho_2}\left(-\frac{r}{t(1-t)}\right)dt,$$

Note: At u = 1/2, where u (1−u) attains its maximum value 1/4, the logarithmic term becomes:

$$\ln\left(e + \frac{\upsilon}{4}\right) = 1 + \ln\left(1 + \frac{\upsilon}{4e}\right),$$

Showing how δ modulates the weight at the most influential point of the integration domain.

Thus, δ controls the presence of the logarithmic deformation, while α and p provide connections to established Mittag-Leffler and exponential Beta extensions. The proposed logarithmic Mittag-Leffler Beta function serves as a unified framework that encompasses all these previous extensions as special cases, while introducing the novel logarithmic modulation through the parameter δ.

Basic Properties and Integral Representations

Property (Symmetry). The logarithmic Mittag-Leffler beta function is symmetric in p_1 and p_2:

$$\mathcal{B}_\alpha^{(\log)}(p_1, p_2; \delta, p) = \mathcal{B}_\alpha^{(\log)}(p_2, p_1; \delta, p).$$

(16)

Integral Representation (Trigonometric form). With the substitution $u = \sin^2\theta$, one obtains

$$\mathcal{B}_\alpha^{(\log)}(p_1, p_2; \delta, p) = \int_0^{\pi/2} (\cos\theta)^{2p_1-1}(\sin\theta)^{2p_2-1} \ln e + \delta\sin^2\theta\cos^2\theta) E_\alpha\left(-\frac{p}{\sin^2\theta\cos^2\theta}\right) d\theta.$$

(17)

Integral Representation (Laplace-type form). With the substitution $u = \frac{v}{1+v}$, one obtains

$$\mathcal{B}_{\alpha}^{(\log)}(p_1, p_2; \delta, p) = \int_0^{\infty} \frac{v^{p_1-1}}{(1+v)^{p_1+p_2}} \ln\left(e + \frac{\delta v}{(1+v)^2}\right) E_{\alpha}\left(-\frac{p(1+v)^2}{v}\right) dv. \qquad (18)$$

Recurrence Relation (First-order)

$$\mathrm{B}_{\alpha}^{(\log)}(p_1, p_2; \delta, p) = \mathrm{B}_{\alpha}^{(\log)}(p_1+1, p_2; \delta, p) + \mathrm{B}_{\alpha}^{(\log)}(p_1, p_2+1; \delta, p).$$

(19)

Proof. By definition,

$$\mathrm{B}_{\alpha}^{(\log)}(p_1, p_2; \delta, p) = \int_0^1 u^{p_1-1}(1-u)^{p_2-1} \ln\left(e + \delta u(1-u)\right) E_{\alpha}\left(-\tfrac{p}{u(1-u)}\right) du.$$

For convenience, set $W(u) := \ln\left(e + \delta u(1-u)\right) E_{\alpha}\left(-\tfrac{p}{u(1-u)}\right)$. then,

$$\mathcal{B}_{\alpha}^{(\log)}(p_1, p_2; \delta, p) = \int_0^1 u^{p_1-1}(1-u)^{p_2-1} W(u)\, du.$$

Now, using the identity 1 = u + (1 − u), we write

$$u^{p_1-1}(1-u)^{p_2-1} = u^{p_1}(1-u)^{p_2-1} + u^{p_1-1}(1-u)^{p_2}.$$

Multiplying by W(u) and integrating yields

$$\mathcal{B}_{\alpha}^{(\log)}(p_1, p_2; \delta, p) = \int_0^1 u^{p_1}(1-u)^{p_2-1} W(u)\, du + \int_0^1 u^{p_1-1}(1-u)^{p_2} W(u)\, du.$$

By definition, these are

$\mathcal{B}_{\alpha}^{(\log)}(p_1+1, p_2; \delta, p)$ and $\mathcal{B}_{\alpha}^{(\log)}(p_1, p_2+1; \delta, p)$. Hence the recurrence is established.

Binomial-type Identity. For n ∈ N,

$$\mathcal{B}_{\alpha}^{(\log)}(p_1, p_2; \delta, p) = \sum_{m=0}^{n} \binom{n}{m} \mathcal{B}_{\alpha}^{(\log)}(p_1+m, p_2+n-m; \delta, p).$$

(20)

Proof. Using the binomial expansion $(u + (1-u))^n = 1$, we write

$$1 = \sum \binom{n}{m} u^m (1-u)^{n-m}.$$

Multiplying both sides by $u^{p1-1}(1-u)^{p2-1}W(u)$ and integrating over [0,1] gives

$$\mathcal{B}_{\alpha}^{(\log)}(p_1, p_2; \delta, p) = \sum_{m=0}^{n} \binom{n}{m} \int_0^1 u^{p_1-1+m}(1-u)^{p_2-1+(n-m)} W(u)\, du.$$

Recognizing each term as

$\mathcal{B}_{\alpha}^{(\log)}(p_1+m, p_2+n-m; \delta, p)$ Yields the stated formula.

Note. The case n = 1 reduces to the first-order recurrence relation

$$\mathcal{B}_{\alpha}^{(\log)}(p_1, p_2; \delta, p) = \mathcal{B}_{\alpha}^{(\log)}(p_1+1, p_2; \delta, p) + \mathcal{B}_{\alpha}^{(\log)}(p_1, p_2+1; \delta, p).$$

For general n, the identity can also be obtained by applying this recurrence repeatedly. Thus the binomial-type identity admits a natural proof by mathematical induction on n.

Series Expansions

In this section, we derive the series representations for the logarithmic Mittag-Leffler extended Beta function. defined earlier in (15).

Expansion I: Generating Function (Shift in First Parameter)

For $|\tau| < 1$, the following generating function relation holds:

$$\int_0^1 \frac{u^{p_1-1}(1-u)^{p_2-1}\ln\left(e+\delta u(1-u)\right)}{1-\tau u} E_{\alpha}\left(-\frac{p}{u(1-u)}\right) du = \sum_{r=0}^{\infty} \tau^r \mathrm{B}_{\alpha}^{(\log)}(p_1+r, p_2; \delta, p).$$

(21)

Proof. Using the geometric series expansion

$$\frac{1}{1-\tau u} = \sum \tau^r u^r, \quad |\tau u| < 1,$$

Which converges uniformly for u ∈ [0,1] when $|\tau| < 1$, we substitute this expansion into the integral in (15). The interchange of summation and integration is justified by uniform convergence on [0, 1] and the absolute integrability of the kernel. Each term in the resulting series corresponds exactly to $\mathcal{B}_{\alpha}^{(\log)}(p_1+r, p_2; \delta, p)$, yielding (21).

Expansion II: Generating Function (Shift in Second Parameter)

For $|\tau| < 1$, the following generating function representation holds:

$$\int_0^1 \frac{u^{p_1-1}(1-u)^{p_2-1}\ln\left(e+\delta u(1-u)\right)}{1-\tau(1-u)} E_{\alpha}\left(-\frac{p}{u(1-u)}\right) du = \sum_{r=0}^{\infty} \tau^r \mathrm{B}_{\alpha}^{(\log)}(p_1, p_2+r; \delta, p).$$

(22)

Proof. We use the geometric series expansion

$$\frac{1}{1-\tau(1-u)} = \sum \tau^r (1-u)^r, \quad |\tau(1-u)| < 1,$$

Which converges uniformly on [0, 1] whenever $|\tau| < 1$. Substituting this expansion

into the definition (15) and interchanging the order of summation and integration, we obtain

$$\sum_{r=0}^{\infty}\tau^r\int_0^1 u^{p_1-1}(1-u)^{p_2+r-1}\ln\big(e+\delta u(1-u)\big)E_\alpha\left(-\frac{p}{u(1-u)}\right)du.$$

Each integral in this sum equals $B_\alpha^{(\log)}(p_1, p_2+r;\delta, p)$, which proves (22).

Corollary: Connection with the Shadab-type Extension

Setting δ = 0 removes the logarithmic factor and yields

$$\mathcal{B}_\alpha^{(\log)}(p_1,p_2;0,p)=\int_0^1 u^{p_1-1}(1-u)^{p_2-1}E_\alpha\left(-\frac{p}{u(1-u)}\right)du =: \mathcal{B}_\alpha(p_1,p_2;p)$$

,

Which represents the Mittag–Leffler–damped Beta function considered by Shadab et al.

In this case, the generating functions (21) and (22) reduce to

$$\int_0^1 \frac{u^{p_1-1}(1-u)^{p_2-1}}{1-\tau u}E_\alpha\left(-\frac{p}{u(1-u)}\right)du=\sum_{r=0}^{\infty}\tau^r\,\mathcal{B}_\alpha(p_1+r,p_2;p)$$

,

$$\int_0^1 \frac{u^{p_1-1}(1-u)^{p_2-1}}{1-\tau(1-u)}E_\alpha\left(-\frac{p}{u(1-u)}\right)du=\sum_{r=0}^{\infty}\tau^r\,\mathcal{B}_\alpha(p_1,p_2+r;p).$$

Furthermore, when $p \to 0^+$, the Mittag–Leffler kernel tends to unity, and the classical Beta function is recovered: $\lim B_\alpha(p_1,p_2;p) = B(p_1,p_2)$.

Thus, the present logarithmic extension generalizes the Mittag–Leffler–damped Beta function proposed by Shadab et al., and in the limiting case, it reduces to the classical Beta function.

Mellin Transform

Define the Mellin transform of $\mathcal{B}_\alpha^{(\log))}$ with respect to p by,

$$\mathcal{M}\{\mathcal{B}_\alpha^{(\log)}(p_1,p_2;\delta,p);s\} := \int_0^\infty p^{s-1}\,\mathcal{B}_\alpha^{(\log)}(p_1,p_2;\delta,p)\,dp,\ \ \Re(s) > 0. \quad (23)$$

Theorem 7.1. Let $\Re(p_1) > 0$, $\Re(p_2) > 0$, $\alpha > 0$, $\delta \geq 0$, and $0 < \Re(s) < \alpha$. Then

$$M = \Gamma(s)\Gamma\left(1-\frac{s}{\alpha}\right)\int_0^1 u^{p_1+s-1}(1-u)^{p_2+s-1}\ln\left(e+\delta u(1-u)\right)du. \qquad (24)$$

Proof (sketch). Starting from the definition,

$$B_\alpha^{(\log)}(p_1, p_2; \delta, p) = \int_0^1 u^{p_1-1}(1-u)^{p_2-1}\ln\left(e+\delta u(1-u)\right)E_\alpha\left(-\tfrac{p}{u(1-u)}\right)du.$$

We substitute this into the Mellin transform and, using Fubini's theorem (valid since $\Re(p_1),\Re(p_2) > 0$ and $0 < \Re(s) < \alpha$ ensure absolute convergence), obtain

$$M = \int_0^1 u^{p_1-1}(1-u)^{p_2-1}\ln\left(e+\delta u(1-u)\right)\left[\int_0^\infty p^{s-1}E_\alpha\left(-\frac{p}{u(1-u)}\right)dp\right]du.$$

Let $c = u(1-u) \in (0, \frac{1}{4})$ and substitute p = ct. Then the inner integral becomes

$$c^s \int_0^\infty t^{s-1}E_\alpha(-t)\,dt.$$

From the classical Mellin transform identity of the Mittag-Leffler function (see Kilbas and Saigo [14]; Haubold, Mathai, and Saxena [15]) ,

$$\int_0^\infty t^{s-1}E_\alpha(-t)\,dt = \Gamma(s)\,\Gamma\left(1-\frac{s}{\alpha}\right), \quad 0 < \Re(s) < \alpha,$$

We obtain

$$I(s) = c^s\,\Gamma(s)\,\Gamma\left(1-\frac{s}{\alpha}\right)$$

.

Substituting this result back gives the stated expression (24).

Remark (log-weighted Beta form): The u-integral in (24) is a Beta-type integral with a logarithmic weight. Thus, the Mellin transform represents a logarithmically weighted Beta-type function. In the limiting case $\delta = 0$, the logarithmic factor disappears and

$$\mathcal{M} = \Gamma(s)\,\Gamma\left(1-\frac{s}{\alpha}\right)B(p_1+s,\, p_2+s),$$

Recovering the classical Gamma–Gamma–Beta structure associated with the exponential kernel models of Chaudhry and Zubair.

Differential Relations

Derivative relations of $\mathcal{B}_\alpha^{(\log)}$ with respect to its parameters can be obtained by differentiating under the integral sign.

Derivative with respect to p_1 by definition,

$$\mathrm{B}_{\alpha}^{(\log)}(p_1, p_2; \delta, p) = \int_0^1 u^{p_1-1}(1-u)^{p_2-1}\ln\left(e+\delta u(1-u)\right)E_{\alpha}\left(-\tfrac{p}{u(1-u)}\right)du.$$

Differentiating with respect to p_1 yields

$$\frac{\partial}{\partial p_1}\mathrm{B}_{\alpha}^{(\log)}(p_1, p_2; \delta, p) = \int_0^1 u^{p_1-1}(1-u)^{p_2-1}\ln(u)\ln\left(e+\delta u(1-u)\right)E_{\alpha}\left(-\tfrac{p}{u(1-u)}\right)du.$$

(25)

Derivative with respect to p_2. Similarly,

$$\frac{\partial}{\partial p_2}\mathrm{B}_{\alpha}^{(\log)}(p_1, p_2; \delta, p) = \int_0^1 u^{p_1-1}(1-u)^{p_2-1}\ln(1-u)\ln\left(e+\delta u(1-u)\right)E_{\alpha}\left(-\tfrac{p}{u(1-u)}\right)du.$$

(26)

Derivative with respect to δ, since δ only appears in the logarithmic factor,

$$\frac{\partial}{\partial \delta}\mathrm{B}_{\alpha}^{(\log)}(p_1, p_2; \delta, p) = \int_0^1 \frac{u^{p_1}(1-u)^{p_2}}{e+\delta u(1-u)}E_{\alpha}\left(-\tfrac{p}{u(1-u)}\right)du.$$

(27)

Derivative with respect to p, differentiating inside the kernel gives,

$$\frac{\partial}{\partial p}\mathrm{B}_{\alpha}^{(\log)}(p_1, p_2; \delta, p) = -\frac{1}{\alpha}\int_0^1 u^{p_1-2}(1-u)^{p_2-2}\ln\left(e+\delta u(1-u)\right)E_{\alpha-1,\alpha}\left(-\tfrac{p}{u(1-u)}\right)du,$$

(28)

Where $E_{\alpha-1,\alpha}(z)$ denotes the two parameter Mittag–Leffler function.

Remark.

Derivatives with respect to p_1 and p_2 introduce additional logarithmic weights ln(u) and ln(1 − u) in the integrand.
The derivative with respect to δ has a rational form, which shows how the logarithmic kernel deforms smoothly as δ varies.
The derivative with respect to p connects the one-parameter Mittag-Leffler kernel to

its two-parameter generalization, thereby linking the function to fractional calculus.

Remark. The inclusion of differential relations is standard in the theory of special functions, since they reveal how the function responds to variations of its parameters. For the present extension, derivatives with respect to p_1 and p_2 naturally introduce logarithmic weights, while the derivative with respect to p links the one-parameter Mittag-Leffler kernel to its two-parameter generalization. Such formulas are not only consistent with earlier works on extended Beta and Gamma functions (e.g., Chaudhry-Zubair, Shadab –Jabee-Choi), but also serve as a foundation for applications in fractional calculus, hypergeometric type series, and statistical modelling. Hence, their inclusion ensures that the study of $B_{\alpha}^{(\log)}$ is both complete and comparable with existing literature.

Statistical Distribution Associated with the LML– Beta Function

In probability theory, the Beta function serves as the normalizing constant of the Beta distribution. Since our definition introduces the Logarithmic Mittag–Leffler Beta (LML– Beta) function, we can naturally construct a corresponding probability distribution on the interval (0,1).

Definition 9.1 (LML–Beta Distribution)**.** Let $\alpha > 0$, $\delta \geq 0$, $p \geq 0$, and $\Re(p_1), \Re(p_2) > 0$. A random variable X is said to follow the LML–Beta distribution with parameters $(p_1, p_2; \alpha, \delta, p)$ if its probability density function (pdf) is given by

$$f_X(x; p_1, p_2; \alpha, \delta, p) = \frac{x^{p_1-1}(1-x)^{p_2-1} \ln\left(e + \delta x(1-x)\right) E_{\alpha}\left(-\frac{p}{x(1-x)}\right)}{B_{\alpha}^{(\log)}(p_1, p_2; \delta, p)}, \qquad 0 < x < 1,$$

(29)

and $f_X(x) = 0$ otherwise.

Here, ln denotes the natural logarithm (base e), ensuring the kernel is positive on (0,1) and equals unity when $\delta = 0$. The denominator $\mathcal{B}_{\alpha}^{(\log)}(p_1, p_2; \delta, p)$ serves as the normalizing
Constant, ensuring that $\int_0^1 f_X(x)\, dx = 1$.

Moments

Let $r > -p_1$. The r-th raw moment of X is defined by

$$\mathbb{E}[X^r] = \int_0^1 x^r\, f_X(x; p_1, p_2; \alpha, \delta, p)\, dx,$$

where f_X is the pdf of the LML–Beta distribution. Substituting the definition of f_X

gives

$$\mathrm{E}[X^{r}] = \frac{1}{\mathrm{B}_{\alpha}^{(\log)}(p_1, p_2; \delta, p)} \int_0^1 x^{p_1+r-1}(1-x)^{p_2-1} \ln\left(e + \delta x(1-x)\right) E_{\alpha}\left(-\tfrac{p}{x(1-x)}\right) dx.$$

Recognizing the integral in the numerator as $\mathrm{B}_{\alpha}^{(\log)}(p_1 + r, p_2;\delta,p)$, we obtain

$$\mathbb{E}[X^{r}] = \frac{\mathcal{B}_{\alpha}^{(\log)}(p_1 + r,\ p_2; \delta, p)}{\mathcal{B}_{\alpha}^{(\log)}(p_1,\ p_2; \delta, p)}.$$

(30)

The condition $r > -p_1$ ensures convergence of the moment integral near $x = 0$, since the integrand behaves as x^{p_1+r-1} in that region. In particular, the mean and variance follow by taking $r = 1$ and combining the cases $r = 1,2$, respectively.

Mean and Variance

The mean is obtained as a special case of the raw moment:

$$\mu = \mathbb{E}[X] = \frac{\mathcal{B}_{\alpha}^{(\log)}(p_1 + 1,\ p_2; \delta, p)}{\mathcal{B}_{\alpha}^{(\log)}(p_1,\ p_2; \delta, p)}.$$

(31)

The variance is then

$$\sigma^2 = \frac{\mathcal{B}_{\alpha}^{(\log)}(p_1 + 2,\ p_2; \delta, p)}{\mathcal{B}_{\alpha}^{(\log)}(p_1,\ p_2; \delta, p)} - \left(\frac{\mathcal{B}_{\alpha}^{(\log)}(p_1 + 1,\ p_2; \delta, p)}{\mathcal{B}_{\alpha}^{(\log)}(p_1,\ p_2; \delta, p)}\right)^2.$$

(32)

Moment Generating Function

The moment generating function (mgf) of X is given by the series

$$M_X(t) = \mathbb{E}[e^{tX}] = \sum_{n=0}^{\infty} \frac{t^n}{n!} \frac{\mathcal{B}_{\alpha}^{(\log)}(p_1 + n,\ p_2; \delta, p)}{\mathcal{B}_{\alpha}^{(\log)}(p_1,\ p_2; \delta, p)}.$$

(33)

Cumulative Distribution Function

Define the incomplete LML–Beta function by

$$\mathrm{B}_{\alpha}^{(\log)}(z; p_1, p_2; \delta, p) = \int_0^z x^{p_1-1}(1-x)^{p_2-1} \ln\left(e + \delta x(1-x)\right) E_{\alpha}\left(-\tfrac{p}{x(1-x)}\right) dx.$$

(34)

Then the cumulative distribution function (CDF) of X is

$$F_X(z) = \frac{\mathcal{B}_{\alpha}^{(\log)}(z;\, p_1, p_2; \delta, p)}{\mathcal{B}_{\alpha}^{(\log)}(p_1, p_2; \delta, p)}, \qquad 0 < z < 1.$$

(35)

Thus, the LML–Beta distribution extends the classical Beta distribution through additional parameters (α,δ,p), providing greater flexibility in modeling skewness and tail behavior.

Remark. For δ = 0, the LML Beta distribution reduces to the Mittag–Leffler Beta distribution studied by Shadab et al., and for δ = p = 0, it coincides with the classical Beta distribution.

Hypergeometric Extensions

It is worth noting that the newly introduced Logarithmic Mittag-Leffler Beta function $\mathcal{B}_{\alpha}^{(\log)}(p_1, p_2; \delta, p)$ can also serve as a kernel to define extended Gauss hypergeometric and

Confluent hypergeometric functions in both series and integral forms. Such extensions would parallel the constructions given in the works of Chaudhry, Shadab, Talha and others, where the classical Beta function is replaced by its generalizations. A detailed study of these extensions is left for future work.

Generating Functions

The following logarithmic Mittag–Leffler extension of the Gauss hypergeometric series is defined by

$${}_2F_1^{\log,\alpha}(a,b;c;z) = \sum_{m=0}^{\infty} \frac{(a)_m (b)_m}{(c)_m\, m!} \frac{\mathrm{B}_{\alpha}^{(\log)}\left(b+m, c-b; \delta, p\right)}{B\left(b, c-b\right)} z^m, \qquad |z| < 1,$$

(36)

Theorem 1. If $\Re(c) > \Re(b) > 0$ and $|t| < 1$, then

$$\sum_{n=0}^{\infty} \frac{(b)_n}{n!}\, {}_2F_1^{(\log)}(a, b+n; c+n; z)\, t^n = (1-t)^{-b}\, {}_2F_1^{(\log)}\left(a, b; c; \tfrac{z}{1-t}\right).$$

(37)

Proof (coefficient extraction).

Expand the right-hand side using $(1-t)^{-b} = \sum_{n\geq 0} \frac{(b)_n}{n!} t^n$ and $(1-t)^{-m} = \sum_{k\geq 0} \frac{(m)_k}{k!} t^k$.

From (36) with $z \to z/(1-t)$,

$$ {}_2F_1^{(\log)}\left(a,b;c;\tfrac{z}{1-t}\right)=\sum_{m\ge 0}\frac{(a)_m(b)_m}{(c)_m m!}\frac{\mathrm{B}_\alpha^{(\log)}(b+m,c-b)}{B(b,c-b)}z^m(1-t)^{-m}. $$

Multiplying by $(1-t)^{-b}$ and taking the Cauchy product, the coefficient of t^n equals

$$ \frac{(b)_n}{n!}\sum_{m=0}^{\infty}\frac{(a)_m(b)_m}{(c)_m m!}\frac{\mathrm{B}_\alpha^{(\log)}(b+m,c-b)}{B(b,c-b)}z^m(m)_n. $$

Using $(m)_n=\frac{(b+n)_m}{(b)_m}$ (a standard Pochhammer shift identity), this coefficient becomes

$$ \frac{(b)_n}{n!}\sum_{m=0}^{\infty}\frac{(a)_m(b+n)_m}{(c+n)_m m!}\frac{\mathrm{B}_\alpha^{(\log)}\left(b+n+m,c-b\right)}{B(b,c-b)}z^m., $$

Which is precisely ${}_2F_1^{(\log)}(a,b+n;c+n;z)$ multiplied by $(b)_n/n!$. Summing over n yields (37).

Remark. No auxiliary "scaling lemma" is needed; the argument only uses binomial expansions and the identity $(m)_n=\frac{(b+n)_m}{(b)_m}$.

Confluent hypergeometric of ${}_1F_1$

Define confluent case,

$$ {}_1F_1^{(\log)}(a;c;z)=\sum_{m=0}^{\infty}\frac{(a)_m}{(c)_m m!}\frac{\mathrm{B}_\alpha^{(\log)}(a+m,c-a;\delta,p)}{\mathrm{B}(a,c-a)}z^m. $$

(38)

Theorem 2. For $|t|<1$,

$$ \sum_{n=0}^{\infty}\frac{t^n}{n!}\,{}_1F_1^{(\log)}(a+n;c+n;z)=e^t\,{}_1F_1^{(\log)}\ a;c;\tfrac{z}{1-t}). $$

(39)

Proof (coefficient extraction). Expand $e^t=\sum_{n\ge 0}\frac{t^n}{n!}$, *and* $(1-t)^{-m}=\sum_{k\ge 0}\frac{(m)_k}{k!}t^k$ in

$$ {}_1F_1^{(\log)}\left(a;c;\tfrac{z}{1-t}\right)=\sum_{m\ge 0}\frac{(a)_m}{(c)_m m!}\frac{\mathrm{B}_\alpha^{(\log)}(a+m,c-a)}{\mathrm{B}(a,c-a)}z^m(1-t)^{-m}. $$

The coefficient of t^n the right side of (39) is

$$ \sum_{m=0}^{\infty}\frac{(a)_m}{(c)_m m!}\frac{\mathrm{B}_\alpha^{(\log)}(a+m,c-a)}{\mathrm{B}(a,c-a)}z^m(m)_n=\sum_{m=0}^{\infty}\frac{(a+n)_m}{(c+n)_m m!}\frac{\mathrm{B}_\alpha^{(\log)}(a+n+m,c-a)}{\mathrm{B}(a+n,c-a)}z^m, $$

Which equals ${}_1F_1^{(\log)}(a+n;c+n;z)$ Summing over n gives (39).

Conclusion and Future Work

In this study, we have formulated a new logarithmic Mittag-Leffler (LML) framework that extends the classical Beta and Gamma functions through the inclusion of a logarithmic kernel coupled with the one-parameter Mittag-Leffler function. This compact formulation preserves key analytical properties such as symmetry, recurrence, and integral consistency, while naturally collapsing to the Eulerian Beta and Gamma functions in the limiting case $\delta \to 0$.

The developed approach successfully integrates the Mittag-Leffler structure with logarithmic modulation, providing a unified analytic setting that connects traditional exponentialtype extensions with fractional type generalizations. Its versatility is further demonstrated through the derivation of related hypergeometric and confluent hypergeometric functions, as well as the construction of generating functions that exhibit a clear functional dependence on the newly introduced parameters (α,δ,p).

Future Work: Potential extensions include multi-parameter Mittag-Leffler generalizations, statistical applications, and studies of related fractional differential equations.

Overall, the proposed logarithmic Mittag-Leffler extension provides a flexible analytic foundation that not only generalizes well-known classical functions but also opens new directions for research in fractional analysis, applied mathematics, and mathematical physics.

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