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Innovations and Research in Science and Technology

Volume II

Editors:
Dr. D. Brighty
Dr. Deepak Shelke
Ms. Radhika P. Karad
Dr. Sujata A. Mankar

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PREFACE

The rapid transformations in the global scientific landscape are driven by relentless innovation, interdisciplinary collaboration, and a shared commitment to advancing human knowledge. *Innovations and Research in Science and Technology* is a collective effort to present contemporary developments, emerging trends, and progressive ideas from diverse scientific fields. This volume brings together researchers, academicians, and practitioners who contribute valuable insights and novel perspectives that have the potential to influence future scientific inquiry and technological advancement.

Science and technology today are no longer confined to isolated domains; major breakthroughs emerge at the interface of disciplines. From materials science, biotechnology, environmental studies, and computational modeling to applied physics, chemistry, and engineering, this book reflects a broad spectrum of ongoing research. Each chapter aims to bridge theoretical concepts with practical applications, highlighting how innovative thinking and experimental approaches are shaping solutions for complex real-world challenges.

The contributors have undertaken significant efforts to present current research trends, well-supported analyses, and meaningful interpretations. This compilation will serve as a useful reference not only for students and researchers but also for industry professionals and policymakers seeking to understand the expanding horizons of science and technology. We hope that the ideas presented here will inspire further exploration, foster academic dialogue, and encourage collaborative endeavors across institutional and geographical boundaries.

We express our sincere appreciation to all the authors for their dedication and timely contributions. We also extend our thanks to our editorial team, reviewers, and all those who supported the publication of this volume. Their collective efforts have made this book possible.

It is our sincere belief that *Innovations and Research in Science and Technology* will stimulate curiosity, promote research culture, and serve as a platform for intellectual growth in the scientific community.

- Editors

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ISOLATION AND PURIFICATION OF PHOSPHATASE ENZYME FROM *VIGNA* SPECIES: A COMPREHENSIVE REVIEW

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Abstract:
Leguminous crops are known for their rich content of essential nutrients and enzymes that support plant development, with phosphatases especially acid phosphatases playing a central role in phosphate metabolism and mobilization. This function is particularly crucial during seed germination and the early stages of seedling growth. Among these crops, black gram (*Vigna mungo*) and mung bean (*Vigna radiata*) are notable both for their nutritional significance and agricultural importance. Investigating their enzyme systems not only advances our understanding of plant biochemistry but also offers valuable insights for industrial biotechnology applications. Acid phosphatases, which operate most efficiently in acidic conditions, are widespread in plant tissues and contribute to phosphate recycling, stress adaptation, and metabolic control. Previous studies on *Vinga mungo* and *Vinga radiata* have outlined detailed purification methods and kinetic characterizations, revealed the presence of multiple isoenzymes and shed light on their regulation.

Keywords: Phosphatase, *Vigna mungo,* Chromatography, Purification

1. Introduction:

Phosphatases represent a heterogeneous ensemble of enzymes that are indispensable for cellular signalling, metabolic regulation, and the preservation of phosphate homeostasis. The leguminous species *Vigna mungo* and *Vigna radiata* constitute not only significant sources of protein within human nutrition but also function as invaluable models for the investigation of enzyme activity and purification methodologies. Recent scholarly inquiries have increasingly concentrated on the isolation and purification of phosphatases derived from these legumes, attributable to their prospective applications within the realms of biotechnology and agriculture. The utilization of phosphatase enzymes in plant systems is paramount for a multitude of physiological processes, notably in the acquisition of phosphorus (P) and the modulation of signalling pathways. These enzymes facilitate the hydrolytic cleavage of phosphate esters, thereby enabling plants to harness inorganic phosphate and mineralize organic phosphorus, which are critical for their growth and

metabolic functions. The ensuing sections will elucidate the roles and applications of phosphatases in plant systems.

Acid Phosphatases Functions in Phosphorus Acquisition:

These enzymes catalyse the hydrolysis of phosphate esters, thereby playing an essential role in the metabolism of phosphate within plant tissues. They are particularly significant in the scavenging of inorganic phosphate from vacuole compartments (Sharma *et al.,* 2023).

1. **Response to Phosphorus deficiency**: The activity of phosphatases exhibits an elevation under conditions of phosphorus deficiency, thereby enhancing the bioavailability of organic phosphorus. Empirical studies indicate that mono-esterase activity is markedly inhibited by inorganic phosphorus yet stimulated by the presence of organic phosphorus (Janes-Bassett, 2022).
2. **Role in Protein Phosphatases Signalling Pathways**: These enzymes are integral to the regulation of signalling cascades in response to environmental stressors, such as salinity. They deactivate phosphorylated signalling components, thus sustaining cellular equilibrium and facilitating adaptive stress responses (Banerjee & Roychoudhury, 2020).
3. **Phosphatidic Acid Phosphatases:** These enzymes participate in lipid biosynthesis and signalling mechanisms, converting phosphatidic acid into diacylglycerol, which is vital for membrane integrity and signalling pathways (Nakamura & Ohta, 2010). While phosphatases are crucial for plant growth and responses to stress, their enzymatic activity can be modulated by a variety of environmental factors, including nutrient availability and soil conditions. A comprehensive understanding of these dynamics is essential for the optimization of agricultural practices and the enhancement of crop resilience.

Bacterial Phosphatases:

Bacterial phosphatases are integral to cellular signalling and metabolic processes, influencing a wide range of biological functions. Their roles in bacterial pathogenesis, biotechnology, and metabolic regulation underscore the importance of continued research in this area. As studies advance, a deeper understanding of bacterial phosphatases may pave the way for novel therapeutic approaches and biotechnological applications, emphasizing the need for ongoing exploration of these fascinating enzymes (Brynhildur Thors *et al.,* 2008). The identification of phosphatase interactomes has provided insights into the broader regulatory networks in which these enzymes operate, revealing potential crosstalk between different signalling pathways.

The regulation of phosphatase activity in bacteria is complex and involves various mechanisms, including post-translational modifications and protein-protein interactions. Studies have indicated that the activity of bacterial phosphatases can be modulated by the phosphorylation of specific

residues, which can either enhance or inhibit their enzymatic function (Mijakovic and Deutscher, 2015). This fine-tuning of phosphatase activity is crucial for bacterial cells to respond appropriately to internal and external signals. Phosphatases can localize to diverse cellular compartments, including vacuoles, cytosol, plasma membrane, and extracellular spaces such as the rhizosphere, reflecting their multifaceted physiological roles and interactions with both internal cellular metabolism and external soil environments. *Vigna* species are recognized for their diverse bioactive compounds that contribute to both plant health and human nutrition. These legumes, including *Vigna radiata, Vigna unguiculata*, and *Vigna umbellata*, are rich in proteins, phenolic compounds, and saponins, which have been shown to possess various health-promoting properties (Mubarak, 2005).

Phosphatases are crucial enzymes in the phosphorus cycle, facilitating the conversion of organic phosphorus into inorganic forms that plants can readily absorb. This enzymatic activity is particularly vital in phosphorus-deficient soils, where the availability of nutrients is limited. Phosphatases are produced by both plants and soil microorganisms, including mycorrhizal fungi, and they play significant roles in enhancing plant nutrition, growth, and defence against pathogens. Using small molecules to hit phosphatases is turning into a promising way to make new medicines. A number of inhibitors have already moved into human trials, but it's still hard to precisely dial these enzymes up or down in patients (Guo *et al.,* 2023).

Plant Phosphatases:

The enzymes referred to as acid phosphatases (EC 3.1.3.2) have significance for the metabolism of phosphate in tissues due to them catalyse transphosphorylation activities and encourage the hydrolysis of many different orthophosphate esters in acidic conditions. Intracellular and secretory acid phosphatases play important roles in the exploitation and detoxification of inorganic phosphate (Pi) as well as the turnover of Pi-rich sources present in plant vacuoles. (Sharma *et al.,* 2023). Therefore, for a full comprehension of the role of acid phosphatases in plant energy metabolism, one must have a detailed grasp of the structural features, specificity, and physiochemical properties of these enzymes.

Vigna species highlight the significance of understanding their endogenous enzymatic machinery, specifically phosphatases (Prazeres *et al.,* 2017) These enzymes may directly enhance nutrient bioavailability by breaking down anti-nutritional substances like phytic acid, or they may indirectly influence metabolic pathways that lead to the creation of beneficial bioactive chemicals. This relationship between fundamental enzyme research and the practical health and nutritional value of these crops is a tempting subject for additional inquiry.

Material and Methods for Isolation of Phosphatase:

Part of plant	Extraction method	Key findings	Reference
Seeds *Vigna radiata* (mung bean)	1) Ammonium sulphate precipitation 2) DEAE cellulose ion-exchange chromatography	Purified acid phosphatase from germinating *V. radiata* seeds. Two isoenzymes (29 kDa and 18 kDa) were resolved by SDS-PAGE. The 29 kDa form was characterized further, including kinetics, pH optimum, inhibition, etc.	Sadia Nadir *et al., 2012*
Leaves *Phaseolus vulgaris* (common bean)	1) Induction under phosphate starvation; 2) Enzyme activity assays; partial purification (details)	In *"Induction of a Major Leaf Acid Phosphatase"* they investigate how P starvation boosts a major leaf ACP. They did partial purification and characterized induction kinetics.	X. Yan, *et al.,* 2001
Roots (cell wall bound) *White clover*	Extraction of cell wall associated fraction (1 M NaCl)	Used a three-step FPLC protocol: first hydrophobic chromatography, then further purification, to isolate multiple acid phosphatase isoforms associated with the cell wall of *white clover* roots.	Researchers at Massey University
Shoots (similar to leaves) *Vigna aconitifolia*	1) Ammonium sulfate precipitation 2) DEAE-cellulose ion-exchange 3) Chromatography Sephadex G-200 gel filtration	They purified acid phosphatase from the shoot of *Vigna aconitifolia*. Achieved ~60-fold purification, characterized pH optimum (~5.4), studied active-site residues (via inhibition, mechanism), kinetics, etc.	Mohammed A. Al-Omair *et al.,* 2010

Seeds:

The seeds were soaked overnight in water, drained, spread out on paper towels, and kept for germination. The seeds were allowed to germinate at room temperature or at 37°C for an average of four days. (Lightle *et al.,* 2021)

The germinated mung bean seeds were crushed with pre-chilled mortar and pestle and mixed uniformly in 25 ml precooled extraction buffer (0.1M citrate buffer pH 5.2, 40 mM EDTA). The

homogenate was filtered through four layers of muslin cloth and centrifuged at 10000 rpm for 30 min at 4°C. The filtrate was used for partial purification of acid phosphatase. (Roy *et al.,* 2012).

Extraction Methods:

Various types of extraction techniques exist. Typically, seeds are sterilized using sodium hypochlorite and mercury chloride (Asaduzzaman *et al.,* 2011). Homogenization can be performed using different methods, such as a mortar and pestle. This is a widely used technique. Other methods include blender homogenization and the use of liquid nitrogen. Certain buffers are utilized for extraction (Nadir *et al.,* 2012). Filtration is employed to eliminate large debris found in phosphatase enzyme. Muslin cloth and Whatman filter paper are used for this process. The centrifugation technique is also applied.

Partial Purification:

The supernatant was purified by dissolving 0.166 g ammonium sulphate per ml of enzyme extract to bring the final concentration of 30%. The mixture was kept on ice for 30 min and centrifuged (REMI C-24 cooling centrifuge) at 10000 rpm for 30 min at 4°C. The precipitate was removed and supernatant was again brought to 70% final concentration by addition 0.253 g ammonium sulphate per ml of extract. The mixture was kept on ice for 30 min and centrifuged at 12000 rpm for 30 min. The pellet was dissolved in 2 ml extraction buffer and dialyzed against the same buffer with constant stirring for overnight. It was used for acid phosphatase activity assay (Roy *et al.,* 2012).

Determination of Acid Phosphatase Activity:

The amount of p-nitrophenol (pNP) produced from the substrate p-nitrophenyl phosphate (pNPP) was used to assess acid phosphatase activity. (Campbell *et al.,* 1978). The reaction mixture containing pNPP (2mM), crude enzyme (50 µl) and citrate buffer (50 mM, pH 5.0) was incubated at 300C for 30 min, then 2 ml of sodium hydroxide (0.1N) was added to stop the reaction and absorbances were measured at 430 nm using a spectrophotometer. pNP was used as standard. One unit of enzyme (U) is defined as amount of enzyme release 1ìmol of pNP per min under experimental conditions. Specific activity of acid phosphatase is defined as the amount of enzyme that liberates 1.0 µM p-nitrophenol/ minute/mg of protein.

Results and Discussion:

When we utilized PNPP as a substrate for *Vigna mungo* and *Vigna radiata*, Na it resulted in a relative activity of 100%. Nadir *et al.* (2012). Acid Phosphatase Isolation, Purification, and Characterization from Vigna radiata Seed Germination.

The molecular weight varies between 18 kDa and 34.5 kDa. Xu, Q., *et al.* (2025). The optimal pH for specific activity is between 5 and 5.5. The temperature needed to demonstrate peak activity ranges from 50 to 70 degrees (Xu *et al.,* 2025; Kumar and Singh, 2010).

There are several methods for isolating the phosphatases enzyme, but it *Vigna mungo* especially *Vigna radiata* include a significant amount of the enzyme. Although there are methods for extraction and purification, homoginazation and mortal and pestel provide more effective enzymes. 30% of ammonium sulphate precipitation is attained, followed by 70%. There are still some contaminants in this precipitation, so we use chromatography to get rid of them. Gel filtration yields a yield of 65.3%, and ion exchange chromatography yields a yield of 21.9% for a particular activity. (Assaduzzaman *et.al* 2011) Additionally, the yield after gel filtration is 65.3%.

Another method is gel excision from SDS-PAGE. Electrophoresis is carried out in sodium dedecyl sulphate PAGE. (Hailey Elizabeth Lightle, 2021) Proteins were sorted using their molecular weight and size in SDS-PAGE.

Conclusion:

Phosphatases exist in microbial, plant, fungal, and mammalian systems. Research on their separation and purification continuously demonstrates that these enzymes are extremely different but have similar biochemical behaviours. A well-established purification framework is highlighted in the literature, which starts with crude extraction and concentration stages like ammonium sulphate precipitation. Chromatographic techniques including ion-exchange, gel filtration, and affinity chromatography come next. Recombinant expression with affinity tags and aqueous two-phase partitioning are two more recent techniques that have improved purity, yield, and scalability while cutting down on the time needed for processing. The significance of thorough biochemical characterisation following purification can be observed by the fact that purified phosphatases show distinctive pH and temperature optima, metal-ion sensitivities, and substrate specificities that reflect their biological origins across examinations.

The compromise between high purity and acceptable yield, the stability of enzymes during multi-step purification, and the complexity of separating closely related isoforms are among the major obstacles that still need to be overcome despite recent advancements. Promising ways to get around these restrictions include membrane-based purification techniques, tailored recombinant phosphatases, and monolithic columns. Overall, the amount of research indicates that phosphatase purification has become effective and versatile, allowing for widespread use in agriculture, biotechnology, environmental remediation, and medical diagnosis. The scientific and industrial applications of these vital enzymes will continue to grow as scalable and economical techniques are developed.

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MULTI-MODAL CLIMATE PARAMETERS CHANGING THE CROP YIELD PRODUCTION

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Abstract:

Climate parameters have the significant impact on crop growth and variations in yield gap became one of the most critical challenges in agriculture field. Yield gaps across selected regions, driven by climatic variability and soil moisture availability. The growing gap between potential and actual crop yields intensifies food insecurity for staple crops such as wheat. It is essential to identify and quantify the drivers of yield gaps to develop effective, evidence-based strategies for yield improvement and sustainable agricultural practices. Quantified gaps form a compact foundation for further process-based crop modeling designed at identifying specific agronomic, environmental, and socioeconomic yield gap drivers.

This paper focuses on assessing wheat yield variability across major wheat-producing regions in India and impact of climate factors. The analysis incorporates historical yield records, soil moisture data, and climatic variables such as precipitation and temperature. The analysis performed using linear regression model to evaluate the variation in wheat yield and climate variables. The regression analysis performance is measured with a root mean square error (RMSE), mean absolute error (MAE) and coefficient of determination (R^2) the model's ability to explain wheat yield variability at the national level.

Keywords: Climate, Wheat, Yield Gap, Climate, Linear Regression, Food Security.

Introduction:

Agriculture plays a vital role in global food provider in the world, with wheat being one of the most significant staple crops in India. However, wheat yield prediction and adaptive farming strategies face challenges due to dynamic climate conditions, soil variability, and fluctuating market trends. The strategic change is essential to increase the crop yield production to provide universal health, wealth, or environmental sustainability to communities around the world (Kerry *et al.,* 2021). Globally, more than two billion people experience food insecurity and obesity arising health problems. Agriculture systems playing dual role as a vehicle to crop yield production as well as the driver to provide the food security (Malhi *et al.,* 2021). The crop yield

production is to be impacted by temperature variations, irregular rainfalls, soil moisture, extreme weather conditions and climate change. This is aggravated by yield production depending on the seasonal and regional environment situations which are ultimately affecting on the increase in crop yield and market prices (Amit *et al.,* 2022). Farmers need to be aware in advance about the variations about the climate changes through smart technologies for helping and providing solutions along with planned agronomic management.

The existing yield prediction techniques are depending on the statistical methods and which often fail to adapt to real-time environmental variations. Similarly, the static decision-making approaches in farming do not optimize crop management strategies dynamically, leading to the economic losses. Various existing machine learning methods are available for crop yield prediction and estimation. This paper focus on the analysis of wheat yield data and yield predictions of major wheat-producing regions in India (Di *et al.,* 2022; Iqbal *et al.,* 2024). The findings with the established that machine learning model has a better fit and were functional for studying the complex non-linear relationships (Nayana *et al.,* 2022). This paper focus on the various multi-modal climate parameters such as temperature, rainfall, soil condition and their impact on the wheat crop yield production (Aslıhan *et al.,* 2025). Machine learning approach, linear regression model was used for climate variability analysis and computed the wheat crop yield gap.

Climate change is a continuous process in the weather arrays across a global world. The climate variability has an influence on the sustainability of agricultural sectors concerning the scenario of food production and food supplies. It is challenging to theose countries with agriculture as an integral part of their economy and total productivity (Kashif *et al.,* 2022). The changes in climatic parameters such as temperature variations and rainfall significantly affect the crop growth as well as production. The increase in temperature has directly affect to reduce the soil moisture and eventually decrease the yield production. While the increase in precipitation controls the increase in temperature. Crop productivity depends on adaptation abilities of the crop, climate scenario, and CO_2 fertilization effects (Satyendra *et al.,* 2023). The net revenue of farmers is found to be decreasing significantly with a decrease in precipitation or increase in temperature. The effects of seasonal weather variables and extremes on the mean yield and yield variability in India changes due to large rainfall variation. However, considering the importance of water availability to crop yields, the study suggests improving irrigation and water reallocation and management to reduce the verity of seasonal climate impacts on crop yields (Souryabrata *et al.,* 2024).

Climate change has emerged the challenges in modern agriculture, with potential implications for global food production and security. The influence of climatic conditions shows the variations in

wheat crop yield which has concerns about future food production (Fallen *et al.,* 2024). By integrating historical climate data, and wheat-yield datasets, the analysis aims to provide the insights into how to reduce the yield gap due to climate variation impacts. Machine learning models used include multiple linear regression, boosted tree, random forest, ensemble models, and several types of Artificial Neural Networks have been applied for analysis purpose. The ensemble model demonstrated outstanding yield performance whereas Random Forest Regression and boosting tree observed to be outperforming (Sandeep *et al.,* 2024).

Additionally, our findings highlighted that ensemble approaches leveraging multiple model strengths offer more accurate and reliable predictions under varying climate scenarios. This suggests a significant potential for integrating machine learning in developing climate-resilient agricultural practices for future sustainable food prediction and security solutions (Kashif *et al.,* 2022).

By analyzing the effect of the multiple factors on wheat yield the paper presents the novel insight into potential impacts of climate change on wheat production in India (Godara *et al.,* 2024). The datasets consist of weather data; soil data and crop yield data collected from the environmental and agronomic factors from all over India. Crop yield gaps observed for the selected regions of India are found to be strongly correlated with rainfall, temperature fluctuation and the Soil moisture and positively correlated throughout the growing period. Although Wheat yield forecasting and estimation on a regional scale remains difficult in real time scenarios (Cheng E *et al.,* 2022). To solve the limitations of estimating wheat yield and regional forecasting, two strategies were presented paper; one is long short-term memory (LSTM) and other is reinforcement learning (RL) for agricultural production (Dhivya *et al.,* 2020).

This study aims to address these research gaps by providing a comprehensive analysis of the impact of weather and soil parameters on wheat yield in India. Through the integration of regional-level data of climate and crop yield information, this study quantifies the relative contributions of environmental factors to wheat yield production across different regions of India (Shen *et al.,* 2022). The paper extends the study to estimates the economic impact of the quantified yield gaps, providing an important perspective on the farmers efforts to increase the wheat production revenue.

Related Work

India is covering approximately 3.3 million sq. km area located in the northwest part of South Asia between $8^0$4', $37^0$6' latitudes north of equator and $68^0$7', $97^0$25' longitudes east of it. The Indian peninsula is separated from mainland Asia by the Himalayas (National, 2025). It is surrounded by Bay-of-Bengal to the east, Arabian Sea on the west and Indian Ocean on the south.

The country is divided into 36 administrative regions composed of 28 states and 8 union territories.

The climates in India exhibits high variations in temperature. The central India regions, face the extreme heat approximately 25°C to 45°C during summer March to June. Whereas, during Monsoon season, June to September exhibit high humidity approximately 20°C to 35°C. Northern India is having colder winter approximately 0°C to 25°C. Himalayan regions show sub-zero temperatures during winter and mild temperature during summer. The coastal areas influenced by sea region winds exhibits moderate temperature throughout the year.

India's climate is heavily influenced by the monsoon winds, which are originating over the Arabian seas driven by the interaction of land and sea temperatures (Know India, 2025). The Southwest Monsoon winds bring heavy rainfall from the Arabian Sea and Bay of Bengal during the months June to September. The Northeast monsoon mainly affects southeastern India during October to December months. The Western monsoon wind brings winter rain and snow to northwestern India, benefiting for wheat crops during December to February. The complex weather patterns coming from variations in Pacific Ocean affects climate temperatures in the eastern tropical Pacific, significantly affect monsoon behavior, leading to droughts or excess rainfall.

The climate variations exert effect on the major agriculture Rabi and Kharif crops like wheat, rice, maize, millet, pulses and cotton, etc. growing in winter and mid-summer. The temperature cools down between 10^0–25°C due to the winter rainfall in western regions. Kharif crops like rice growing in monsoon undergo the rise in temperature and can reduce the yield. Warm temperature and weak monsoon can lead the lower yield production of kharif and rabi crops like maize & millet respectively. Rabi & kharif can cause fungal infections due to excess rainfall. Warm climate, moderate rainfall, well-drained soil and adequate water supply are appropriate to nurture the rabi & kharif crops in India (Department of Agriculture and Welfare, 2025). High temperature, unseasonal rainfall, excess moisture, high humidity can cause crop diseases & crop damage resulting in reduced crop yield. This paper focuses on India’s wheat crop production which is an important staple food for the country’s population, food security, agriculture and economy.

India's diverse climate and geographical location create significant variations in agriculture sector. The monsoons play a critical role in determining crop yields, while temperature extremes, droughts, and unseasonal rainfall impacts on food security and farmers' livelihoods. The effects of climate change are becoming more visible, leading to shifts in sowing patterns, increasing use of drought-resistant crops and AI-based yield predictions for better adaptation (Nishu *et al.,*

2021). The spatial distribution of the average wheat yield estimation across six major wheat producing regions in India are identified for year 2024-25. Figure 1 shows the India's historical area and year-wise average wheat crop production from year 2015-16 to 2023-24.

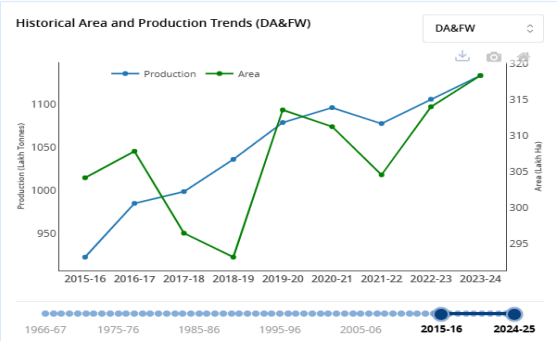

Figure 1: Historical Area-wise wheat production

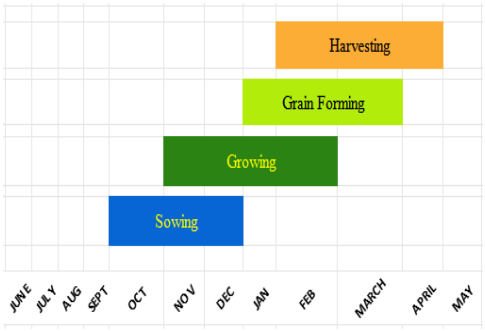

Figure 2: Wheat Crop Calendar in India

Material and Methods:

Various datasets and crop calendars are used to understand the seasonal agricultural activities in different regions of India. Figure 1 illustrates India's historical area-wise and year-wise average wheat crop production since year 2015-16 to 2023-24. The actual and potential Wheat yield data collected from the historical yields of agricultural census statistics is given in table 1 for the major crop, wheat. The analyses of crop yield datasets is collected from the portal and compared with the datasets published earlier (R Krishnan et al.,2020). Consequently, these historical yield data are having better quality and covered more extended period to meet the scientific research needs. This study considers the wheat yield historical datasets as the actual yield focusing on India's primary wheat-producing regions. The data were pre-processed and extracted from the wheat growing areas in India and the accuracy of the wheat yield data was evaluated using a reliable crop yield datasets from the selected primary region of India.

Department of Agriculture & Farmer Welfare, Unified Portal for Agricultural Statistics (UPAg) is an advanced agricultural data management platform designed to generate crop estimates and agriculture statistics such as real time information on crop yield production, market trends, price, and other vital agricultural data analysis (Department of Agriculture and Welfare, 2025). Its aim is to allow the participants in the agriculture sector, including policymakers, researchers, and farmers, by supporting them with comprehensive insights to support the informed decision-making and to create awareness about the scenarios in agriculture fields.

The India Meteorological Department (IMD), Pune Maharashtra is an Indian government agency, provides the platform on its portal for datasets and related information. IMD runs a network of weather stations, observatories, and other equipment of all over India (Li Z *et al.,* 2024). It

provides daily, monthly and yearly datasets, region-wise and season-wise of all the regions of India. It includes a comprehensive collection of meteorological metrics recorded across all weather stations throughout the state of Maharashtra during years 2001 to 2024 with further data for the current year. Datasets are available for rainfall, temperature, wind speed, evaporation, sunlight hours, gull strength, dust accumulations, and storm frequency; and free for research and study purpose (Mostafaeipour *et al.,* 2020). Table 1 shows the summary of some of the datasets parameters along with its sources.

Table 1: Summary of datasets used in study

Datasets	Data Description	Data Type	Souces
Yield	Actual yield data	Spatial and temporal	Ministry of Agriculture & Farmers Welfare
Wheat Area	Wheat harvested area	Spatial	Ministry of Agriculture & Farmers Welfare
Climate - precipitation	Wheat-growing season precipitation	Spatial and temporal	India Meteorological Department
Climate - Temperature	Wheat-growing season temperature	Spatial and temporal	India Meteorological Department
Cliamate & Environment	Cliamate variability	Spatial and temporal	India Climate & Energy Dashboard
Other	Wheat-growing season soil moisture	Spatial and temporal	Indian Council of Agriculture Research

The datasets collected from the various sources such as IMD, Agmarknet, ICAR, etc. the detail information of main parameters. Weather datasets consist of the parameters like rainfall, temperature, humidity, wind speed, etc. Soil datasets consist of the parameters like moisture, pH, nutrients, etc. Crop yield datasets consist of the information of historical wheat yield in different regions (Climate Research & amp; Services, 2025). The datasets are cleaned through pre-processing steps such as handling missing values using interpolation and mean imputation methods. Then the datasets are normalized using the MinMaxScaler technique. Finally, these datasets are converted using the time-series data into a supervised format by applying the sliding windows. The collected datasets are divided into two parts, one titled as training datasets and other titled as testing datasets before applying to the deep learning models (Dhivya *et al.,* 2020).

Wheat crop cultivation takes place in rabbi season that is starting in the winter from the month of October and harvesting happens in summer season that is in the month of April, shown in above

figure 2, wheat crop calendar in India. Region-wise variations are noted for the growing periods, the northern states growing period is during cooler climate, western and central states gets faster maturity due to warm weather whereas southern states having limited wheat cultivation with early harvesting (Kerry *et al.,* 2021). Sowing begins after the monsoon season during the month of October to December at temperature 10- 20 ºC. The wheat crop growing establishes the roots and leaves at temperature 15 º - 25 ºC and keeps moderate soil moisture during November to February. The flowering and grain formation takes place during the warmer days and cool nights at 15º - 25 ºC temperature during February to March. Wheat crop harvesting starts when the grains become hard at high temperature 25º-35 ºC and speed up maturity during March to April months.

The information collected from the agricultural census statistics, IMD, ICAR, satellite and produced the datasets of historical yield data of wheat crop around the whole country regions (ICAR-Indian Agricultural Research Institute, 2025). The historical yield data are having better quality and longer period meets the research needs. The study is analyzed for current yield and impact of climate, precipitation and soil moisture on the crop growth and yield production.

1. Climate Data

All India annual tropospheric temperature information is obtained from radio stations, it shows 500hPa from the surface, increasing trend during period 1971–2015 and it is observed that warming is unevenly distributed across India. The spatial distribution of annual and seasonal trends in °C per decade during years 1986–2015 are observed such as minimum temperature, mean temperature and maximum temperature (Naresh *et al.,* 2014). Spatial distribution of observed annual and seasonal trends of temperature °C per decade is divided season-wise into four labels. The annual temperature is labeled as “ANN”; temperature during winter season starting from December–February is labeled as “DJF”; temperature during pre-monsoon period starting from March–May is labeled as “MAM”, temperature during monsoon season starting from June–September is labeled as “JJAS”; and temperature during post-monsoon period starting from October–November is labeled as “ON” seasons.

The largest increase in annual mean temperature observed is more than 0.2 °C per decade in some areas of north India during years 1986 to 2015 whereas in some parts of the west coast, it is observed weaker warming in southern peninsula with mean temperature increase less than 0.1 °C per decade (Agricultural Meteorology Division, 2025). The winter warming is limited to peninsular India and pre-monsoon season shows more than 0.5 °C per decade over north India. Summer monsoon season warming is close to the eastern parts of the Indo-Gangetic plains and adjoining central India. The post-monsoon season warming pattern is similar to the pre-monsoon

season, but the smaller magnitude and more uniformly distributed across the country than other seasons. These estimates of warming across India are based on a simple linear trend which is found to be similar to the earlier assessments of temperature trends derived using non-stationary approach (Avik *et al.,* 2019). This data was interpolated using the nearest neighbor method into a regular cell of 0.5°×0.5° to be consistent with yield production data. By dividing the growing season into the calendar months, it is possible to pinpoint the most sensitive phases for wheat crops concerning climate variability.

2. Seasonal Rainfall Distribution

India is located in tropical monsoon zone and receives plenty of rainfall mostly occurs in monsoon season of every year. It can be observed the significant changes in rainfall patterns such as intensity and frequency of extreme rainfall events caused by climate change (Department of Agriculture and Welfare, 2025). The analysis reports of annual rainfall during years 1971 - 2020, bring some statistical information regarding the spatial distribution of annual, seasonal and monthly rainfall. This includes the climatic rainfall patterns, coefficient of variation of rainfall, and the contribution of seasonal rainfall towards annual rainfall and frequency of occurrence of different rainfall categories over the entire Indian region for monthly, seasonal and annual timescales (Climate Research and Services, 2025). The rainfall during monsoon season of four months, June, July, August and September is categorized as JJAS, pre-monsoon season of three months, March, April, May is categorized as MAM, post-monsoon season of three months, October, November, December is categorized as OND, winter season of two months, January, February is categorized as JF. Annual Rainfall realized over India for the year 2024 is distributed into four seasons such as winter, pre-monsoon, monsoon and post-monsoon (Wang *et al.,* 2022). The rainfall monitored during winter season is 68% of LPA, during the pre-monsoon season is 97% of LPA, during monsoon season is 108% of its LPA and during post-monsoon is 97% of its LPA.During all the seasons, the received rainfall was at large excess, excess, normal and deficient all over the regions of India.

3. Soil Moisture Data

Soil moisture is the major parameter which has the impact on crop growth and yield production in agricultural (Agricultural Meteorology Division, 2025). Soil moisture depends on the precipitation and temperature reflected on the agriculture ground surface. IMD regularly monitors the soil moisture and estimate daily using Soil Water Balance Model. In addition to realized estimation of soil moisture, IMD has started soil moisture forecast twice in a week using the T1534 model, available at meteorological centers. The soil moisture datasets are collected from sources like IMD, department of irrigation and drainage engineering, Mahatma Phule Krishi

Vidyapeeth Rahuri India (National Portal of India, 2025). These datasets are aggregated over the wheat-growing seasons during months November to May and monthly time steps. It provides daily surface soil moisture content at a spatial resolution of 0.25° and has a higher correlation, lower error validated against ground measurements. This data can be used to calculate the Soil Moisture Condition Index (SMCI).

4. Harvested Area

Irrigated and rain fed crop area data is collected from Unified Portal for Agricultural Statistics (UPAg) and used to determine the wheat harvesting data of all over India during years 2015 - 2024 (Department of Agriculture and Welfare, 2025). Seasonal state-wise area-wise wheat yield and production datasets collected from Department of Agriculture & Farmer welfare (DAFW) portal. Figure 3 shows the yearly seasonal area-wise wheat crop yield and production for duration 2015-16 to 2023-24. Unified Portal for Agricultural Statistics (UPAg) provides real time information on crop production and statistics such as price, trade, procurement, stock, etc. Which can be used for study and analysis. The portal supports to empower stakeholders in the agriculture sector, policymakers, researchers, and farmers by providing them the comprehensive insights to support decision-making system.

5. Data Analyses

The study utilized the data analysis pipeline focused on the datasets such as climate, rainfall, soil moisture, harvesting and the adaptive machine learning models (Nandini *et al.,* 2024). The analysis influences the wheat crop yield datasets and prepares insights for integration into ML prediction models. The analysis of fusion datasets is a powerful approach to combine multiple modalities and discover the hidden interactions within data. Supervised and unsupervised ML models can be used to improve the adaptive wheat crop forecasting and estimation (Andrew *et al.,* 2018). Different data fusion approaches such as concatenating features, merging features are applied on datasets from each modality and models for each modality. The complex cross-modal interactions between the multi-modal fusion analysis involves the features of climate, rainfall, soil and harvesting datasets for wheat crop yield prediction (Cheng *et al.,* 2024). The fused features like soil moisture and average rainfall are to be found the powerful predictors whereas soil NPK index found the impact on crop yield when rainfall is irregular. Temperature deviations during sowing months correlates with lower productivity whereas high soil moisture with rainfall lead to over saturation and lower nutrients. This study utilized the topically adjusted regression model trending method to eliminate the effects of technological advancements, errors in reporting during the study period, as well as non-climatic factors, such as genetics and agronomic improvements, before analyzing the impact of climate on wheat yield production (Nayana *et al.,*

2022). The same approach was employed by related studies suitable for short time series and long short-term memory (LSTM) (Joshi *et al.,* 2025). After the trend was fitted, the yield production time series were trended using the additive method that involves combination of LSTM and reinforcement learning (RL) for wheat crop forecasting. The hybrid model approach in combination with convolution neural network (CNN) and LSTM found suitable for the fused data and RL to be found suitable for multi-modal data to optimize crop production estimation and yield prediction (Petteri *et al.,* 2019; Sonal *et al.,* 2021).

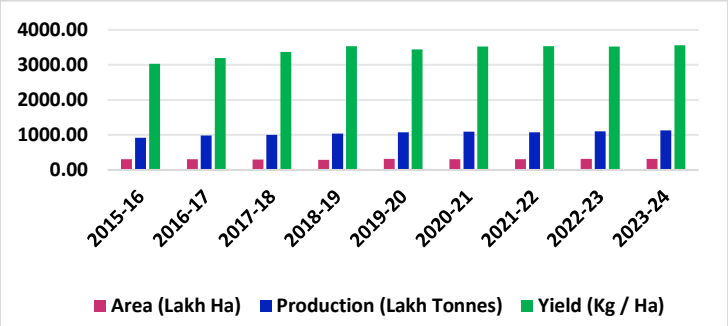

Figure 3: Yearly Seasonal Area-wise wheat Crop Yield & Production

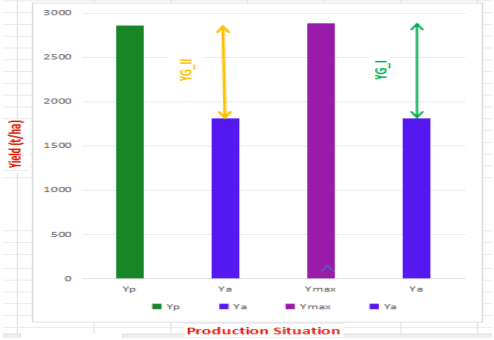

Figure 4: Wheat Yield Gap Analysis

The ensemble learning methods involve computing the trended time series as the ratio of the original data and trend line values. The objective is to find the model that strikes the best balance between explaining the data and avoiding over fitting with a reasonable balance of complexity. This work deployed with a statistical method used to choose between different models. It measures the balance between a model's best -fit and complexity characteristics, providing a way to compare competing model performance.

Wheat Yield Gap Quantification and Analysis

As wheat crop is the global staple food, there is huge demand from all over world and farmers are suppliers it on reasonable price. It became essential to increase wheat production and focus on the parameters like actual yield, maximum production, predicted yield, etc. The analysis is performed to calculate the difference between average yield production, expected yield production and maximum yield production based on the collected datasets, this is referred as the wheat yield gaps. These gaps are detected, identified and quantified to fulfil the demand and supply of wheat yield crops. Wheat yield gaps were quantified in two ways as presented in Figure 4, first yield gap is calculated by equation (1), YG_I give difference between the average achieved yield Ya and the highest achievable yield of the Ymax in the time series (Ymax). The second type of yield gap is YG_II, it is the difference between the average achieved yield Ya and estimated yield Yp given in equation (2).

$$YG\_I = Y_{\max} - Y_a \quad (1)$$

$$\text{YG\_II} = \text{Y}_\text{p} - Y_a \quad (2)$$

Further the parameter correlation is performed during the crop growing seasons to estimate the relationships between monthly cumulative precipitation and the soil moisture condition index (SMCI) calculated using following equation 3, and detrended wheat yield.

$$SMCI = \frac{SM_i - SM_{\min}}{SM_{\max} - SM_{\min}} * 100 \quad (3)$$

SMCI is soil moisture condition index used to normalize the current soil moisture value (SMi) relative to historical extremes to provide an indicator of dryness. SMi is scaled as a percentage between the minimum (SM_{min}) and maximum (S_{Mmax}) soil moisture values from long-term records. The calculated value of SMCI ranges from minimum value 0% at the extreme dry end to the maximum value 100% at the saturated upper limit. This standardized metric quantifies moisture condition compared to past wet and dry extremes that help for monitoring the agricultural drought (Muhamad *et al.,* 2025). The relationship between climate variables and detrended wheat yield is evaluated using Pearson's correlation function.

The correlation coefficient's sign and magnitude help to uncover the relationship's nature and the strength. The effects of minimum, average, maximum temperature and precipitation on wheat yield is analyzed using linear regression equation (4). This study highlights the impacts of climatic factors like precipitation, temperature, soil on wheat crop yield and provides a comprehensive assessment of the historical yield gaps (Godara *et al.,* 2024). Linear regression models is used to analyze the significant effect of climatic variables on crop wheat yield during the entire growing season and considered annual as well as monthly parameters.

$$\begin{aligned} Y = {} & \beta_0 + \sum(\beta_i \times X_i, T_{\min}) + \sum(\beta_j \times X_j, T_{avg}) + \sum(\beta_k \times X_k, T_{\max}) \\ & + \sum(\beta_l \times X_l, precipication) \\ & + \sum(\beta_m \times X_m, soilmoisture) + \varepsilon. \end{aligned} \quad (4)$$

Equation (4) represents the multiple linear regression model with parameters such as Y is wheat yield, β_0 is the model's intercept, and coefficients β_i, β_j, β_k are the minimum, average and maximum temperatures respectively. Coefficient β_l is related to precipitation and β_m corresponds to the average soil moisture, with ε is representing the error term capturing unexplained variability. For the monthly analysis, Y continues to represent the wheat yield. The coefficients β_i, β_j, β_k β_l β_m are used for monthly data and analyzed the effect of month's climatic conditions on wheat yield. This analysis presents the model's annual variability property and the significant impact on wheat yield prediction during the growing season (Krishna *et al.,* 2024). The specific β coefficient of the model offers monthly specific insights into the growing season climatic actuation of wheat yield production.

Annual historical wheat yield production data is used as the training datasets for regression model to calculate the model accuracy using coefficient of determination R^2 given by equation-6, the root mean square error (RMSE) given in equation-7 and mean absolute error (MAE) is given in equation-8. In these equations, Y_i is the observed data for the i^{th} year, $\hat{Y}$ is the average data and n is the number of samples of training datasets.

$$R^2 = \frac{1-\sum(Y-\hat{Y}_i)^2}{\sum(Y-\hat{Y}_i)^2} \quad (6)$$

$$RMSE = \sqrt{\frac{(\sum(y_i-\hat{Y})^2}{n}} \quad (7)$$

$$MAE = \frac{\sum|y_i-\hat{Y}|}{n} \quad (8)$$

Wheat yield gap indicates the loss in production which can be the direct impact on the farmer's economy. The regional production costs and wheat price data measures the wheat yield gap and revenue estimation gap (Miti *et al.,* 2024). This effectively isolates the yield-driven revenue variations occurred due to the market price rate fluctuations. Actual revenue was calculated by multiplying the detrended yield by the detrended price of wheat yield production. Expected revenue was determined using the maximum yield and predicted yield, multiplied by the detrended price of wheat production. Yield gap losses were quantified as the difference between expected and actual revenues, based on computation of YG_I and YG_II equations.

Results and Discussion:

Linear Regression model provides an accurate fitting for regional and national wheat yield datasets as shown in figure 5 and display the best model performance among the evaluated regions, with relatively low RMSE value 0.21 t/ha and 0.24 t/ha; MAE values are 0.18 t/ha and 0.15 t/ha; indicating smaller average differences between actual yield and detrended yield (Nayana *et al.,* 2022). MAE values are 0.27 t/ha, 0.38 t/ha, 0.17 t/ha indicates the larger average diference between detrended and actual crop yield. Whereas RMSE values 0.33 t/ha, 0.47 t/ha, 0.27 t/ha shows the higher results indicating larger average differences between detrended and actual yield.

Table 2 presents the quantifiable measures of trend fitting results of the model. National level and regional level demonstrate superior performance; other regions show varying performance model accuracy for wheat yield datasets. At the national level, the model achieves acceptable performance with an RMSE of 0.53 t/ha and an MAE of 0.40 t/ha. The R^2 values demonstrate the model's ability to explain yield data variability, with values 0.30 t/ha and 0.24 t/ha observed at regional and national levels, respectively. The model shows promising results, capturing localized trends and patterns with varying levels of accuracy across different scales. Based on

these finding, we can incorporate this dataset into our analysis at the regional level, as it offers significant insights into regional patterns and trends in wheat yield prediction.

Rainfall data of selected states are collected on daily basis and analyzed the trend variability and mean rainfall patterns (Satyendra *et al.,* 2023). Monthly average rainfall during four months, June, July August and September has computed for selected states and observed the rainfall trend and variability for annual and monsoon seasons. Based on this analysis, the rainfall intensity trends are identified and the coefficient of variation (CV) is calculated using following equation (9) with standard deviation (SD) and mean.

$$CV = \frac{SD}{mean} \text{X}100 \qquad (9)$$

The observed data of monsoon rainfall variability for selected six regions; Uttar Pradesh, Haryana, Punjab, West Bengal, Madhya Pradesh and Maharashtra is presented in following table 3, table contains the values of coefficient of variation and mean rainfall for selected regions and four monsoon months, June July August and September. The Maharashtra state gets monsoon rainfall highest 34% in September month whereas in June month it's 30% of the monsoon rainfall. During July-August it received 26% and 14% annual respectively with 14% rainfall variability.

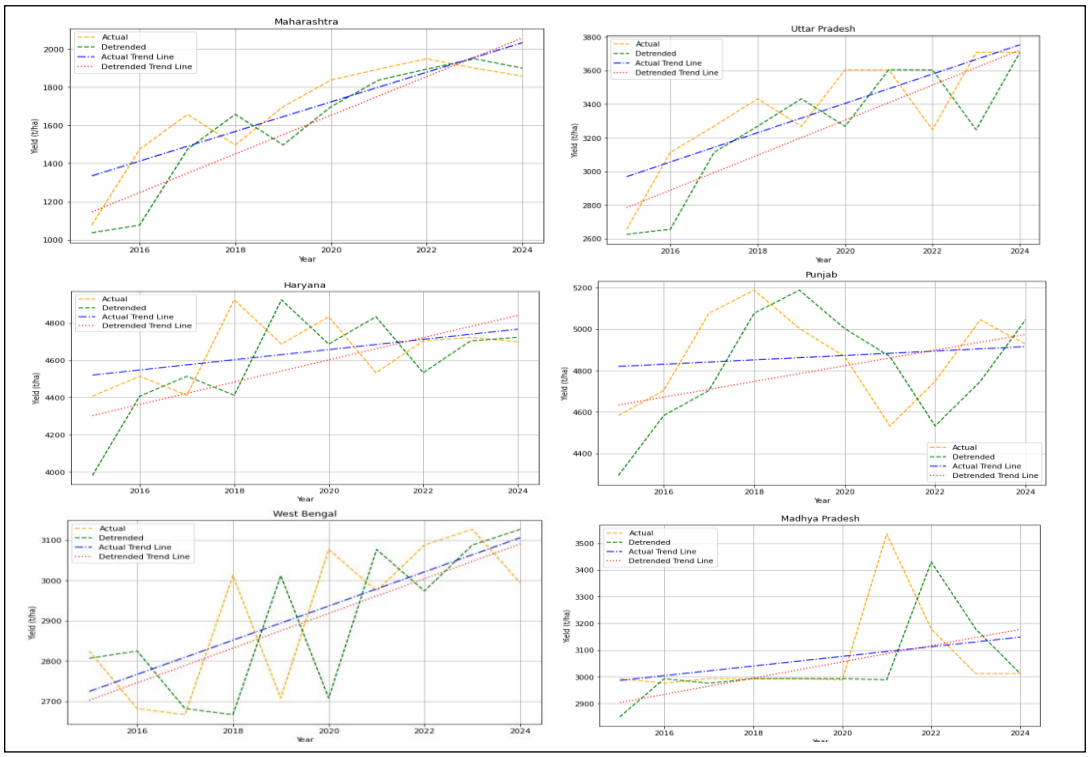

Figure 5: Actual and Detrended wheat yield of selected states

The soil Moisture is an important parameter playing the significant role in variability of wheat yield production over the entire growing season. The soil moisture at different depths up to root

zone can be used for monitoring drought help in determining the right timing and amount of irrigation at critical crop growth stages during sowing of kharif crops (Shook *et al.,* 2021). The R_2 Values ranged from 30.0% to 84.0%, with adjusted R_2 values is slightly lower, indicating substantial model fit and explanatory power. The rainfall during the monsoon period is effective to maintain the soil moisture positively influence on the wheat yield growth. While max and mean temperature having the negative effect on wheat yield during the growing season (Lahcen *et al.,* 2024).

Table 2: Trend fitting Results

Region	**RMSE (t/ha)**	**MAE (t/ha)**	**R^2 (t/ha)**
National	0.53	0.40	0.24
Regional	0.47	0.39	0.30
Uttar Pradesh	0.24	0.19	0.40
Haryana	0.95	0.73	0.11
Punjab	0.13	0.12	0.65
West Bengal	0.21	0.12	0.20
Madhya Pradesh	0.25	0.18	0.36

Table 3: Mean rainfall and coefficient of variation of the state for the monsoon Months and Annual

State	**Parameters**	**June**	**July**	**August**	**Sept.**	**JJAS**	**Annual**
Maharashtra	**Mean**	218.6	341.4	281.1	179.5	1020.7	1146.5
	C V	30.5	26.3	26.3	34.8	14.4	14.0
Uttar Pradesh	**Mean**	96.1	238.6	219.0	142.9	696.7	784.1
	C V	60.9	29.5	34.5	49.8	20.8	18.7
Haryana	Mean	57.7	130.9	137.5	84.5	410.6	499.7
	C V	70.6	52.7	58.4	64.8	31.0	27.2
Punjab	**Mean**	60.2	149.1	139.6	79.1	427.9	538.6
	C V	64.6	40.4	54.8	65.1	28.8	25.4
West Bengal	**Mean**	318.0	431.8	361.1	307.7	1418.7	1851.4
	C V	20.3	19.1	18.9	28.2	13.9	13.5
Madhya Pradesh	**Mean**	127.4	323.6	304.3	166.2	921.4	997.8
	C V	53.6	26.6	28.0	53.8	18.0	17.6

Table 4: Temperature Variations during the Seasonal Monsoon Months

Years	Parameters	June	July	August	September
2024	T_{max}	35.62ºC	32.31ºC	31.31ºC	31.96ºC
	T_{min}	25.47ºC	24.99ºC	24.27ºC	23.79ºC
	T_{mean}	30.55ºC	28.65ºC	27.79ºC	27.88ºC
2023	T_{max}	34.60ºC	31.91ºC	32.19ºC	32.22ºC
	T_{min}	25.39ºC	24.90ºC	24.70ºC	24.22ºC
	T_{mean}	29.99ºC	28.40ºC	28.45ºC	28.23ºC
2022	T_{max}	34.12ºC	31.65ºC	31.44ºC	31.76ºC
	T_{min}	25.06ºC	24.68ºC	24.45ºC	23.89ºC
	T_{mean}	29.59ºC	28.17ºC	27.94ºC	27.82ºC
2021	T_{max}	33.34ºC	32.17ºC	31.75ºC	31.52ºC
	T_{min}	24.81ºC	24.88ºC	24.39ºC	24.06ºC
	T_{mean}	29.07ºC	28.52ºC	28.07ºC	27.79ºC

The linear regression analysis of the relationship between temperature and wheat yield has determined that the effect of minimum, average and maximum temperature during the wheat growing season. Table 4 shows, temperature Variations during the Seasonal monsoon months of monthly average maximum, average minimum and mean temperature values over the country as a whole for the seasonal months of June, July, August, September during four years 2021 – 2024. Table contains the information of highest temperature records with corresponding top ranks along with year of occurrence for $T_{Max,}$ T_{Min} and T_{Mean}. The average maximum, temperature in month June, July, August and September was was 35.62ºC, 32.31ºC, 31.44ºC and 32.22ºC respectively all over region of the India. Whereas the average minimum temperature in month June, July, August and September was was 24.81ºC, 24.68ºC, 24.27ºC and 23.79ºC respectively.

Conclusion:

This study analyzed wheat yield gaps across all over India for selected production regions and examined the impact of climate variability on yields. The findings observed significant yield variations, with yield gaps; first yield gap is the difference between the average achieved yield and the highest achievable yield in the time series. The second yield gap is the difference between the averages achieved yield and estimated yield. The climate parameters precipitation, soil moisture, and temperature are the influencing factors yield production. Increase in temperature during wheat crop growing season reduces the soil moisture and impairing wheat grain

development. To overcome this challenge, there is need to address the issue with a multifaceted approach.

Artificial intelligence techniques are essential in the agriculture fields optimize yield production and provide support for security. Strengthening agricultural research and extension services is crucial to addressing study limitations and disseminating climate-smart farming practices. Developing region-specific adaptation strategies tailored to the unique challenges of different regions within India. These findings and recommendations provide a foundation for future process-based modeling studies to evaluate the impact of various parameters on yield and gap analysis to increase the crop yield production in India. Ultimately, implementing such strategies can majorly contribute to sustainable and economical progress of wheat production which help close yield gaps and enhance food security in the face of climate variability.

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ANTIMICROBIAL ACTIVITIES OF CALOTROPIS PROCERA LINN. ON SELECTED PATHOGENIC MICROORGANISMS – A PRELIMINARY STUDY

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Abstract:

Methanol and Ethyl acetate extract of leaves of *Calotropis procera* Linn. were subjected to analyse the potential antibacterial activities. The extract was subjected to its effectiveness against both Gram-positive and Gram-negative bacteria in agar diffusion method. The zones of inhibition produced by the crude methanol and ethyl acetate extract against few sensitive strains were measured and compared with those of standard antibiotic Tetracycline. It is evident that both extracts are active against the bacteria even at low concentrations. The obtained results provide a support for the use of this plant in traditional medicine and suggest its further advance investigation.

Introduction:

Calotropis procera is a widely recognized medicinal plant belonging to the family Asclepiadaceae. It is distributed throughout India and various tropical regions of the world. The plant is known by several common names, including Arka, Akanal, Madar, and Akanda. Morphologically, the leaves of *C. procera* are typically ovate, obovate, ovate-oblong, or elliptical in shape (Kumar and Basu, 1994).

The medicinal value and pharmacological properties of *C. procera* are primarily attributed to its latex, which contains a variety of bioactive compounds. Traditionally, the leaves are used as an antidote against snakebite and in the treatment of burns, rheumatism, mumps, and bacterial infections. Phytochemical analysis of the leaf powder has revealed the presence of several important groups of secondary metabolites, including cardenolides, steroids, tannins, glycosides, terpenoids, flavonoids, alkaloids, and saponins. Similarly, phytochemical screening of the bark also indicates the presence of diverse secondary metabolites (Dewan *et al.,* 2000).

The latex of *C. procera* is particularly rich in calotropaine, a cardiotoxic proteolytic enzyme, along with calotropin, a less toxic proteolytic component. Beyond its medicinal value, the plant also exhibits significant ecological importance, functioning as a natural phytoremediator and

contributing to soil quality improvement (Verma, 2014). *Calotropis procera* holds considerable global significance owing to its diverse medicinal, chemical, and ecological benefits.

Materials and Methods

Collection and Processing of Plant materials

The healthy and mature leaves of *C. procera* for the proposed study were collected from the nearby areas of Palode, Trivandrum district. The collected leaves were washed thoroughly with tap water and subsequently dried under shade to prevent the degradation of active compounds. The shade-dried leaves were then ground into a fine powder using an electric blender and sieved through a 0.5 mm mesh to obtain uniform particle size. The resulting powdered samples were stored in clean, airtight containers for further analysis.

Extraction of Plant Materials

Methanol and Ethyl acetate extracts of plant leaves were prepared using Soxhlet apparatus. The leaf extracts were concentrated at 50°C and the residues obtained were weighed and stored in different airtight containers at 4°C and used for further investigations.

Phytochemical Screening

Phytochemical analysis of all crude extracts was carried out according to the methods described by Ayoola (2008).

Antibacterial Activity

The crude extracts were screened for antibacterial activity using agar well diffusion method described by Russel and Furr (1977). Test organisms used were, *Staphylococcus aureus, E. coli* and *Salmonella typhi*. The cultures were spread evenly onto Mueller–Hinton agar plates. Wells of 0.6 mm diameter were made using a sterile well cutter, and different extracts were added to the wells at a fixed volume of 100 µL. Appropriate controls were included, with tetracycline serving as the positive control and DMSO as the negative control. All plates were incubated at 37°C for 24 hours. The antimicrobial activity of the extracts was assessed by measuring the diameter of the zones of inhibition formed around the wells.

Statistical Analysis

Results of the experiments are expressed as mean ±S. E. M. All experiments were repeated three times. Microsoft excel was used for statistical analysis.

Results and Discussion:

The leaf extracts in different solvents were screened for the presence of various bioactive phytochemical compounds. The analysis revealed the presence of alkaloids, cardiac glycosides, saponins, flavonoids, reducing sugars and terpenoids in most prominent amount while tannins and steroids in lesser amount. Results were documented in Table 1.

Table 1: Phytochemical analysis of bioactive compounds in Methanol and Ethyl acetate extracts of *Calotropis procera* leaves.

Phytochemicals	Extracts	
	Methanol	Ethyl acetate
Alkaloids	+	+
Cardiac glycosides	+	+
Saponins	-	+
Tannins	+	-
Flavonoids	+	+
Terpenoids	+	+
Reducing sugars	+	+
Anthraquinone	-	-
Steroids	+	-
Phenol	+	+

In vitro antimicrobial sensitivity assay of Methanolic and Ethyl acetate extracts of *C. procera* leaves were evaluated using five pathogenic microbial strains namely, *Escherichia coli*, *Bacillus cereus, Staphylococcus aureus, Klebsiella pneumonia* and *Vibrio cholera*. Agar well diffusion is used for the study and minimum inhibitory zone (mm) were analysed.

Table 2: Antimicrobial activity of Methanol extract of *Calotropis procera leaves* on selected microbes

Test organisms	Zone of Inhibition (mm)				
	Methanol Extract (ug/ml)			PC	NC
	500	750	1000		
Escherichia coli	11±0.2	10± 0.5	15 ± 0.4	20 ± 0.0	-
Bacillus cereus	10 ±0.0	9± 1	9 ± 0.5	22 ± 0.1	-
Staphylococcus aureus	9 ±0.0	11 ± 0.1	14 ± 0.0	17 ± 0.5	-
Klebsiella pneumoniae	10± 0.5	12 ± 0.5	14 ± 0.0	16 ± 0.5	-
Vibrio cholerae	9 ± 0.1	10 ± 0.2	11 ± 0.1	21 ± 0.0	-

All the values are expressed in mean ± standard deviation.

PC- positive control; NC- negative control;

Zone of inhibition not include well diameter. (well diameter – 6 mm)

The results revealed that, different concentrations of methanolic and ethyl acetate extracts of *C. proceraa* leaves showed better zone of inhibition in *Escherichia coli*. Better antibacterial activity

showed by the selected extracts might be due to the presence of phytochemical compounds in the candidate leaves. Compared to other strains, *Staphylococcus aureus, Klebsiella pneumoniae and Vibrio cholera* showed better zone of inhibition in methanolic extracts than ethyl acetate. This may be due to the presence of high amount of bioactive compounds in it. These were depicted in tables (2, 3) and graphs (1,2).

Table 3: Antimicrobial activity of Ethyl acetate extract of *Calotropis procera leaves* on selected microbes

Test organisms	Zone of Inhibition (mm)				
	Ethyl Acetate Extract (ug/ml)			PC	NC
	500	750	1000		
Escherichia coli	9 ± 0.0	12 ± 0.2	13 ± 0.2	24 ± 0.1	-
Bacillus cereus	8 ± 0.5	9 ± 0.0	10 ± 0.2	22 ± 0.0	-
Staphylococcus aureus	9 ± 0.1	10 ± 0.0	11 ± 0.5	18 ± 0.1	-
Klebsiella pneumoniae	7 ± 0.0	8 ± 0.4	9 ± 0.1	16 ± 0.1	-
Vibrio cholerae	9 ± 0.5	10 ± 0.5	11 ± 0.0	24 ± o.o	-

All the values are expressed in mean ± standard deviation.

PC- positive control; NC- negative control

Zone of inhibition not include well diameter. (well diameter – 6 mm)

Conclusion:

The present study reveals the existence of considerable amounts of phytochemical and antimicrobial substances in *Calotropis procera leaves* and further studies are required to find out the active components of medicinal properties in this valuable plant. The novelty of this work lies in its comprehensive and up-to-date evaluation of *Calotropis procera*, emphasizing its broad pharmacological potential as demonstrated through extensive in vivo and in vitro studies. This review uniquely bridges traditional medicinal knowledge with contemporary scientific evidence, providing an integrated assessment of the plant's therapeutic efficacy across a wide range of health conditions.

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BIOINFORMATICS TOOLS FOR GENOMIC DATA ANALYSIS

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Abstract:

The rapid expansion of genomic technologies has transformed biological research into a data-intensive discipline, creating an essential need for efficient computational tools to manage, analyze, and interpret large-scale genomic datasets. Bioinformatics has emerged as the critical interface between sequencing platforms and meaningful biological discovery, enabling researchers to convert raw sequence reads into actionable insights. This chapter provides a comprehensive overview of the major bioinformatics tools and analytical frameworks used in genomic data analysis, beginning with an introduction to genomic data types and the workflow that guides data processing from initial quality control to downstream functional interpretation. Key tool categories—including quality assessment platforms, sequence alignment programs, genome assembly algorithms, variant detection pipelines, and gene prediction and annotation tools—are discussed in detail with their core functionalities, strengths, and limitations. Additional emphasis is placed on data management resources, databases, visualization platforms, and the computational infrastructures that support genomics-driven research. The chapter also highlights emerging trends such as long-read sequencing, cloud-based analytics, machine learning–driven predictions, and integrative multi-omics, along with the challenges posed by increasing data volume, complexity, and the need for standardization. By integrating conceptual explanations with tool-based examples and workflow diagrams, this chapter serves as a practical guide for students, researchers, and practitioners seeking to understand and apply bioinformatics.

Keywords: Genomic Data Analysis, Bioinformatics Tools, Genome Assembly, Sequence Alignment, Variant Calling, Gene Annotation

Introduction:

Genomic Data and Bioinformatics

Genomics is the comprehensive study of an organism's entire genetic material, encompassing the structure, function, evolution, and regulation of all genes within a genome. With the advent of next-generation and third-generation sequencing technologies, genomics has transformed from

studying individual genes to analyzing whole genomes at an unprecedented scale. These advanced platforms generate vast datasets that enable genome assembly, variant detection, gene annotation, comparative analysis, and large-scale functional studies across species. As genomic data volume continues to grow exponentially, the challenge in modern biological research is not merely sequencing DNA but interpreting the complex information embedded within it.

Bioinformatics plays a pivotal role in meeting this challenge by providing computational tools, algorithms, and analytical frameworks that convert raw sequencing data into meaningful biological insights. It supports every stage of the genomic workflow (Fig. 1), including quality checking of reads, sequence alignment, genome assembly, variant calling, gene prediction, annotation, and comparative genomics. Through techniques drawn from computer science, statistics, mathematics, and molecular biology, bioinformatics enables researchers to manage large datasets, ensure data accuracy, and uncover relationships that would be impossible to detect manually. With advances in high-performance computing, machine learning, cloud-based platforms, and user-friendly software, bioinformatics has become indispensable for maximizing the value of genomic data across disciplines.

This chapter focuses on exploring the major bioinformatics tools used in genomic data analysis and their applications in modern research. It outlines the essential steps involved in processing and interpreting genomic datasets and discusses the most widely used tools for each stage. The chapter also highlights the strengths, limitations, and appropriate uses of these tools, helping readers make informed decisions when designing genomic experiments or analyzing sequencing data. Overall, it provides a clear foundation for understanding how genomics and bioinformatics work together to drive discoveries in agriculture, biotechnology, medicine, and evolutionary biology.

Genomic Data Analysis Workflow

The genomic data analysis workflow is a systematic sequence of steps that transforms raw sequencing reads into biologically meaningful interpretations. Although specific projects may vary in complexity, most genomic studies follow a broadly similar pipeline that begins with data generation and ends with downstream functional or comparative analyses. The workflow typically starts with the acquisition of raw sequence data produced by platforms such as Illumina, PacBio, or Oxford Nanopore. This initial stage is crucial because the quality of sequencing reads directly influences the accuracy and reliability of later analyses. Once the raw data is obtained, it undergoes rigorous quality assessment to identify and remove low-quality reads, adapter contamination, or sequencing artifacts. Quality control ensures that only high-confidence data proceeds to subsequent steps, thereby reducing errors and improving analytical precision.

After quality filtering, the next major phase involves aligning the processed reads to a reference genome or assembling them de novo when no reference is available. Sequence alignment places each read in its correct genomic position, enabling the identification of structural features, gene locations, and differences between the sample and reference. In contrast, genome assembly reconstructs the genome from scratch by stitching together overlapping reads to form contigs and scaffolds. Both approaches rely heavily on efficient computational tools and algorithms that can handle large-scale datasets while maintaining accuracy.

Once alignment or assembly is completed, variant calling and gene prediction become essential components of the workflow. Variant calling detects genetic differences such as single nucleotide polymorphisms (SNPs), insertions, deletions, or structural variations, providing insights into genetic diversity, evolution, or trait-related mutations. Gene prediction algorithms identify coding regions, regulatory elements, and functional motifs within the genome, laying the foundation for annotation. The annotation step integrates data from similarity searches, functional databases, and predictive models to assign putative biological roles to genes, pathways, and genomic features. This stage transforms raw sequence information into a structured biological dataset that can be interpreted in a meaningful context.

The final phase of the genomic data analysis workflow (Fig. 1) involves downstream analyses such as comparative genomics, phylogenetics, population genomics, and functional enrichment. These analyses help researchers explore evolutionary relationships, identify conserved or unique genomic elements, understand gene family expansions, and relate genetic variations to phenotypic traits. Visualization tools and statistical models further support interpretation by presenting complex genomic patterns in intuitive formats, including genome browsers, heat maps, interactive plots, and network diagrams. Together, these steps form a cohesive and iterative workflow in which each stage builds upon the previous one, enabling researchers to generate robust, reproducible, and biologically relevant conclusions from large-scale genomic datasets.

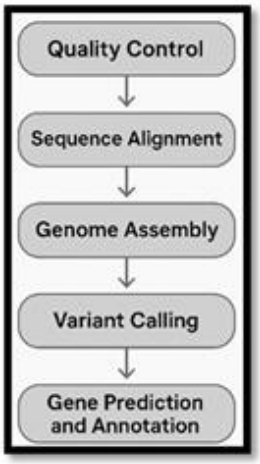

Figure 1: Genomic Data Analysis Workflow

Types of Genomic Data

Table 1: Types of Genomic Data and Their Key Characteristics

Genomic Data Type	Source / Basis	What It Represents	Key Features	References
Raw Sequence Data	NGS/long-read sequencing platforms	Unprocessed reads (short or long)	Contains base calls, quality scores, and sequencing artifacts	Metzker, 2010
Assembled Genome Data	Processed sequence reads	Complete or draft genome structure	Provides genomic architecture, gene locations, and structural organization	Myers *et al.,* 2000
Transcriptomic Data (RNA-seq)	mRNA, total RNA	Gene expression profiles	Reveals expression dynamics, differential regulation, and transcript structure	Wang, *et al.,* 2009
Variant Data (SNPs, Indels, SVs)	Whole-genome or targeted sequencing	Genetic differences across individuals	Useful for population studies, trait mapping, and evolutionary analyses	Altshuler *et al.,* 2010
Epigenomic Data	DNA methylation, histone marks, chromatin accessibility	Regulation of gene expression	Shows epigenetic control mechanisms and stress or developmental adaptations	Lister *et al.,* 2009
Metagenomic Data	Microbial communities in soil, water, gut, etc.	Mixed population genomic content	Captures diversity, abundance, and functional potential of microbiomes	Wooley *et al.,* 2010
Proteogenomic Data	Integrated DNA, RNA, and protein data	Cross-validation of gene and protein products	Enhances annotation accuracy and validates functional elements	Nesvizhskii *et al.,* 2014

Genomic data encompasses a wide range of information generated through molecular, sequencing, and computational approaches. Each type of genomic data captures a different layer of biological organization, enabling researchers to study the genome from structural, functional, and evolutionary perspectives. At the most basic level, raw sequence data represents the direct

output of sequencing instruments, containing millions of short or long reads that form the foundation for all downstream analyses. Once processed and assembled, this data leads to genome sequences that reveal the complete DNA blueprint of an organism, including genes, regulatory elements, and structural variations. Another major category is transcriptomic data, derived from RNA sequencing, which reflects the dynamic activity of genes across tissues, stages, or conditions. This data type helps in understanding gene expression patterns and regulatory networks.

Beyond sequence-based data, variant data captures genetic differences within or between populations. It includes single nucleotide polymorphisms, insertions, deletions, and structural variants that contribute to phenotypic diversity, evolutionary relationships, and trait inheritance. Epigenomic data provides another important dimension by documenting modifications such as DNA methylation, histone modifications, and chromatin accessibility. These signatures help explain how gene activity is regulated without altering the underlying DNA sequence. Metagenomic data expands the scope of genomics further by analyzing genetic material from complex microbial communities in environmental or biological samples, offering insights into ecosystem composition and functional potential. Together, these diverse data types (Table 1) form a comprehensive toolkit for exploring genomic structure, function, regulation, and interactions across biological systems (Table 1).

Quality Control (QC) Tools

Quality control (QC) is a critical first step in any genomic data analysis workflow, ensuring that the sequencing reads are accurate, reliable, and suitable for downstream analyses such as alignment, assembly, and variant calling. High-throughput sequencing technologies produce massive volumes of raw data, but these data often contain sequencing errors, adapter contamination, low-quality bases, overrepresented sequences, and PCR duplicates. If not removed or corrected, these issues can lead to false variant calls, fragmented assemblies, or misinterpretation of genomic features. Therefore, QC is essential to maintain the integrity of the analysis pipeline and to reduce noise that can obscure biological signals.

QC begins with the assessment of raw reads, where tools generate detailed reports on base quality scores, GC distribution, per-base sequence content, duplication levels, and k-mer patterns. These diagnostic metrics allow researchers to identify anomalies from sequencing runs or sample preparation steps (Table 2). Following this assessment, the next stage involves cleaning and filtering the data. This may include trimming low-quality bases, removing adapters, filtering short reads, and eliminating duplicated sequences. By ensuring that only high-quality reads progress to later steps, QC minimizes computational burden and maximizes analytical confidence (Table 4).

Modern QC tools are designed to be fast, user-friendly, and compatible with multiple sequencing platforms. Many provide graphical summaries, making it easier to identify deviations from expected quality profiles. They also integrate seamlessly with downstream workflows and can be automated in pipelines for large-scale projects. Together, these QC tools form the foundation of robust genomic data analysis by improving the accuracy of sequence alignment, enhancing assembly continuity, and increasing the reliability of variant discovery.

Table 2: Commonly Used Quality Control Tools for Genomic Data

Tool	Type	Key Features	Typical Applications	References
FastQC	Quality Assessment	Per-base quality scores, GC content, sequence duplication, adapter detection	Initial QC check for raw Illumina reads	Andrews, (2010).
MultiQC	QC Summary Aggregation	Combines results from FastQC, Cutadapt, Trimmomatic, and others; generates unified report	Large projects requiring consolidated QC reports	Ewels *et al.,* 2016
Fastp	Assessment + Trimming	Quality profiling, adapter trimming, filtering, UMI processing, FASTQ correction	All-in-one QC and trimming for Illumina/Nanopore reads	Chen *et al.,* 2018
Trimmomatic	Read Trimming	Adapter removal, sliding-window trimming, length filtering	Pre-processing for alignment and variant calling	Bolger *et al.,* 2014
Cutadapt	Adapter Trimming	Highly accurate adapter detection and removal	RNA-Seq, small RNA, and whole-genome data	Martin 2011
PRINSEQ	Filtering + QC	Removal of duplicates, poly-N sequences, quality filtering	Dataset cleanup for metagenomics and WGS	Schmieder *et al.,* 2011
NanoPlot	QC for Long Reads	Statistics and visualization for Nanopore/PacBio reads	Long-read QC and run performance assessment	De Coster *et al.,* 2018
FASTX Toolkit	Basic QC Tools	Quality filtering, trimming, nucleotide distribution	Smaller datasets and basic read cleanup	Hannon 2010

Sequence Alignment Tools

Sequence alignment is a fundamental step in genomic data analysis, enabling researchers to determine the degree of similarity between nucleotide or protein sequences, identify conserved regions, detect evolutionary relationships, and support downstream applications such as phylogenetic analysis, gene prediction, and functional annotation. Sequence alignment can be broadly classified into two categories: pairwise alignment and multiple sequence alignment (MSA). Pairwise alignment compares two sequences using local or global alignment algorithms, while MSA aligns three or more sequences simultaneously to reveal conserved motifs and structural or functional domains. Modern alignment tools use dynamic programming, heuristic methods, or progressive alignment strategies to efficiently handle large and complex datasets generated by high-throughput sequencing platforms.

Table 3: Common Pairwise Sequence Alignment Tools

Tool Name	Type of Alignment	Key Features	Applications	References
BLAST	Local alignment	Fast, heuristic, database search	Homology search, gene identification	Altschul *et al.,* 1990
FASTA	Local alignment	Sensitive similarity detection	Identifying homologues, sequence comparison	Pearson *et al.,* 1998
EMBOSS Needle	Global alignment	Implements Needleman–Wunsch algorithm	Complete sequence comparison	Needleman *et al.,* 1971
EMBOSS Water	Local alignment	Implements Smith–Waterman algorithm	Detecting highly similar regions	Smith *et al.,* 1970
SIM	Pairwise local alignment	Outputs detailed similarity plots	Analyzing nucleotide/protein similarity	Huang *et al.,* 1990

In pairwise alignment workflows (Table 3), tools such as BLAST, FASTA, and EMBOSS Needle are widely used for their accuracy and ease of use. These tools help identify homologous regions, detect mutations, or compare sequences against large biological databases. For larger datasets or genome-scale comparisons (Table 4), advanced aligners such as BWA, Bowtie2, HISAT2, and minimap2 provide efficient alignment of millions of short or long reads to reference genomes. These algorithms use indexing methods like the Burrows–Wheeler Transform (BWT) and FM-indexing to drastically reduce computational time and memory requirements while maintaining high alignment accuracy.

Table 4: Genome-Scale Sequence Alignment Tools (Short/Long Reads)

Tool Name	Read Type	Algorithm	Key Strengths	Common Use	References
BWA	Short reads	BWT-based	Fast, accurate, widely used	Mapping NGS reads to reference genomes	Li, & Durbin (2009).
Bowtie2	Short reads	FM-index, seed alignment	Very fast, memory-efficient	Large-scale genome alignment	Langmead & Salzberg (2012).
HISAT2	Short reads	Graph FM-index	Excellent for spliced alignment	RNA-seq read alignment	Kim *et al.,* 2019
minimap2	Long reads	Seed chaining + DP	Handles noisy long reads	PacBio/Nanopore alignment & assembly	Li, 2018
STAR	RNA-seq reads	Spliced junction alignment	Extremely fast for transcriptomes	Gene expression and isoform detection	Dobin, *et al.* (2013).

Table 5: Multiple Sequence Alignment (MSA) Tools – *Updated to Include ClustalW*

Tool Name	Algorithm Type	Key Features	Applications	References
ClustalW	Progressive alignment	Classical, widely used, phylogeny support	MSA for genes/proteins, phylogenetic analysis	Thompson *et al.,* 1994
Clustal Omega	Progressive + HMM	Highly scalable, good for large datasets	Genome-wide MSA	Sievers *et al.* (2011)
MUSCLE	Iterative refinement	High accuracy, speed	Phylogenetics, motif analysis	Edgar, 2004.
MAFFT	FFT-based iterative	Extremely fast on large datasets	Large gene families, viral genomes	Katoh *et al.,* 2002
T-Coffee	Consistency-based	Very accurate alignments	High-quality MSA, structural alignments	Notredame *et al.,* 2000

Multiple sequence alignment tools (Table 5) are essential for evolutionary studies, phylogenomics, and motif discovery. Tools such as ClustalW, Clustal Omega, MUSCLE, MAFFT, and T-Coffee use progressive, iterative, or consistency-based approaches to generate high-quality alignments even for large sets of sequences. ClustalW, in particular, is one of the most widely used and historically significant tools, known for its progressive alignment method and user-friendly interface. These MSA tools help identify conserved residues, functional domains, regulatory motifs, and structural similarities across diverse species. Combined with visualization and editing programs like Jalview or BioEdit, sequence alignment tools form the backbone of comparative genomics and molecular evolutionary research.

Genome Assembly Tools

Genome assembly is a fundamental component of genomic data analysis, enabling the reconstruction of an organism's genome from fragmented sequencing reads. Since sequencing platforms generate millions of short or long reads rather than continuous chromosomes, assembly tools use sophisticated algorithms to piece together these fragments into larger sequences known as contigs and scaffolds. The choice of an assembly tool depends largely on the type of sequencing data, genome size, heterozygosity, repeat content, and the specific objectives of the study. Short-read technologies such as Illumina require assemblers optimized for high accuracy but shorter read length, whereas long-read platforms like PacBio and Oxford Nanopore benefit from assemblers capable of resolving complex genomic regions and long repetitive structures.

Assembly approaches can broadly be categorized into *de novo* assembly and *reference-guided* assembly. De novo assembly constructs the entire genome without prior knowledge, using graph-based algorithms such as de Bruijn graphs or overlap-layout-consensus methods. This approach is essential for organisms lacking a reference genome or for exploring genomic novelty. Reference-guided assembly, in contrast, aligns reads to an existing reference genome and uses the known structure to guide contig placement. While faster and computationally less intensive, its accuracy depends heavily on the quality and similarity of the reference genome.

Modern assembly tools incorporate error correction, scaffolding, polishing, and sometimes hybrid assembly strategies that integrate both short and long reads to maximize accuracy and contiguity. Post-assembly polishing tools further refine the genome by correcting residual errors, improving base accuracy, and closing gaps. The emergence of high-throughput sequencing has led to the development of several powerful assemblers, each with unique strengths tailored to specific data types. The following tables summarize the most widely used genome assembly tools, providing an overview of their algorithms, supported platforms, and key applications (Table 6).

Table 6: Overview of Major Genome Assembly Tools

Tool	Type of Assembly	Supported Data	Algorithm/Approach	Key Features	Applications	References
SPAdes	De novo	Illumina, Ion Torrent, hybrid	De Bruijn graph	Error correction, hybrid assembly, plasmid and metagenome versions	Bacterial genomes, plasmids, metagenomics	Bankevich *et al.* (2012).
Velvet	De novo	Illumina	De Bruijn graph	Fast assembly for short reads	Microbial genome assembly	Zerbino & Birney (2018)
SOAPdenovo2	De novo	Illumina	De Bruijn graph	Highly memory-efficient	Large eukaryotic genomes	Luo, *et al.* (2012)
ABySS	De novo	Illumina	Distributed de Bruijn graph	Scalable for large genomes using multiple nodes	Plant and animal genome projects	Simpson, *et al.* (2009).
MEGAHIT	De novo	Illumina, metagenomic data	Succinct de Bruijn graph	Very fast, low memory usage	Metagenomic assemblies	Li *et al.,* (2015)
Canu	De novo	PacBio, Nanopore	Overlap-Layout-Consensus	Self-correction of noisy long reads	Long-read microbial and eukaryotic genomes	Koren, *et al.* (2017).
Flye	De novo	PacBio, Nanopore	Repeat graph	Excellent repeat resolution, fast	Large genomes, structural variant studies	Kolmogorov *et al.,* (2019)
HGAP	De novo	PacBio	Hierarchical assembly	Error correction + assembly + polishing	High-quality microbial genomes	Chin, *et al.* (2013).
MaSuRCA	Hybrid/De novo	Illumina + long reads	Hybrid assembly pipeline	Integrates long and short reads effectively	Complex plant and animal genomes	Zimin, *et al.* (2013).
Unicycler	Hybrid	Illumina + Nanopore/PacBio	Hybrid SPAdes + bridging	Produces circular bacterial genomes	Complete bacterial genome assembly	Wick *et al.,* 2017

Table 7: Widely Used Variant Calling Tools and Their Key Features

Tool	Type of Variants Detected	Best For	Key Features	Platform/ Read Type	References
GATK Haplotype Caller	SNPs, Indels	High-quality germline variant calling	Local de novo assembly, high accuracy	Illumina short reads	McKenna *et al.,* 2010; Van der Auwera *et al.,* 2013
FreeBayes	SNPs, Indels	Population-level analysis	Haplotype-based Bayesian caller	Short reads	Garrison & Marth, 2012
SAMtools/BCFtools	SNPs, Indels	Fast, lightweight workflows	Pileup-based calling, widely used	Short reads	Li *et al.,* 2009
DeepVariant (Google)	SNPs, Indels	High-accuracy ML-based calling	Deep learning–driven classification	Illumina, PacBio, ONT	Poplin *et al.,* 2018
Strelka2	SNPs, Indels	Germline + somatic variants	Very sensitive to low allele frequency	Short reads	Kim *et al.,* 2018
VarScan2	SNPs, Indels	Somatic mutation detection	Works with low coverage, tumor–normal	Short reads	Koboldt *et al.,* 2012
Mutect2 (GATK)	Somatic variants	Cancer genomics	Statistical tumor–normal comparisons	Short reads	Cibulskis *et al.,* 2013
LoFreq	SNPs, Indels	Viral & microbial variant calling	Quality-aware variant detection	Short reads	Wilm *et al.,* 2012
NanoVar	Structural variants	ONT long reads	Long-read optimized, large SV detection	Long reads	Tham *et al.,* 2020
Sniffles	Structural variants	PacBio/ONT reads	Detects large SVs using long-read signatures	Long reads	Sedlazeck *et al.,* 2018

Variant Calling Tools

Variant calling is a crucial step in genomic data analysis that identifies genetic differences between a sample and a reference genome. These differences include single nucleotide polymorphisms (SNPs), small insertions and deletions (indels), copy number variations (CNVs), and structural variations (SVs). The accuracy of variant detection depends not only on the quality of the input sequence data but also on the efficiency of the computational tools used for analyzing aligned reads. Variant calling tools typically rely on probabilistic models, statistical frameworks, and machine-learning algorithms to distinguish true biological variants from sequencing errors, mapping ambiguities, and technical noise (Table 7).

Table 8: Commonly Used Gene Prediction Tools

Tool Name	Type	Key Features	Applications	References
AUGUSTUS	Ab initio & evidence-based	Highly accurate; supports many organisms; integrates extrinsic hints	Eukaryotic gene prediction, annotation pipelines	Stanke & Waack, 2003; Stanke *et al.,* 2006
GeneMark / GeneMark-ES	Ab initio	Self-training models; widely used for prokaryotes and eukaryotes	Prokaryotic and eukaryotic gene structure prediction	Besemer & Borodovsky, 2005; Lomsadze *et al.,* 2005
Glimmer	Ab initio	Uses interpolated Markov models; optimized for microbial genomes	Bacterial and archaeal gene prediction	Delcher *et al.,* 1999; Delcher *et al.,* 2007
SNAP	Ab initio	Trainable HMM-based model	Large genome annotation projects	Korf, 2004
FGENESH	Ab initio	High accuracy for plant and animal genomes	Commercial genome annotation and prediction	Salamov & Solovyev, 2000
BRAKER2	Evidence-based + ab initio	Integrates RNA-Seq and protein homology with GeneMark and AUGUSTUS	Automated eukaryotic genome gene prediction	Bruna *et al.,* 2021

The variant calling workflow generally begins after sequence alignment. Aligned reads in BAM or SAM format are processed to remove duplicates, recalibrate base quality scores, and improve mapping precision. Variant callers then analyze the depth of coverage, base quality, allele frequencies, and read mapping patterns to determine whether a genomic position differs from the reference. Some tools are optimized for high-depth short-read data, while others are designed for long-read platforms that capture larger structural changes. Modern variant callers also integrate Bayesian inference, hidden Markov models, graph-based genomic representations, and machine-learning classifiers to improve variant accuracy.

The identified variants are typically output in Variant Call Format (VCF), a standardized file that documents variant position, allele type, genotype, quality metrics, and annotations. These variant datasets can be further analyzed for population-level diversity, evolutionary patterns, trait associations, or clinical/functional significance. Overall, variant calling tools play a central role in connecting raw genomic information to meaningful genetic interpretation, enabling researchers to investigate genomic diversity, evolutionary relationships, and molecular mechanisms underlying traits.

Gene Prediction and Annotation Tools

Gene prediction and annotation represent one of the most critical steps in genomic data analysis, as they translate the raw sequence information into biologically meaningful insights. Once a draft genome is assembled, researchers need to identify which regions of the genome encode proteins, functional RNAs, regulatory sequences, or other genomic elements. Gene prediction typically involves two complementary approaches: *ab initio prediction* and *evidence-based prediction*. Ab initio methods use computational models, such as Hidden Markov Models (HMMs), to detect coding regions purely from sequence patterns like codon usage, open reading frames (ORFs), and splice site motifs. Evidence-based prediction, on the other hand, integrates external data such as transcriptome (RNA-Seq) reads, expressed sequence tags (ESTs), and homology to known genes from related species to refine gene models. Modern tools often combine both strategies to improve accuracy (Table 8).

Annotation goes beyond identifying genes; it assigns biological meaning by linking predicted genes to known functions, pathways, and protein domains. Functional annotation utilizes large databases such as UniProt, Pfam, KEGG, and GO, allowing researchers to characterize genes based on conserved domains, molecular functions, biological processes, and cellular components (Table 16). Gene annotation pipelines typically include steps such as sequence similarity searches (BLAST), protein domain detection, pathway mapping, and ontology assignment. Automated annotation systems streamline the annotation of large genomes while ensuring consistency,

scalability, and integration with existing biological knowledge. High-quality gene prediction and annotation are essential for comparative genomics, evolutionary studies, trait analysis, and molecular breeding applications.

Downstream Analysis and Interpretation

Downstream analysis and interpretation represent the final and most biologically meaningful phase of genomic data analysis. Once the genome has been assembled, aligned, and annotated—and variants or functional elements have been identified—the next step is to understand what these results imply in a broader biological, evolutionary, or applied context. This stage integrates statistical analysis, functional genomics approaches, comparative methods, and visualization techniques to translate raw genomic information into actionable insights.

One of the primary components of downstream interpretation is functional analysis**,** where genes, proteins, and variants are mapped onto biological pathways, ontologies, and molecular networks. Tools such as GO annotation, KEGG pathway mapping, and gene set enrichment analysis help determine whether certain biological processes, enzymatic pathways, or molecular functions are overrepresented in the dataset. These analyses enable researchers to uncover the biological significance behind differentially expressed genes, structural variants, or genomic regions of interest.

Another essential aspect involves comparative genomics, which examines similarities and differences between genomes from different species, varieties, or populations. Comparative analyses can reveal evolutionary relationships, identify conserved domains, detect gene family expansions and contractions, and highlight genomic signatures of selection. In population genomics, downstream analysis includes estimating allele frequencies, genetic diversity, population structure, and demographic history using tools such as STRUCTURE, PCA, and phylogenetic reconstruction.

Downstream interpretation also incorporates variant impact prediction, where computational tools assess whether SNPs or structural variants are likely to alter protein structure or function. This is particularly important in medical genomics, breeding programs, and functional gene studies. Visualization platforms such as genome browsers, phylogenetic trees, heatmaps, volcano plots, PCA scatterplots, and interaction networks further support interpretation by presenting the data in intuitive, biologically meaningful formats.

Overall, downstream analysis transforms processed genomic data into biological knowledge. By combining computational predictions with functional insights and comparative perspectives, researchers can draw conclusions about gene function, adaptive evolution, trait associations, lineage history, and molecular mechanisms underlying phenotypic variation.

Data Management and Resources

Effective data management is a foundational component of genomic research, as the volume, complexity, and diversity of genomic datasets continue to grow at an unprecedented rate. Modern sequencing platforms generate gigabytes to terabytes of raw data in a single experiment, requiring robust strategies for data storage, organization, processing, and long-term preservation. Proper data management ensures that genomic analyses remain accurate, reproducible, and transparent, and that datasets are easily accessible for future research or re-analysis. It also supports compliance with international standards for data sharing and contributes to the broader scientific community by enabling collaborative discovery.

A key aspect of genomic data management is the use of standardized file formats. Common formats such as FASTQ for raw reads, BAM/SAM for alignments, VCF for variants, GFF/GTF for annotations, and FASTA for assembled sequences serve as universal languages that allow tools, pipelines, and computational platforms to interact seamlessly. Consistency in file structure reduces errors during analysis and improves interoperability among bioinformatics tools. In addition to formats, metadata—descriptions that accompany datasets—is equally important. Metadata provides essential contextual information such as sample source, sequencing platform, library preparation method, and experimental conditions. High-quality metadata increases dataset transparency and enables meaningful comparison across studies, while poor or incomplete metadata often results in ambiguous interpretations and reduced scientific value.

Data storage infrastructure plays a crucial role in handling genomic datasets. High-performance computing (HPC) clusters, network-attached storage (NAS), cloud-based platforms (AWS, Google Cloud, Microsoft Azure), and institutional servers are widely used to store sequencing data and run computationally intensive analyses. Cloud platforms have become particularly important due to their scalability, cost-efficiency, and ability to host integrated bioinformatics environments. Many genomic workflows now run fully on cloud services, reducing the need for local hardware while enabling global collaboration.

Another essential component of genomic data management is data sharing. Public repositories such as NCBI's Sequence Read Archive (SRA), Gene Expression Omnibus (GEO), ENA, and DNA Data Bank of Japan (DDBJ) provide secure, standardized platforms for depositing sequencing data. These repositories ensure long-term preservation, enable worldwide access to datasets, and support the FAIR principles—Findability, Accessibility, Interoperability, and Reusability. Journals and funding agencies increasingly mandate data deposition to ensure transparency and reproducibility.

Finally, specialized databases and knowledge resources contribute to the interpretation and enrichment of genomic data. Databases such as Ensembl, UCSC Genome Browser, UniProt, Pfam, KEGG, and InterPro provide curated information on gene structure, protein domains, molecular pathways, and evolutionary relationships. Integration of such resources with computational pipelines strengthens biological interpretation, elevates the quality of downstream analysis, and enables multi-layered insights.

Together, these components—file formats, metadata standards, storage systems, repositories, and biological databases—form the backbone of genomic data management. They ensure that data remains organized, interpretable, reusable, and secure throughout the entire lifecycle of genomic research.

Future Trends and Challenges

The field of genomic data analysis is advancing rapidly, driven by improvements in sequencing technologies, computational power, and analytical methodologies. As genomes become easier and more affordable to sequence, the scale, complexity, and diversity of genomic datasets will continue to expand, creating new opportunities and challenges for bioinformatics tools. One of the most significant future trends is the integration of multi-omics data, where genomics is combined with transcriptomics, proteomics, epigenomics, and metabolomics to generate a comprehensive systems-level understanding of biological processes. Such integrative analyses require sophisticated computational platforms capable of handling heterogeneous data types, managing batch effects, and performing cross-layer correlations. Machine learning and artificial intelligence (AI) are also poised to play a major role, offering new possibilities for automated pattern recognition, variant interpretation, genome annotation, and predictive modelling in personalized medicine and precision agriculture.

Another emerging trend is the accelerated shift toward real-time and portable genomic analysis using technologies such as nanopore sequencing, mobile bioinformatics applications, and edge computing. These advancements will allow genomic data to be generated and analyzed outside traditional laboratories, supporting applications like rapid disease diagnostics, field-based biodiversity assessments, and on-site environmental monitoring. Cloud computing will continue to expand as a dominant infrastructure for large-scale genomic data storage, high-performance computation, collaborative analysis, and reproducibility. In addition, the increasing use of graph-based genome representations, pangenomes, and long-read assemblies will reshape genome reference frameworks and require new standards for tool development and data interpretation.

Despite these promising directions, several challenges must be addressed to fully realize the potential of future genomic bioinformatics. Data volume and storage demands are growing

exponentially, placing pressure on computational infrastructure and costing models. Ensuring data privacy, ethical handling, and secure sharing of genomic information remains a global concern, particularly in human genomics. Another major challenge is the lack of universal standards for data formats, workflow interoperability, and benchmarking, which complicates tool comparison and integration. Moreover, as tools become more complex, there is an increasing need for user-friendly interfaces, capacity-building, and training to ensure that researchers across disciplines can efficiently use advanced bioinformatics platforms. Finally, maintaining reproducibility in computational analyses—given the rapid evolution of software versions, dependencies, and pipelines—remains an ongoing issue.

Overall, the future of genomic data analysis will be shaped by continued innovation in sequencing technologies, computational approaches, data integration, and usability. Addressing the associated challenges will be essential for ensuring that bioinformatics remains robust, accessible, and capable of supporting the growing demands of research, industry, healthcare, and agriculture.

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RECENT ADVANCES IN PLANT FUNGAL DISEASE MANAGEMENT

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Abstract:

Fungal diseases are one of the major challenges in agriculture because they damage different parts of the plant and reduce both yield and quality. Their fast spread and increasing resistance to chemical fungicides make them difficult to control with traditional methods alone. This chapter explains the main types of fungal diseases affecting roots, leaves, stems, fruits, and seeds, and why they are important for farmers. It also discusses common management methods such as crop rotation, sanitation, proper irrigation, and careful use of fungicides. In recent years, safer and more effective solutions have become available. Beneficial microbes like *Trichoderma* and *Bacillus* help naturally suppress harmful fungi, while Integrated Disease Management (IDM) combines different approaches for better control. Modern biotechnology such as marker-assisted selection, speed breeding, RNA interference, CRISPR gene editing, and nanotechnology has helped in developing resistant crop varieties and detecting diseases early. Overall, this chapter highlights how combining traditional practices with modern scientific tools can create sustainable, long-term, and eco-friendly ways to protect crops from fungal diseases.

Keywords: Fungal Diseases, Integrated Disease Management (IDM), Marker-Assisted Selection, RNA Interference, CRISPR/Cas9, Resistant Varieties, Sustainable Agriculture.

1. Introduction:

Fungal diseases are like unseen thieves in our farms. They quietly attack our crops and take away a large part of the food we grow every year. These diseases spoil the leaves, roots, stems, and fruits of many plants, making them weak and reducing both yield and quality. This is a big concern for farmers and our food security. Due to climate change, which brings more warm and wet weather, among all disease-causing agents, fungal pathogens are the most widespread and economically damaging, affecting nearly every crop species cultivated today. According to recent estimates, fungal diseases alone account for approximately 15–20% of annual global crop losses. Major fungal pathogens such as *Fusarium spp.*, *Alternaria spp.*, *Colletotrichum spp.*, *Sclerotium rolfsii*, and *Magnaporthe oryzae* cause destructive diseases including wilts, blights, rusts, rots,

and mildews that severely reduce both yield and quality. Fungal diseases are spreading even faster and becoming harder to control (Savary *et al.,* 2019).

For many years, farmers have mainly used chemical fungicides (chemical sprays) to fight these diseases. These sprays act quickly and help save crops in the short term. But with time, major problems have appeared:

i. **Fungi become stronger and resistant.** When the same chemicals are used again and again, the fungi start adapting it. (Ishii, 2006).
ii. **Chemicals harm the environment and health.** Over use of fungicides can pollute soil and water and may also affect the health of humans, animals, and beneficial insects.

Because of these issues, scientists are now focusing on safe, eco-friendly, and long-term solutions to manage plant diseases. One such way is biological control, which uses natural enemies of the fungus, like beneficial bacteria or fungi, to stop their growth. Another important approach is biotechnology, where scientists use advanced tools to develop disease-resistant crop varieties.

Modern technologies like marker-assisted selection (MAS), transgenic crops, and genome editing tools such as CRISPR-Cas9 helps to create plants that can fight fungal infections on their own. These methods also help us understand how fungi attack plants and how plants defend themselves.

Today, the aim is not just to kill the fungus but to create a balanced and sustainable system where crops can protect themselves naturally, without harming the environment or human health.

This chapter will discuss the main types of fungal diseases in plants, the different management methods—including chemical, biological, and biotechnological approaches—and the latest advances and future strategies that can make farming healthier and more sustainable.

2. Types of Fungal Diseases in Plants

Fungal diseases are among the most common and serious problems faced by crops around the world. These diseases are caused by microscopic organisms called fungi, which succeed in warm and humid conditions (Agricos, 2005). They can spread easily through air, water, soil, insects, or even contaminated tools. Once a fungal spore reaches a plant surface, it germinates and infects the tissues, showing symptoms such as spots, rotting, yellowing, or powdery patches on various plant parts (Dean *et al.,* 2012).

Fungi can attack almost every part of a plant — from roots and stems to leaves, fruits, and seeds. Depending on where and how they infect, fungal diseases are grouped into the following main categories:

2.1. Root Diseases

Root-infecting fungi mainly attack the underground portions of plants. They interfere with the uptake of water and nutrients, leading to stunted growth, yellowing, and wilting (Singh *et al.,*

2020).

Examples:

- *Sclerotium rolfsii* – causes collar rot or stem rot in soybean, groundnut, and chickpea (Sharma *et al.,* 2018).
- *Fusarium oxysporum* – responsible for Fusarium wilt in tomato, banana, and cotton (Fravel *et al.,* 2003).
- *Rhizoctonia solani* – causes root rot and damping-off in vegetables and cereals (Taheri & Tarighi, 2011).
- *Pythium spp.* – causes damping-off and root rot in seedlings of vegetables, ornamentals, and cereals
- *Phytophthora spp.* – responsible for root and crown rot in pepper, tomato, and citrus crops
- *Macrophomina phaseolina* – causes charcoal rot in soybean, sorghum, and sunflower, especially under drought stress

2.2 Leaf Diseases

Fungi infecting leaves reduce the plant's ability to perform photosynthesis, leading to poor growth and yield (Agrios, 2005). The symptoms are usually easy to recognize—spots, blights, patches, or a powdery layer on leaf surfaces.

Examples:

- *Alternaria spp.* – causes leaf spot and blight in mustard, cotton, and tomato (Meena *et al.,* 2016).
- *Puccinia spp.* – responsible for rust diseases in wheat and other cereals (Kolmer, 2005).
- *Erysiphe spp.* – causes powdery mildew, which appears as white powder on leaves (Glawe, 2008).
- *Colletotrichum* spp. – Causes Anthracnose, characterized by dark, sunken lesions on leaves and stems of crops like beans and mango (Hyde *et al.,* 2009).
- *Peronospora* spp. / *Plasmopara* spp. – Cause Downy Mildew, which typically shows yellow spots on the top of the leaf and fuzzy growth underneath
- *Cercospora* spp. – Responsible for Cercospora Leaf Spot, often resulting in "frogeye" spots with pale centers on plants like soybean (Crous *et al.,* 2022).
- *Septoria* spp. – Causes Septoria Leaf Spot, identified by spots that contain small black reproductive structures (pycnidia) (McDonald & Mundt, 2016).
- *Phytophthora infestans* – Causes Late Blight, leading to rapid, destructive dark, water-soaked patches on potato and tomato leaves (Fry, 2008)

2.3. Stem and Fruit Diseases

These fungi attack the above-ground parts of plants such as stems, flowers, and fruits. They reduce quality and make fruits unmarketable (Dean *et al.,* 2012).

Examples:

- *Colletotrichum spp.* – causes anthracnose, leading to dark spots and fruit rot in mango, chili, and beans (Cannon *et al.,* 2012).
- *Botrytis cinerea* – responsible for gray mold on flowers, fruits, and vegetables (Williamson *et al.,* 2007).
- *Phytophthora infestans* (an oomycete fungus-like pathogen) – causes late blight in potato and tomato (Fry, 2008).

2.4. Seed and Grain Diseases

Some fungi infect seeds in the field or during storage. They lower seed germination and may produce mycotoxins, which are harmful to humans and animals (Pitt & Hocking, 2009).

Examples:

- *Aspergillus flavus* – produces aflatoxins in groundnut and maize (Amaike & Keller, 2011).
- *Penicillium spp.* – causes blue mold on stored fruits and grains (Pitt & Hocking, 2009).
- *Ustilago spp.* – causes smut diseases in cereals like maize and wheat (Brefort *et al.,* 2009).
- *Fusarium graminearum* – causes Head Scab or Blight in wheat and barley, often producing the mycotoxin deoxynivalenol (DON) (Leslie & Summerell, 2006).
- *Claviceps purpurea* – responsible for Ergot disease, replacing kernels in rye and other grasses with hard, toxic black masses known as sclerotia (Tudzynski & Hölter, 1998).
- *Tilletia laevis* and *T. caries* – cause Bunt or Stinking Smut in wheat, replacing the grain with foul-smelling spore masses (Kuhn & Cromey, 2002).
- *Rhizopus* spp. – causes rapid Storage Rot or Mold on high-moisture grains and seeds (Pitt & Hocking, 2009)

Why Understanding Fungal Diseases Matters

Identifying the type of fungal disease is the first and most important step in managing it effectively. Each pathogen has a different life cycle some can survive in soil for several years, while others spread quickly through air or water (Agrios, 2005). Understanding how they attack, helps in selecting the best management method, whether chemical, biological, or biotechnological.

3. Plant Disease Management Strategies

Plant disease management refers to all the practices and technologies used to reduce the impact of pathogens (like fungi, bacteria, and viruses) on crops. The goal is not always to eliminate the pathogen completely, but to keep it below a level where it causes serious damage. Among all

plant pathogens, fungi are the most destructive, causing nearly 70–80% of recorded plant diseases globally (Dean *et al.,* 2012).

Fungal disease management has evolved from simple cultural practices to advanced biotechnological interventions. Today, plant protection combines traditional knowledge with modern science to achieve long-term and sustainable disease control.

3.1 Chemical Control

Chemical control is one of the most common strategies used by farmers. It involves **the application of fungicides** — chemicals that either kill fungi or prevent their growth.

a. Types of Fungicides

i. **Protectant Fungicides:** Applied before infection, forming a barrier on plant surfaces (e.g., *Mancozeb*, *Copper oxychloride*).
ii. **Systemic Fungicides:** Absorbed by the plant and act from inside to stop infection (e.g., *Carbendazim*, *Propiconazole*).
iii. **Curative Fungicides:** Used shortly after infection to stop disease development.

b. Advantages and Limitations

Chemical fungicides provide quick and visible results, protecting crops during critical stages. However, continuous use leads to fungicide resistance, where fungi evolve to survive chemical exposure (Hawkins *et al.,* 2019).

They may also contaminate soil and water, affect non-target organisms, and pose health risks to humans. Therefore, modern disease management prefers rational fungicide use combined with biological and cultural practices under Integrated Disease Management (IDM).

3.2 Biological Control

Biological control means using living organisms such as beneficial bacteria, fungi, or other microbes to suppress plant pathogens. It is a safe and sustainable alternative to chemicals.

a. Mechanisms of Biocontrol

i. **Antibiosis:** Beneficial microbes produce natural antibiotics that kill or inhibit pathogens (*Bacillus subtilis*, *Streptomyces*).
ii. **Competition:** Beneficial microbes compete with pathogens for nutrients and space (*Trichoderma harzianum* is a classic example).
iii. **Parasitism and Mycoparasitism:** Some fungi directly attack and consume other fungi (*Trichoderma spp.* attacking *Sclerotium rolfsii*).
iv. **Induced Systemic Resistance (ISR):** Certain microbes “train” the plant’s immune system to respond faster and stronger against future infections.

b. Popular Biocontrol Agents

- **Trichoderma spp.** – effective against soil-borne pathogens such as *Rhizoctonia* and *Sclerotium*.
- **Pseudomonas fluorescens** – colonizes roots and suppresses fungal pathogens through antibiotics and ISR.
- **Bacillus subtilis** – produces antifungal compounds and promotes plant growth.

Biocontrol agents can be used as seed treatments, soil drenches, or foliar sprays, often in combination with reduced doses of fungicides (Saravanakumar *et al.,* 2020).

3.3 Cultural Control Methods

Cultural practices are the oldest and most natural form of disease management. They aim to create conditions that depress fungal growth or break the disease cycle. These methods are often low-cost and form the first line of defense in Integrated Disease Management (IDM).

a. Crop Rotation

Growing the same crop every year allows soil-borne fungi such as *Sclerotium rolfsii* or *Fusarium oxysporum* to survive and build up in the soil. Rotating crops with non-host species (for example, cereals followed by legumes) helps reduce inoculum levels and break the infection cycle (Bockus and Shroyer, 1998).

b. Field Sanitation

Removing infected crop residues, weeds, and helper plants minimizes fungal survival. Proper composting or burning of infected debris reduces the spread of spores. Farmers are also advised to clean farm tools and machinery after each use to avoid contamination.

c. Proper Irrigation and Drainage

Excessive soil moisture creates a perfect environment for fungi such as *Pythium* and *Rhizoctonia*. Controlled irrigation, raised beds, and good drainage systems help prevent such diseases.

d. Use of Healthy Seed Material

Seeds can carry dormant fungal spores. Using certified, pathogen-free seeds and seed treatments with fungicides or biological agents ensure healthy crop establishment.

3.4 Integrated Disease Management (IDM)

IDM is a holistic approach that combines different management strategies to control diseases in an eco-friendly way. It does not depend on a single method but integrates chemical, biological, cultural and genetic tools for long-term success.

For example, in managing *Sclerotium rolfsii* of soybean:

- Crop rotation reduces soil inoculum.
- Seed treatment with *Trichoderma harzianum* suppresses early infection.

- Judicious fungicide use at flowering stage ensures protection.

This combination provides effective and sustainable control while minimizing environmental risks

4. Biotechnological Interventions in Plant Disease Management

Biotechnology has transformed the way we understand and control plant diseases. It allows scientists to study the plant–pathogen relationship at the molecular level and use this knowledge to develop resistant crops, eco-friendly bio-products, and precise diagnostic tools. In the past, farmers mostly relied on chemical fungicides, but now biotechnology provides safer and smarter solutions that protect crops without harming the environment.

4.1 Importance of Biotechnology in Disease Management

Traditional methods such as crop rotation, fungicide use, and biological control help manage diseases, but they have **certain limitations**:

- They may not provide long-term protection.
- Pathogens can develop resistance to chemicals.
- Some methods are slow or not effective against newly emerging pathogens.
- Biotechnological tools overcome these limitations by providing disease resistance at the genetic level, rapid detection of pathogens, and precise control strategies that are specific and sustainable (Collinge *et al.,* 2010).

4.2 Developing Disease-Resistant Cultivars

Developing resistant varieties is one of the most effective and sustainable ways to manage fungal diseases. Biotechnology speeds up this process through several modern techniques.

4.2.1 Traditional Breeding Approaches

Conventional plant breeding has long been used to develop disease-resistant varieties. It involves crossing a resistant parent with a susceptible one and selecting offspring that show resistance.

However, this process takes many years and depends heavily on environmental conditions. Sometimes, resistance genes are lost or masked during hybridization. Modern biotechnology improves this by combining classical breeding with molecular markers and genomic tools, making selection faster and more accurate.

4.2.2 Marker-Assisted Selection (MAS)

Marker-Assisted Selection (MAS) is one of the most significant advances in plant biotechnology. Molecular markers are specific DNA sequences that are linked to disease resistance genes.

Instead of waiting for visible symptoms, breeders can now screen plants at the DNA level, identifying resistant genotypes even in the seedling stage (Varshney *et al.,* 2014).

For example:

- In wheat, MAS has been used to incorporate resistance genes against *Puccinia* spp. (rust fungi).
- In rice, MAS is used to transfer blast resistance genes (*Pi* genes).

This technique saves time, increases accuracy, and helps in pyramiding multiple resistance genes, giving long-lasting protection.

4.2.3 Speed Breeding

Speed breeding is an advanced method that uses controlled environmental conditions such as extended daylight and optimal temperature to produce multiple crop generations per year (Watson *et al.,* 2018).

This drastically reduces breeding time. For example, in wheat and chickpea, researchers can produce 6–8 generations per year instead of just one.

By combining speed breeding with MAS, scientists can quickly introduce and test new fungal resistance genes from wild or resistant relatives, accelerating the release of improved varieties.

4.2.4 Genomic Selection and Genome-Wide Association Studies (GWAS)

Modern genomics has made it possible to study entire plant genomes to find resistance-related regions.

Genomic Selection (GS) uses statistical models and DNA data to predict which plants carry beneficial genes, even before they are grown in the field.

GWAS identifies gene regions associated with resistance traits, helping scientists target specific loci during breeding (Crossa *et al.,* 2017).

These methods allow precise and large-scale selection, saving years of field testing.

4.3 Transgenic Approaches for Disease Resistance

Transgenic or genetically modified (GM) plants carry genes from other organisms that give them new traits, such as resistance to fungal infection.

This approach became popular in the 1990s and has provided major breakthroughs in managing difficult fungal diseases.

4.3.1 Pathogenesis-Related (PR) Proteins

Plants naturally produce PR proteins such as chitinases and glucanases when attacked by fungi. These enzymes break down the fungal cell wall, stopping infection.

Scientists have transferred genes encoding PR proteins from one plant to another to increase resistance (Punja & Raharjo, 1996).

For instance:

- Transgenic tobacco and rice expressing chitinase genes show resistance against *Rhizoctonia solani* and *Fusarium* spp.
- Overexpression of glucanase enhances defense against *Botrytis cinerea*.

4.3.2 Antifungal Peptides and Enzymes

Genes producing antifungal peptides (AFPs) and enzymes can be introduced into crops to inhibit fungal growth. These peptides work by damaging fungal cell membranes or blocking essential enzymes (Grover & Gowthaman, 2003).

Example:

- Expression of *AFP* from *Aspergillus giganteus* in tomato plants has provided resistance to *Fusarium oxysporum*.

4.3.3 RNA Interference (RNAi) Technology

RNA interference (RNAi) is a natural gene-silencing mechanism. In this technique, plants are engineered to produce small RNA molecules that target and silence essential fungal genes during infection (Nowara *et al.,* 2010).

This approach, known as Host-Induced Gene Silencing (HIGS), has been used successfully in crops like wheat, barley, and maize against pathogens such as *Fusarium graminearum* and *Blumeria graminis*.

RNAi-based crops are considered non-transgenic in some countries since they do not produce new proteins — only RNA molecules that silence target genes.

4.3.4 Genome Editing Using CRISPR/Cas9

The CRISPR/Cas9 system is one of the most revolutionary technologies in modern biotechnology. It allows scientists to edit specific parts of plant DNA with high precision.

By targeting susceptibility (S) genes or enhancing defense genes, plants can be made resistant without introducing foreign DNA (Zhou *et al.,* 2022).

Examples:

- In rice, CRISPR knockout of the *OsERF922* gene improved blast resistance.
- In tomato, editing *Mlo* genes provided resistance to powdery mildew.

CRISPR technology is faster, cheaper, and more acceptable for regulation compared to older transgenic methods.

4.4 Molecular Diagnostics and Pathogen Detection

Early detection is crucial for effective disease control. Biotechnology has enabled accurate and rapid identification of fungal pathogens through DNA and protein-based tools.

i. **PCR and qPCR (Quantitative PCR):** Detects fungal DNA even before symptoms appear.
ii. **ELISA (Enzyme-Linked Immunosorbent Assay):** Uses antibodies to detect specific fungal proteins.
iii. **LAMP (Loop-Mediated Isothermal Amplification):** A field-friendly tool that gives quick results without advanced lab equipment.
iv. **DNA Barcoding and Next-Generation Sequencing (NGS):** Used for identifying unknown fungi and studying pathogen diversity in ecosystems (Lievens & Thomma, 2005).

These diagnostic tools help in early warning systems, guiding farmers to take timely control measures.

4.5 Nanobiotechnology in Disease Management

Nanotechnology provides new opportunities in agriculture by developing nano-sized materials that can deliver fungicides or biomolecules precisely to the infection site (Khot *et al.,* 2012).

- **Nano-fungicides:** Controlled-release formulations that reduce chemical use and increase effectiveness.
- **Nano-sensors:** Detect fungal spores in the air or soil before visible symptoms appear.
- **Nano-encapsulation:** Protects biocontrol agents like *Trichoderma* from heat and UV damage, improving their shelf life.

These innovations promote precision plant protection while minimizing pollution and residue problems.

4.6 Future Prospects

The future of biotechnological plant disease management looks very promising. Some upcoming areas include:

- **Integration of Artificial Intelligence (AI)** for predicting fungal outbreaks using weather and satellite data.
- **Metagenomics** to study soil microbial diversity and discover new beneficial microbes.
- **Synthetic biology** to design customized defense pathways in plants.
- **CRISPR 3.0 and Base Editing tools** for fine-tuned resistance gene modification.

The ultimate goal is to develop resilient, climate-smart crops that can protect themselves naturally with minimal chemical input.

Conclusion:

Fungal diseases continue to threaten global agriculture by reducing crop yield, quality, and farmer income. Although chemical fungicides have provided quick protection for decades, their

excessive use has caused resistance in pathogens and raised environmental and health concerns. Therefore, modern plant disease management is now moving toward safer and more sustainable approaches.

Recent progress in biotechnology has greatly improved our ability to fight fungal infections. Techniques like marker-assisted selection, RNA interference, and CRISPR/Cas9 genome editing allow scientists to develop resistant crop varieties more efficiently. Alongside these, biological control using beneficial microbes such as *Trichoderma* and *Bacillus* offers natural and eco-friendly disease suppression. Nanotechnology and molecular diagnostic tools have also made early detection and precision treatment possible.

The future of fungal disease management lies in combining traditional, biological, and modern biotechnological methods under an integrated and sustainable framework. By focusing on prevention, resilience, and environmental safety, agriculture can achieve healthier crops and long-term food security for future generations.

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SPLINE COMPRESSION TECHNIQUE FOR SYSTEM OF SINGULARLY PERTURBED DELAY DIFFERENTIAL EQUATIONS

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Abstract:
In this work, we develop a spline compression technique for the numerical solution of system of singularly perturbed delay differential equations (SPDDEs). The method constructs a piecewise cubic spline in compression to approximate the solution, effectively balancing accuracy in boundary layers with computational efficiency. Numerical experiments on benchmark problems validate the effectiveness of the proposed technique, showing that it yields highly accurate results even for very small perturbation parameters and significant delays.

Keywords: Compression Spline, Delay Term, Singular Perturbation, Convection-Diffusion.

Introduction:

A more realistic model must account for how a system is influenced not only by its present state but also by its past and even anticipated future states. Therefore, real-world systems are often described using differential equations with delays or advances. Such equations play an important role in mathematical modelling across many disciplines, including Human pupil-light reflex [1], HIV infection [2, 3], Biological oscillators [4], Control systems [5], Neuronal activity [6], Physiological processes [7, 8], Bistable electronic devices [9], Population dynamics [10]. They appear in situations where the system's evolution is determined by present values together with different influences from its previous states.

Over the past twenty years, significant research has been conducted on numerical methods for SPDDEs. While effective numerical techniques have been developed for single SPDDEs, there are only a limited number of results available in the literature for systems of such equations. Subburayan and Ramanujam [11, 12] came up with two approaches: the initial value technique and the asymptotic numerical method: to tackle convection-diffusion and reaction-diffusion equations. Meanwhile, Selvi and Ramanujam [13] proposed an iterative numerical method tailored for a coupled system of SPDDEs.

Here, we derived a fitted compression spline approximation scheme to solve systems of SPDDEs. Traditional methods tend to stumble when ε (perturbation parameter) gets tiny compared to the grid width h used in discretization. Our goal is to prove that cubic spline in compression can deliver solid, accurate results whether ε is small or large relative to h. Splines techniques were first introduced by Schweikert [14] to reduce spurious oscillations that often occur in cubic spline curve fitting. This concept was later explored and developed further by researchers such as Pruess [15], de Boor [16], and others.

In developing ε-uniform methods, one effective approach is the fitted operator method. This technique was initially proposed by Allen *et al.* [17] for modelling viscous fluid flow past a cylinder. A comprehensive overview of ε-uniform fitted operator methods can be found in the work of Miller and Riordan [18]. Further contributions were made by Kadalbajoo and Sharma [19], who applied an ε-uniform fitted operator method to boundary value problems involving singularly perturbed delay differential equations.

The objective of the present study is to construct an ε-uniform numerical scheme for solving boundary value problems arising from coupled systems of SPDDEs. To achieve this, we employ a fitted operator method in conjunction with cubic splines in compression to effectively handle such complex problems.

Statement of the Problem:

Consider the following system of SPDDEs of convection-diffusion type:

$$\begin{cases} -\varepsilon\, v_1''(x) + p_1(x)v_1'(x) + \sum_{k=1}^{2} q_{1k}(x)v_k(x) + \sum_{k=1}^{2} r_{1k}(x)v_k(x-1) = f_1(x), x \in \Omega \\ -\varepsilon\, v_2''(x) + p_2(x)v_2'(x) + \sum_{k=1}^{2} q_{2k}(x)v_k(x) + \sum_{k=1}^{2} r_{2k}(x)v_k(x-1) = f_2(x), x \in \Omega \\ v_1(x) = \phi_1(x), x\epsilon[-1,0], v_1(2) = l_1, \\ v_2(x) = \phi_2(x), x\epsilon[-1,0], v_2(2) = l_2, \end{cases} \tag{1}$$

where $0 < \varepsilon \ll 1$, the function $p_i,\ q_{ik}, r_{ik}, f_i \in C^4(\Omega), i = 1,2, k = 1,2, \Omega = (0,2), \bar{\Omega} = [0,2],\ \Omega^- = (0,1), \Omega^+ = (1,2)$ and $\phi_i, i = 1,2$ are smooth functions on [-1,0]. It may be noted that problem (1) exhibits a strong boundary layer at x=2.

SPLINE COMPRESSION APPROXIMATION DIFFERENCE SCHEME:

Spline compression approximation difference scheme is developed on a uniform mesh as follows:

Let h is step size and $x_0 = 0, x_{2N} = 2, x_i = ih, i = 1\ to\ 2N-1$.

The functions $S_j(x,\tau) = S_j(x), j = 1,2$ satisfying the following differential equations:

$$S_j''(x) + \tau S_j(x) = [S_j''(x_i) + \tau S_j(x_i)]\ \frac{(x_{i+1}-x)}{h} + [S_j''(x_{i+1}) - \tau S_j(x_{i+1})]\ \frac{(x-x_i)}{h},$$

$$x \in [x_i, x_{i+1}] \quad (2)$$

where, $S_j(x_i) = V_j(x_i) \simeq v_j(x_i), j = 1, 2$ and $\tau > 0$ is termed as compression factor.

Solving Eq. (2), we get

$S_j(x) = C_j \cos\frac{\mu x}{h} + D_j \sin\frac{\mu x}{h} + (\frac{M_{j,i}+\tau V_{j,i}}{\tau})\,(\frac{x_{i+1}-x}{h}) + (\frac{M_{j,i+1}+\tau V_{j,i+1}}{\tau})\,(\frac{x-x_i}{h})$,

where C_j and D_j are the arbitrary constants, whose values are found with the use of interpolatory conditions $S_j(x_{i+1}) = V_{j,i+1}, S_j(x_i) = V_{j,i}$ for $j = 1, 2$.

Take μ=$h\,\tau^{\frac{1}{2}}$ and $M_{j,i} = S_j''(x_i)$, we get

$$S_j(x) = -\frac{h^2}{\mu^2 \sin\mu}\left[M_{j,i+1}\sin\frac{\mu(x-x_i)}{h} + M_{j,i}\sin\frac{\mu(x_{i+1}-x)}{h}\right] + \frac{h^2}{\mu^2}\left[\frac{(x-x_i)}{h}\left(M_{j,i+1} + \frac{\mu^2}{h^2}V_{j,i+1}\right) + \frac{(x_{i+1}-x)}{h}\left(M_{j,i} + \frac{\mu^2}{h^2}V_{j,i}\right)\right]$$

(3)

Differentiating Eq. (3) and taking $x \to x_i$ we obtain

$$S_j'(x_i^+) = \frac{(V_{j,i+1} - V_{j,i})}{h} + \frac{h}{\mu^2}\left[\left(1 - \frac{\mu}{\sin\mu}\right)M_{j,i+1} - (1 - \mu\cot\mu)M_{j,i}\right].$$

Considering the interval (x_{i-1}, x_i) and proceeding similarly, we get

$$S_j'(x_i^-) = \frac{(V_{j,i} - V_{j,i-1})}{h} + \frac{h}{\mu^2}\left[(1 - \mu\cot\mu)M_{j,i} - \left(1 - \frac{\mu}{\sin\mu}\right)M_{j,i-1}\right]$$

Equating the left-hand and right-hand derivatives at x_i, we have

$$\frac{(V_{j,i+1} - V_{j,i})}{h} + \frac{h}{\mu^2}\left[\left(1 - \frac{\mu}{\sin\mu}\right)M_{j,i+1} - (1 - \mu\cot\mu)M_{j,i}\right] = \frac{(V_{j,i} - V_{j,i-1})}{h} + \frac{h}{\mu^2}\left[(1 - \mu\cot\mu)M_{j,i} - \left(1 - \frac{\mu}{\sin\mu}\right)M_{j,i-1}\right]$$

(4)

Thus, we get a tridiagonal system

$$h^2(\mu_1 M_{j,i-1} + 2\mu_2 M_{j,i} + \mu_1 M_{j,i+1} = V_{j,i+1} - 2V_{j,i} + V_{j,i-1}, i = 1\ to\ 2N-1 \quad (5)$$

For j $= 1, 2$, where $\mu_1 = \frac{1}{\mu^2}\left(\frac{\mu}{\sin\mu} - 1\right)$, μ_2=$\frac{1}{\mu^2}(1 - \mu\cot\mu)$, and $M_{j,i} = S_j''(x_i)$,

$$i = 1\ to\ 2N - 1.$$

The equation (5) is consistent if $\mu_1 + \mu_2 = \frac{1}{2}$.

From the boundary conditions $V_{j,i} = \phi_{j,i}, -N \le i \le 0$, $V_{j,2N} = l_j$, where $\phi_{j,i} = \phi_j(x_i)$.

Take the notation

$p_1(x_i) = p_{1,i}, p_2(x_i) = p_{2,i}$, $q_{1j}(x_i) = q_{1j,i}$, $q_{2j}(x_i) = q_{2j,i}, r_{1j}(x_i) = r_{1ji}$, $r_{2j}(x_i) = r_{2ji}$ and $f_j(x_i) = f_{j,i}$.

From Eq. (1), we have

$$\varepsilon M_{1,k} = p_{1,k}V'_{1,k} + q_{11,k}V_{1,k} + q_{12,k}V_{2,k} + r_{11,k}V_1(x_k - 1) + r_{12,k}V_2(x_k - 1) - f_{1,k},$$

$$\varepsilon M_{2,k} = p_{2,k}V'_{2,k} + q_{21,k}V_{1,k} + q_{22,k}V_{2,k} + r_{21,k}V_1(x_k - 1) + r_{22,k}V_2(x_k - 1) - f_{2,k},$$

Substituting $M_{1,k}$ and $M_{2,k}$with $k = i, i \pm 1$ and

$$V'_{j,i} = \frac{V_{j,i+1} - V_{j,i-1}}{2h}, j = 1, 2,$$

$$V'_{j,i+1} = \frac{3V_{j,i+1} - 4V_{j,i} + V_{j,i-1}}{2h}, j = 1, 2,$$

$$V'_{j,i-1} = \frac{-V_{j,i+1} + 4V_{j,i} - 3V_{j,i-1}}{2h}, j = 1, 2.$$

In Eq. (5), we obtain the following system of linear equations in $V_{1,i}$ and $V_{2,i}$,

$$\begin{aligned}
&\{-\varepsilon - 1.5\mu_1 hp_{1,i-1} + \mu_1 h^2 q_{11,i-1} - \mu_2 hp_{1,i} + 0.5\mu_1 hp_{1,i+1})V_{1,i-1} + (2\varepsilon + 2\mu_1 hp_{1,i-1} \\
&\quad + 2\mu_2 h^2 q_{11,i} - 2\mu_1 hp_{1,i+1})V_{1,i} + (-\varepsilon - 0.5\mu_1 hp_{1,i-1} + \mu_2 hp_{1,i} + 1.5\mu_1 hp_{1,i+1} \\
&\quad + \mu_1 h^2 q_{11,i+1})V_{1,i+1} + h^2(\mu_1 q_{12,i-1}V_{2,i-1} + 2\mu_2 q_{12,i}V_{2,i} + \mu_1 q_{12,i+1}V_{2,i+1}) \\
&\quad = h^2[\{\mu_1 f_{1,i-1} + 2\mu_2 f_{1,i} + \mu_1 f_{1,i+1}\} \\
&\quad - \{\mu_1 r_{11,i-1}V_1(x_{i-1-N}) + 2\mu_2 r_{11,i}V_1(x_{i-N}) + \mu_1 r_{11,i+1}V_1(x_{i+1-N})\} \\
&\quad - \{\mu_1 r_{12,i-1}V_2(x_{i-1-N}) + 2\mu_2 r_{12,i}V_2(x_{i-N}) + \mu_1 r_{12,i+1}V_2(x_{i+1-N})\} \\
&\{(-\varepsilon - 1.5\mu_1 hp_{2,i-1} + \mu_1 h^2 q_{22,i-1} - \mu_2 hp_{2,i} + 0.5\mu_1 hp_{2,i+1})V_{2,i-1} + (2\varepsilon + 2\mu_1 hp_{2,i-1} \\
&\quad + 2\mu_2 h^2 q_{22,i} - 2\mu_1 hp_{2,i+1})V_{2,i} + (-\varepsilon - 0.5\mu_1 hp_{2,i-1} + \mu_2 hp_{2,i} + 1.5\mu_1 hp_{2,i+1} \\
&\quad + \mu_1 h^2 q_{22,i+1})V_{2,i+1} + h^2(\mu_1 q_{21,i-1}V_{1,i-1} + 2\mu_2 q_{21,i}V_{1,i} + \mu_1 q_{21,i+1}V_{1,i+1}) \\
&\quad = h^2[\{\mu_1 f_{2,i-1} + 2\mu_2 f_{2,i} + \mu_1 f_{2,i+1}\} \\
&\quad - \{\mu_1 r_{22,i-1}V_2(x_{i-1-N}) + 2\mu_2 r_{22,i}V_2(x_{i-N}) + \mu_1 r_{22,i+1}V_2(x_{i+1-N})\} \\
&\quad - \{\mu_1 r_{21,i-1}V_1(x_{i-1-N}) + 2\mu_2 r_{21,i}V_1(x_{i-N}) + \mu_1 r_{21,i+1}V_1(x_{i+1-N})\}]
\end{aligned}$$

For $i = 1\ to\ 2N - 1$

(6)

Incorporating a fitting factor in Eq. (6), we get

$$\{(-\varepsilon\sigma_1 - 1.5\mu_1 hp_{1,i-1} + \mu_1 h^2 q_{11,i-1} - \mu_2 hp_{1,i} + 0.5\mu_1 hp_{1,i+1})V_{1,i-1}$$
$$+ \left(2\varepsilon\sigma_1 + 2\mu_1 hp_{1,i-1} + 2\mu_2 h^2 q_{11,i} - 2\mu_1 hp_{1,i+1}\right)V_{1,i}$$
$$+ \left(-\varepsilon\sigma_1 - 0.5\mu_1 hp_{1,i-1} + \mu_2 hp_{1,i} + 1.5\mu_1 hp_{1,i+1} + \mu_1 h^2 q_{11,i+1}\right)V_{1,i+1}$$
$$+ h^2\left(\mu_1 q_{12,i-1}V_{2,i-1} + 2\mu_2 q_{12,i}V_{2,i} + \mu_1 q_{12,i+1}V_{2,i+1}\right)$$
$$= h^2[\left\{\mu_1 f_{1,i-1} + 2\mu_2 f_{1,i} + \mu_1 f_{1,i+1}\right\}$$
$$- \left\{\mu_1 r_{11,i-1}V_1(x_{i-1-N}) + 2\mu_2 r_{11,i}V_1(x_{i-N}) + \mu_1 r_{11,i+1}V_1(x_{i+1-N})\right\}$$
$$- \left\{\mu_1 r_{12,i-1}V_2(x_{i-1-N}) + 2\mu_2 r_{12,i}V_2(x_{i-N}) + \mu_1 r_{12,i+1}V_2(x_{i+1-N})\right\}],$$
$$\{(-\varepsilon\sigma_2 - 1.5\mu_1 hp_{2,i-1} + \mu_1 h^2 q_{22,i-1} - \mu_2 hp_{2,i} + 0.5\mu_1 hp_{2,i+1})V_{2,i-1} + (2\varepsilon\sigma_2 + 2\mu_1 hp_{2,i-1}$$
$$+ 2\mu_2 h^2 q_{22,i} - 2\mu_1 hp_{2,i+1})V_{2,i} + (-\varepsilon\sigma_2 - 0.5\mu_1 hp_{2,i-1} + \mu_2 hp_{2,i}$$
$$+ 1.5\mu_1 hp_{2,i+1} + \mu_1 h^2 q_{22,i+1})V_{2,i+1} + h^2(\mu_1 q_{21,i-1}V_{1,i-1} + 2\mu_2 q_{21,i}V_{1,i}$$
$$+ \mu_1 q_{21,i+1}V_{1,i+1})$$
$$= h^2[\left\{\mu_1 f_{2,i-1} + 2\mu_2 f_{2,i} + \mu_1 f_{2,i+1}\right\}$$
$$- \left\{\mu_1 r_{22,i-1}V_2(x_{i-1-N}) + 2\mu_2 r_{22,i}V_2(x_{i-N}) + \mu_1 r_{22,i+1}V_2(x_{i+1-N})\right\}$$
$$- \{\mu_1 r_{21,i-1}V_1(x_{i-1-N}) + 2\mu_2 r_{21,i}V_1(x_{i-N}) + \mu_1 r_{21,i+1}V_1(x_{i+1-N})\}]$$

(7)

where

$$\sigma_j = \frac{p_j(x)\frac{h}{\varepsilon}}{2}\coth\left(\frac{p_j(x)\frac{h}{\varepsilon}}{2}\right), j = 1, 2.$$

We solved the above system by taking $\mu_1 = \frac{1}{18}, \mu_2 = \frac{4}{9}$.

Numerical Examples:

The maximum absolute pointwise errors using the double mesh principle is given by

$$E_{i,\varepsilon}^M = \max_{0\le j\le M}\left|V_{i,j}^M - V_{i,2j}^{2M}\right|, i = 1, 2.$$

The ε - uniform maximum absolute error is given by

$$E_i^M = \max_{\varepsilon} E_{i,\varepsilon}^M, i = 1, 2.$$

The numerical rate of convergence is given by

$$R_i^M = \frac{\log\left(E_i^M/E_i^{2M}\right)}{\log 2}, i = 1, 2.$$

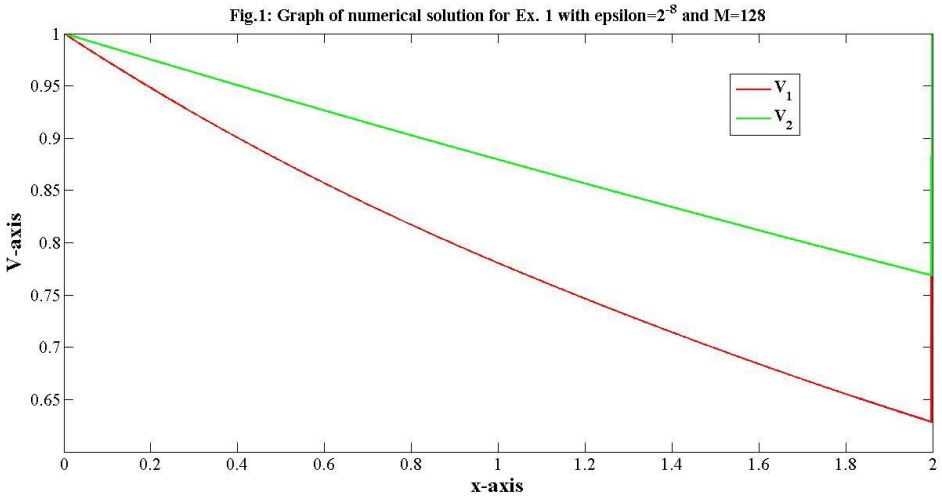
Example 1:

$$-\varepsilon\, v_1''(x) + 11v_1'(x) + 6v_1(x) - 2v_2(x) - v_1(x-1) = 0$$
$$-\varepsilon\, v_2''(x) + 16v_2'(x) - 2v_1(x) + 5v_2(x) - v_2(x-1) = 0$$
$$v_1(x) = 1, if -1 \le x \le 0, v_1(2) = 1$$

$$v_2(x) = 1, if -1 \le x \le 0, v_2(2) = 1$$

Table 1:

$M \rightarrow$	64	128	256	512	1024	2048
E_1^M	5.7950e-04	2.9202e-04	1.4657e-04	7.3424e-05	3.6746e-05	1.8382e-05
R_1^M	0.9887	0.9944	0.9972	0.9986	0.9993	-
E_2^M	1.5066e-04	7.7020e-05	3.8934e-05	1.9573e-05	9.8133e-06	4.9133e-06
R_2^M	0.9680	0.9841	0.9921	0.9960	0.9980	-



Example 2:

$$-\varepsilon\, v_1''(x) + 11v_1'(x) + 10v_1(x) - 2v_2(x) + x^2 v_1(x-1) - xv_2(x-1) = e^x$$

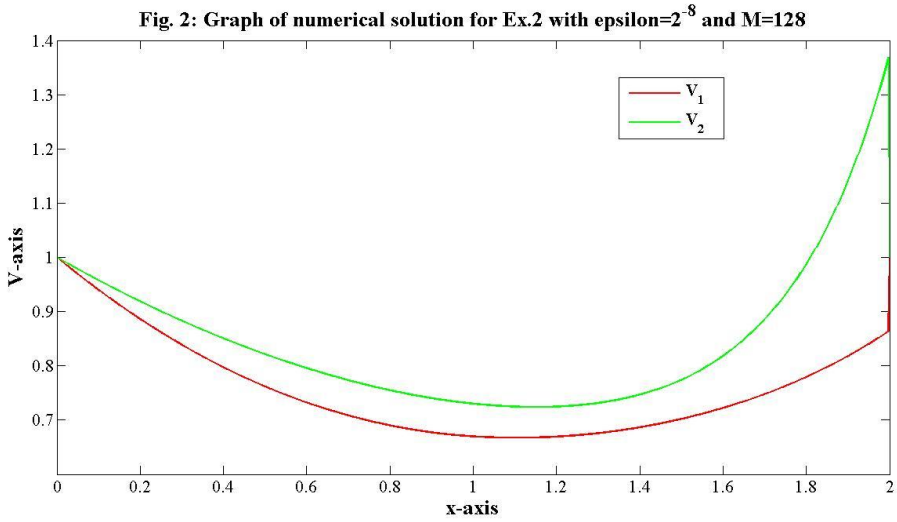
$$-\varepsilon\, v_2''(x) + 16v_2'(x) - 2v_1(x) + 10v_2(x) - xv_1(x-1) - xv_2(x-1) = e^{x^2}$$

$$v_1(x) = 1, if -1 \le x \le 0, v_1(2) = 1$$

$$v_2(x) = 1, if -1 \le x \le 0, v_2(2) = 1$$

Table 2:

$M \rightarrow$	64	128	256	512	1024	2048
E_1^M	5.3221e-03	2.7533e-03	1.4009e-03	7.0667e-04	3.5491e-04	1.7785e-04
R_1^M	0.9508	0.9748	0.9872	0.9935	0.9967	-
E_2^M	2.0320e-02	1.0560e-02	5.3835e-03	2.7181e-03	1.3657e-03	6.8452e-04
R_2^M	0.9443	0.9719	0.9859	0.9929	0.9964	-



Interpretation:

We have proposed a uniform mesh difference scheme using fitted compression spline approximation method that converges consistently. It's designed for a coupled system of SPDDEs of the convection-diffusion type. We've included numerical examples to highlight how well the scheme performs. The results show that our fitted tension spline approximation method delivers oscillation-free solutions for $0 < \varepsilon < 1$ across the entire domain, $0 < x < 2$. We tested it on two examples with varying ε values.

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LITHIUM-ION BATTERIES FOR ELECTRIC VEHICLES: STATE-OF-THE-ART, SUSTAINABILITY, CHALLENGES, AND FUTURE PERSPECTIVES

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Abstract:

Lithium-ion batteries (LIBs) have emerged as the dominant energy storage technology powering modern electric vehicles (EVs) due to their high energy density, long cycle life, and rapid technological evolution. This paper provides a comprehensive review of lithium-ion battery development, emphasizing sustainability, reliability, materials innovation, battery management systems (BMS), and emerging research trends. Drawing from recent literature, the paper synthesizes past progress, current advancements, and future directions. Key challenges related to thermal stability, material scarcity, recyclability, and digital optimization are highlighted.

1. Introduction:

The global transition toward cleaner and more sustainable transportation systems has accelerated the adoption of electric vehicles (EVs) across both developed and developing economies. Governments, policymakers, and industries are increasingly committing to carbon neutrality targets, stringent emission regulations, and the reduction of dependency on fossil fuels. In this evolving landscape**,** lithium-ion batteries (LIBs) have emerged as the dominant energy storage technology, powering nearly all modern EV architectures due to their high energy density, long cycle life, fast charging capabilities, and excellent power-to-weight ratio.

Beyond their technical advantages, LIBs enable significant improvements in vehicular efficiency, range, and performance, making them central to the growth of sustainable mobility. Over the past decade, extensive research has focused on enhancing various aspects of lithium-ion technology, including material innovation, thermal stability, battery safety, and system-level reliability. Scholars and industry experts have also emphasized the importance of evaluating the environmental footprint of LIBs, covering raw material extraction, manufacturing processes, operational emissions, and end-of-life recycling pathways.

Recent advancements have increasingly integrated artificial intelligence, machine learning, and digital twin modeling to optimize battery health estimation, predict degradation, and improve lifecycle performance. At the same time, growing concerns regarding the availability of critical

materials such as lithium, cobalt, and nickel have encouraged the exploration of sustainable alternatives, second-life applications, and circular economy models.

As the demand for EVs continues to rise globally, the study of lithium-ion batteries has become essential for understanding not only their technological progress but also their sustainability challenges, economic implications, and role in future mobility systems. This paper brings together recent state-of-the-art research to provide a comprehensive overview of lithium-ion battery development, challenges, and future directions.

2. Lithium-Ion Battery Fundamentals

Lithium-ion batteries (LIBs) function based on **the** reversible intercalation and deintercalation of lithium ions between the anode and cathode during charge and discharge cycles. This process is facilitated by an electrolyte and separated by a porous membrane known as the separator, ensuring ion flow while preventing electrical short circuits.

The performance, safety, and reliability of LIBs depend significantly on the choice of electrode materials, electrolyte composition, and cell architecture.

2.1 Cathode Materials

Cathodes are typically composed of lithium-containing metal oxides, each offering distinct performance characteristics:

- **Lithium Cobalt Oxide (LCO)** – High energy density, commonly used in portable electronics but less favored for EVs due to cost and thermal risks.
- **Lithium Nickel Manganese Cobalt Oxide (NMC)** – Widely used in EVs for its balanced energy density, safety, and longevity. Variants like NMC 811 offer improved performance through reduced cobalt content.
- **Lithium Iron Phosphate (LFP)** – Known for exceptional thermal stability, long cycle life, and safety; increasingly adopted in EVs, especially for mass-market and commercial segments.
- **Lithium Nickel Cobalt Aluminum Oxide (NCA)** – High specific energy and power density, used in high-performance EV applications.

2.2 Anode Materials

The anode serves as the host for lithium ions during the charging process. Common materials include:

- **Graphite** – The most widely used anode material due to structural stability, low cost, and reliable cycling performance.

- **Silicon-Enhanced Graphite** – Offers significantly higher theoretical capacity but faces challenges related to volume expansion and mechanical degradation.
- **Emerging Materials** – Research continues into silicon-dominant anodes, lithium-metal anodes, and composite structures for next-generation battery systems.

2.3 Electrolyte and Separator

The electrolyte typically consists of lithium salt (e.g., LiPF6) dissolved in organic solvents, enabling ion conduction. Additives are often introduced to enhance stability, suppress dendrite growth, and form a stable solid electrolyte interphase (SEI).

The separator ensures ionic conductivity while preventing physical contact between electrodes, with materials designed to withstand thermal and mechanical stresses.

2.4 Key Performance Metrics

LIB performance is evaluated based on several critical metrics:

- **Energy Density** – Determines driving range in EVs; influenced by electrode materials and cell design.
- **Power Density** – A measure of how quickly energy can be delivered; essential for acceleration and rapid response.
- **Charging and Discharging Capability** – Fast-charging performance depends on ion mobility, electrode kinetics, and thermal regulation.
- **Cycle Life & Degradation Behavior** – Influenced by temperature, charging rates, material stability, and BMS strategies.
- **Safety & Thermal Stability** – Prevention of thermal runaway is crucial, requiring effective thermal management and robust material selection.

Overall, LIB fundamentals form the technological backbone of modern electric vehicle performance, shaping advancements in range, safety, and cost optimization.

3. Technological Advancements (2021–2025)

Between 2021 and 2025, lithium-ion battery research has seen significant breakthroughs driven by the need for higher energy density, improved safety, longer lifecycle, and reduced material dependency. Key advancements include innovations in electrode materials, electrolyte design, structural optimization, and the integration of intelligent battery management technologies.

3.1. Silicon-Dominant and Silicon-Composite Anodes:

Silicon has emerged as a leading anode material due to its theoretical capacity nearly ten times higher than graphite. From 2021 onward, researchers have developed nano-engineered silicon composites, surface-coated silicon particles, and elastic polymer binders to address volume-

expansion issues. These efforts have enabled commercial-grade silicon–graphite anodes with enhanced cycle stability and fast-charging performance.

3.2. Cobalt-Free and Low-Cobalt Cathodes:

To reduce cost and improve supply-chain sustainability, the shift toward cobalt-free cathode chemistries accelerated. High-nickel NMC (NMx) materials, manganese-rich cathodes, and LFP variants have been optimized for greater thermal stability and longer lifespan. Companies and research groups have demonstrated cobalt-free cathodes with competitive energy density, making them suitable for mass-market EVs.

3.3. Electrolyte Engineering and Additive Development:

Advanced electrolyte formulations—such as localized high-concentration electrolytes (LHCEs), fluorinated solvents, and functional additives—have significantly improved SEI stability, high-voltage tolerance, and safety. Gel and hybrid polymer electrolytes gained attention for enabling safer, flame-retardant battery systems while supporting fast ion transport.

3.4. Solid-State Battery Progress:

Solid-state batteries (SSBs) have advanced from early prototypes to more stable pre-commercial designs. Between 2021 and 2025, major developments include sulfide-based and oxide-based solid electrolytes with higher ionic conductivity, reduced interfacial resistance, and improved dendrite suppression. These innovations have boosted interest in SSBs for next-generation EVs due to their potential for superior safety and energy density.

3.5. AI-Driven Optimization and Smart Battery Management:

Artificial intelligence and machine-learning techniques have improved battery diagnostics, state-of-charge (SOC) prediction, and degradation forecasting. AI-based control algorithms optimize fast charging, enhance thermal management, and prolong battery lifecycle. Digital-twin models are increasingly used for real-time performance prediction and operational safety.

3.6. Thermal Stability and Safety Improvements:

New separator materials, flame-retardant electrolytes, and thermal-runaway mitigation strategies have strengthened overall battery safety. Research has focused on ceramic-coated separators, advanced thermal-interface materials, and integrated temperature-sensing layers to improve resilience in high-load EV applications.

4. Sustainability and Environmental Impact

The sustainability of lithium-ion batteries (LIBs) has become a critical research focus as EV adoption accelerates globally. Environmental assessments emphasize that while LIBs enable significant reductions in tailpipe emissions, their lifecycle—from raw material extraction to end-of-life disposal—poses notable ecological challenges.

4.1. Raw Material Extraction and Ethical Concerns:

Key materials such as lithium, nickel, cobalt, and manganese involve environmentally sensitive mining processes. Cobalt extraction, particularly in regions like the Democratic Republic of Congo (DRC), raises concerns about habitat degradation, groundwater contamination, and ethical labor practices. Recent sustainability metrics encourage shifting toward cobalt-free chemistries, improved mining regulations, and the use of secondary raw materials.

4.2. Energy-Intensive Manufacturing:

Battery cell production requires substantial electricity, especially for cathode material synthesis and electrode drying. Studies indicate that the carbon footprint of LIB manufacturing varies significantly depending on the energy mix used in factories. As a result, battery manufacturers are increasingly shifting toward renewable-powered gigafactories and adopting low-temperature electrode processing to reduce CO_2 emissions.

4.3. Lifecycle Emissions and Use-Phase Benefits:

Despite high manufacturing energy requirements, EVs powered by LIBs typically achieve lower lifecycle greenhouse gas (GHG) emissions than internal combustion engine vehicles (ICEVs). Their sustainability benefits improve further when EV charging relies on renewable sources. Innovations in battery durability and high-efficiency thermal management systems also extend battery lifespan, reducing environmental impact per kilometer.

4.4. End-of-Life Challenges and Waste Generation:

Discarded lithium-ion batteries contain hazardous compounds and critical minerals that can cause soil and water pollution if improperly handled. Growing EV adoption is expected to substantially increase global LIB waste volumes after 2030, making effective recycling pathways essential.

4.5. Recycling, Reuse, and Circular Economy Frameworks:

Advanced recycling technologies—such as hydrometallurgical, pyrometallurgical, and direct recycling methods—allow recovery of lithium, nickel, cobalt, and other valuable materials. Circular strategies include second-life applications, where retired EV batteries are repurposed for stationary energy storage. Global initiatives aim to design “recyclable-by-design” batteries, promote closed-loop supply chains, and reduce dependence on virgin mineral extraction.

4.6. Policy and Regulatory Developments:

International policies, including extended producer responsibility (EPR) regulations and battery passport frameworks, encourage traceability, responsible sourcing, and sustainable disposal. Governments and industries are jointly working toward setting minimum recycling efficiency targets and improving end-of-life management infrastructure.

5. Battery Management Systems (BMS)

Battery Management Systems (BMS) play a crucial role in ensuring the safe, reliable, and efficient operation of lithium-ion batteries in electric vehicles. As battery capacities increase and EV architectures become more complex, the functionality and intelligence of BMS technologies have expanded significantly.

5.1. Core Functions of BMS:

A conventional BMS performs several essential tasks, including:

- **State of Charge (SOC) estimation:** Determines the remaining energy in the battery using algorithms such as Kalman filtering, coulomb counting, and machine-learning models.
- **State of Health (SOH) estimation:** Assesses battery degradation by analyzing capacity fade, internal resistance, and historical usage patterns.
- **Cell balancing:** Ensures uniform voltage and charge distribution across cells to prevent overcharging or undercharging.
- **Thermal monitoring:** Tracks temperature at cell/module level to avoid overheating and thermal runaway.
- **Fault detection and protection:** Identifies anomalies such as short circuits, overcurrent, overvoltage, or abnormal temperature rise, and initiates protective control actions.

5.2. AI-Enhanced BMS and Predictive Algorithms:

Recent EV technologies integrate artificial intelligence and machine learning into BMS for enhanced accuracy and decision-making. AI-driven BMS models improve SOC/SOH prediction by learning from real-world battery usage patterns, environmental conditions, and dynamic load behavior. Predictive algorithms enable early detection of degradation modes, allowing preventive maintenance and longer battery lifespan.

5.3. Advanced Thermal and Safety Management:

Modern BMS frameworks incorporate real-time heat distribution mapping, thermal runaway modeling, and active cooling strategies. By continuously monitoring temperature gradients, AI-supported BMS can predict hotspots and adjust cooling mechanisms to enhance safety. This is especially important for fast charging, where batteries experience higher thermal stress.

5.4. Cybersecurity and Communication Protocols:

With increasing EV connectivity, BMS systems now rely on secure communication protocols to prevent unauthorized access or data manipulation. CAN, LIN, and Ethernet-based architectures support high-speed data transmission, while encryption techniques protect system integrity.

5.5. Role in Fire Prevention and Hazard Mitigation:

AI-assisted BMS can identify early signals of internal short circuits, gas generation, or abnormal impedance changes—conditions that may precede thermal runaway. This early intervention capability is crucial for minimizing the risk of battery fires and enhancing passenger safety.

5.6. Integration with Digital Twins and Cloud Platforms:

The next generation of BMS integrates cloud-based analytics and digital twin models to simulate battery behavior under various operating conditions. These digital platforms allow real-time diagnostics, remote updates, and optimization of battery performance throughout the EV's lifecycle.

Conclusion:

Lithium-ion batteries remain the cornerstone of electric vehicle energy storage, driven by continuous advancements in materials, design, and management technologies. Recent progress—from silicon-based anodes and cobalt-free cathodes to AI-assisted Battery Management Systems—has significantly enhanced performance, safety, and lifespan. However, addressing persistent challenges related to sustainability, thermal safety, raw material dependency, and end-of-life recycling remains essential for their long-term viability. As global EV adoption accelerates, coordinated efforts in advanced material research, intelligent BMS development, and circular-economy-based recycling strategies will define the next generation of high-performance, eco-efficient lithium-ion batteries.

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IONIC LIQUIDS AS GREEN SOLVENTS AND CATALYSTS IN ORGANIC TRANSFORMATIONS

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Abstract:
Ionic liquids have gained significant attention as environmentally friendly solvents and catalysts in organic synthesis. Their distinctive properties, such as negligible vapor pressure, high thermal stability, and customizable polarity, make them ideal alternatives to traditional volatile organic solvents. These liquids enhance reaction efficiency and selectivity while reducing harmful emissions and waste. Ionic liquids also function as catalysts or catalytic media, facilitating sustainable processes with easier catalyst recovery and reuse. Due to their tunable nature, ionic liquids can be designed to optimize various organic transformations, including condensations, cycloadditions, and coupling reactions. Their use aligns with green chemistry principles by minimizing environmental impact, improving atom economy, and promoting recycling. Consequently, ionic liquids are valuable tools for advancing eco-friendly methodologies in organic synthesis. [1]

Keywords: Ionic Liquids, Green Solvents, Organic Synthesis, Catalysis, Sustainable Chemistry

Introduction:

Ionic liquids (ILs), a class of organic salts which exist as liquids near or at room temperature, have redefined the landscape of sustainable chemistry in recent decades. Characterized by their negligible vapor pressure, high thermal stability, and extensive tunability in physicochemical properties, ILs present an attractive alternative to conventional volatile organic solvents commonly used in organic synthesis. Their ability to minimize environmental contamination by eliminating air emissions and their enhanced recyclability situate them as quintessential green solvents. This advancement addresses critical environmental and safety issues, aligning chemical processes with the principles of green chemistry to reduce the chemical industry's ecological footprint [1].

Beyond their unique solvent properties, ILs also perform as efficient catalysts or catalytic media in a myriad of organic transformations. Due to their inherent ionic nature, ILs can stabilize charged intermediates and transition states, thereby promoting reaction rates and selectivity under

milder conditions than traditional solvents and catalysts. Moreover, ILs' structural diversity allows fine-tuning of their catalytic activity and selectivity by modifying cation-anion combinations, paving the way for target-specific applications in syntheses such as condensations, cycloadditions, and coupling reactions. Their dual functionality as both solvent and catalyst can simplify reaction setups, reduce waste generation, and facilitate catalyst recovery, further strengthening their role in sustainable organic synthesis [2].

The use of ILs in organic transformations also enables improved processes in biomass conversion and pharmaceutical synthesis, demonstrating broad applicability in industrially relevant reactions. Their high solvation capacity allows effective dissolution of diverse substrates, including polar, nonpolar, and polymeric materials, which often pose challenges in conventional organic solvents. Additionally, ILs can be paired with metal catalysts or immobilized on supports to create recyclable catalytic platforms, thereby enhancing economic viability and reducing environmental impact. As research advances, the design of task-specific ionic liquids aims to tackle challenges like toxicity and biodegradability, ensuring that these green solvents meet the growing demand for eco-friendly and practical synthetic methodologies [3].

In summary, ionic liquids have emerged as promising sustainable media in organic chemistry, offering versatile solvent and catalytic characteristics that promote greener and more efficient synthetic routes. Their role in reducing volatile organic emissions, increasing reaction efficiency, and enabling catalyst recyclability exemplifies the ongoing transformation toward environmentally responsible chemical manufacturing. These innovations not only improve the sustainability profile of diverse organic reactions but also inspire further development of advanced materials and processes aligned with the objectives of green chemistry [1,2,3].

Literature Review:

The utilization of ionic liquids (ILs) in organic reactions has witnessed considerable advancement from 2010 to 2025, positioning ILs as essential components in green chemistry.

In 2010, Rogers and Seddon, through their work titled "Ionic Liquids—Solvents of the Future," underscored the potential role of ILs in promoting green chemistry by reducing volatile organic emissions and enhancing reaction efficiencies, laying foundational insights for sustainable organic synthesis [4]

Welton's 2014 review, "Room-Temperature Ionic Liquids: Solvents for Synthesis and Catalysis," delved into ILs' adaptability across diverse organic transformations, showcasing their influence in catalysis, improved selectivity, and catalyst recycling during complex syntheses [5].

Zhao (2015), through "Functional Ionic Liquids for Catalysis," further pioneered the concept of task-specific ionic liquids (TSILs), which act simultaneously as solvents and catalysts, enhancing reaction selectivity and sustainability [6].

Lei *et al.* (2017) authored "Introduction: Ionic Liquids," providing comprehensive insights into the synthetic versatility of ILs, highlighting their dual solvent-catalyst roles tailored to optimize organic reaction efficiency [7].

Gujjala *et al.* (2024) discussed environmental and catalytic advancements in "Advances in Ionic Liquids: Synthesis, Environmental Effects, and Catalytic Applications," emphasizing sustainable synthetic processes driven by ionic liquids innovations, bridging the gap between IL design and practical green applications [8].

In 2025, Handique's critical analysis titled "Advances in Organic Synthesis on Ionic Liquid Platforms" presented extensive applications of ILs in multi-component and asymmetric syntheses, showcasing their capability to streamline reaction protocols while reducing environmental footprint [9].

Alreshidi's 2025 review, "A Review on the Evolution of Ionic Liquids," traced the trajectory of IL technology, highlighting how innovations have tailored ILs toward more sustainable and efficient organic synthetic methodologies [10].

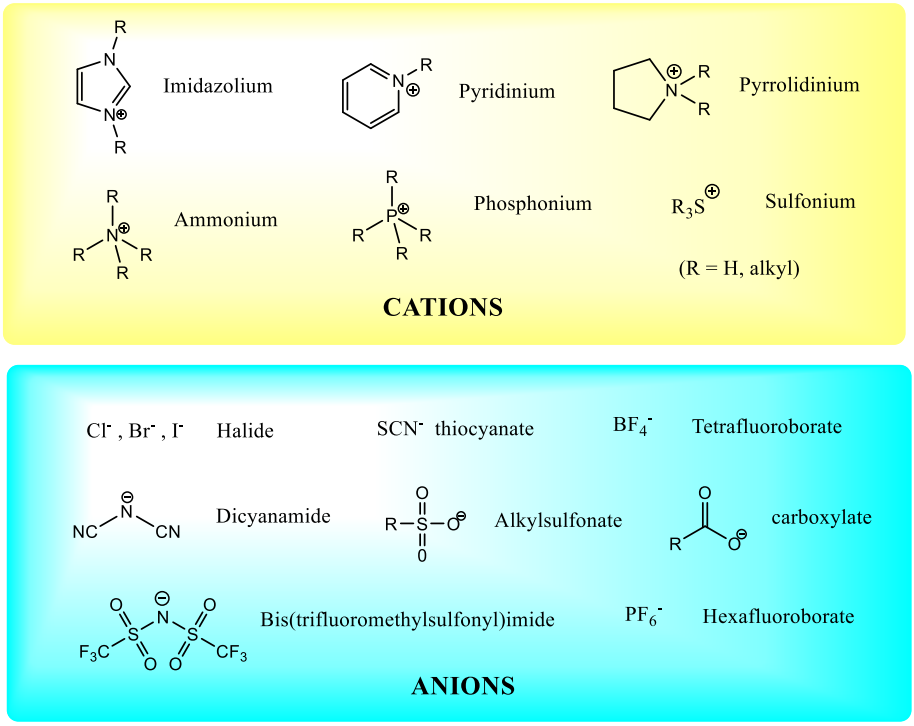
Additionally, Zhao and Welton together contributed to advancing catalytic efficiency and functional design of ILs during this period, reflected in joint symposium proceedings [11].

Collectively, these contributions from leading scientists represent a paradigm shift toward integrating ILs as versatile, sustainable media reshaping modern organic synthesis paradigms.

Classification :

Ionic liquids (ILs) can be classified based on their cationic and anionic components, as well as their temperature of liquidity and functional properties. The most common classification is based on cations, including imidazolium, pyridinium, phosphonium, ammonium, and sulfonium types. For example, imidazolium-based ILs—such as 1-butyl-3-methylimidazolium ($[bmim]^+$)—are widely used in organic synthesis due to their stability and tunable properties.

Anions also vary, with common examples being tetrafluoroborate ($BF4^-$), hexafluorophosphate ($PF6^-$), bis(trifluoromethanesulfonyl)imide ($NTf2^-$), and acetate (Ac^-). ILs are further classified as room-temperature ionic liquids (RTILs) when they remain liquid below 100 °C, and task-specific ionic liquids (TSILs) designed to carry specific functional groups for catalysis or separation processes. This classification reflects the versatile design and application spectrum of ILs in green chemistry [1, 12,13,14].



Advantages:

- Negligible vapor pressure, which makes them essentially non-volatile and minimizes air pollution compared to traditional organic solvents.[15].
- High thermal, chemical, and electrochemical stability allows reactions under harsh conditions [8]
- Tunable polarity and viscosity via cation/anion modification customize solvents for specific reactions [13].
- Dual role as solvents and catalysts increases efficiency, and easy catalyst recovery aids recyclability [16].
- Non-flammability enhances handling safety compared to traditional solvents [17].
- Excellent solubility for a wide range of polar and nonpolar compounds broadens reaction scope [13].
- Low toxicity and potential for biodegradability with proper structural design.

Limitations:

- High viscosity can limit mass transfer, impacting reaction rates [18].
- Relatively high cost restricts large scale application [8].
- Toxicity and environmental persistence of some ILs raise sustainability concerns [19].
- Sensitivity to moisture may affect stability and reproducibility [13].
- Product isolation can be challenging due to IL solvation properties [18].

These factors require careful selection and design for sustainable applications.

Methods for preparing ionic liquids (ILs)

It involves various synthesis routes tailored to achieve desired cation-anion combinations and properties. Key methods include:

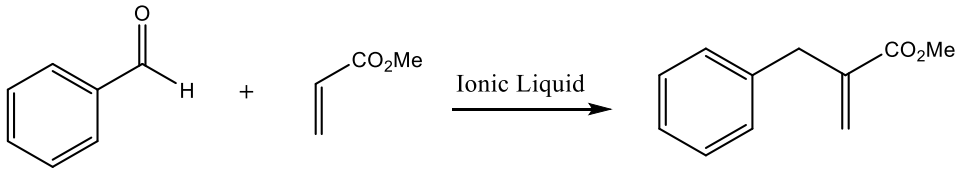
- **Direct Neutralization:** This involves reacting a Brønsted acid with a Brønsted base to form the ionic liquid. For example, protonation of triethylamine with hydrochloric acid produces triethylammonium chloride ionic liquid. This method is straightforward and widely used for ammonium and phosphonium ILs [1].
- **Alkylation of Heterocyclic Compounds:** Imidazolium or pyridinium ILs are typically prepared by alkylation of their corresponding heterocycles with alkyl halides. For instance, 1-butyl-3-methylimidazolium chloride ([bmim]Cl) is synthesized by reacting 1-methylimidazole with butyl chloride [2].
- **Metathesis Reaction:** This method replaces the halide anion in a quaternary ammonium or phosphonium salt with more hydrophobic anions like hexafluorophosphate ($PF6^{-}$) or bis(trifluoromethanesulfonyl)imide (NTf_2^-) via anion-exchange reactions. For example, the chloride anion of [bmim]Cl can be exchanged with PF_6– to yield [bmim]PF_6 [13].
- **Electrochemical Synthesis:** This is a modern approach where ILs are generated through electrochemical reactions, offering control over purity and composition. It is less common but applied for functional ILs with tailored properties [20].

Organic Transformations involving Ionic Liquids:

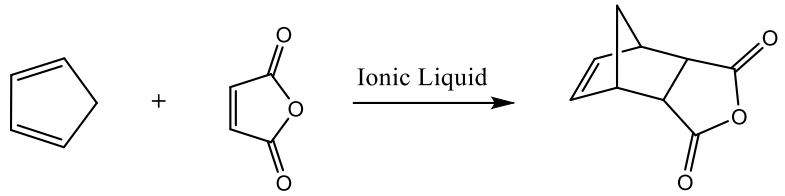
Ionic liquids (ILs) have proven to be exceptional media in diverse organic reactions due to their unique properties. Below are eight important organic reactions involving ILs, with illustrative examples:

1. Morita–Baylis–Hillman Reaction: Enhanced reaction rate and selectivity in imidazolium-based ILs such as [bmim][PF6], catalyzing aldehyde and acrylate coupling under mild conditions with recyclability.

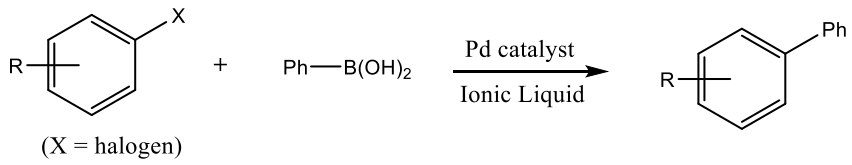
   Example: Benzaldehyde + Methyl acrylate → Baylis–Hillman adduct [21]



2. Diels–Alder Cycloaddition: Using chiral ionic liquids as dual solvent-catalysts improves conversion and stereoselectivity of cycloadditions between cyclopentadiene and maleic anhydride. [2, 22]

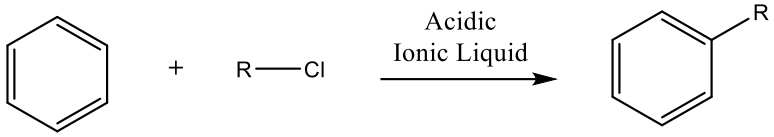


3. Suzuki Cross-Coupling: Ionic liquids stabilize Pd catalysts promoting coupling between aryl halides and boronic acids with high yields and catalyst reuse. [13, 23]

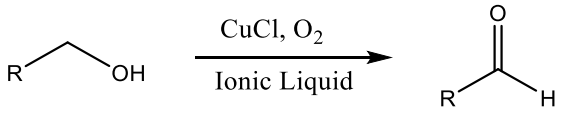


(X = halogen)

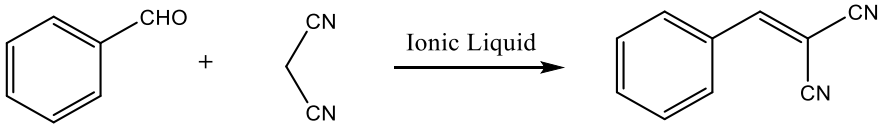
4. Friedel-Crafts Alkylation: Acidic ionic liquids replace volatile acid catalysts in alkylation of benzene with chloroalkanes, enabling easy catalyst recovery. [23]



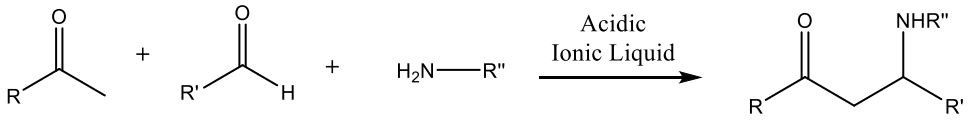
5. Oxidation of Alcohols: CuCl-catalyzed aerobic oxidation of primary alcohols to aldehydes proceeds with better selectivity in [bmim][BF4]. [23]



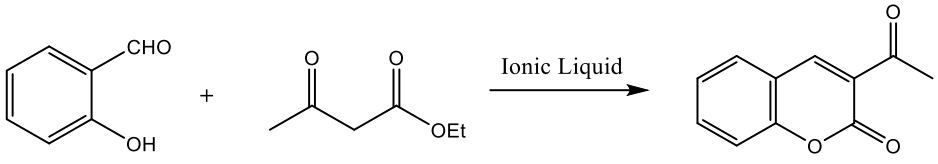
6. Knoevenagel Condensation: Mild, solvent-free Knoevenagel condensation between aldehydes and malononitrile catalyzed by basic functionalized ILs yields products in high purity. [22]



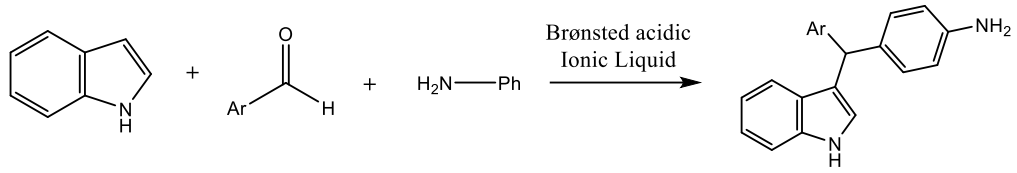
7. Mannich Reaction: One-pot, multicomponent Mannich reaction catalyzed by Brønsted acidic ILs producing β-amino carbonyl compounds efficiently under green conditions[24].



8. Synthesis of Coumarins: Cyclization of salicylaldehydes and activated methylene compounds catalyzed by acidic ILs under solvent-free conditions yielding coumarins [25].

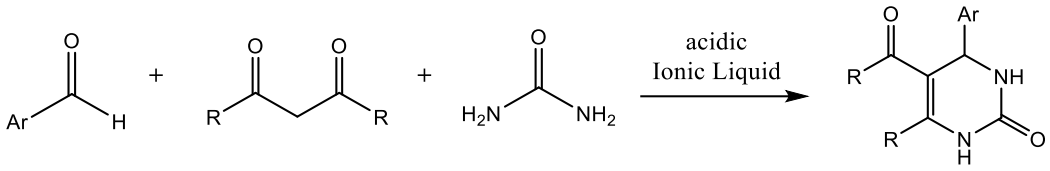


9. Aza-Friedel–Crafts Reaction: Brønsted acidic ILs catalyze one-pot three-component reactions of anilines, indoles, and aldehydes in aqueous media to synthesize indolylmethanes with high yield and recyclability [26].



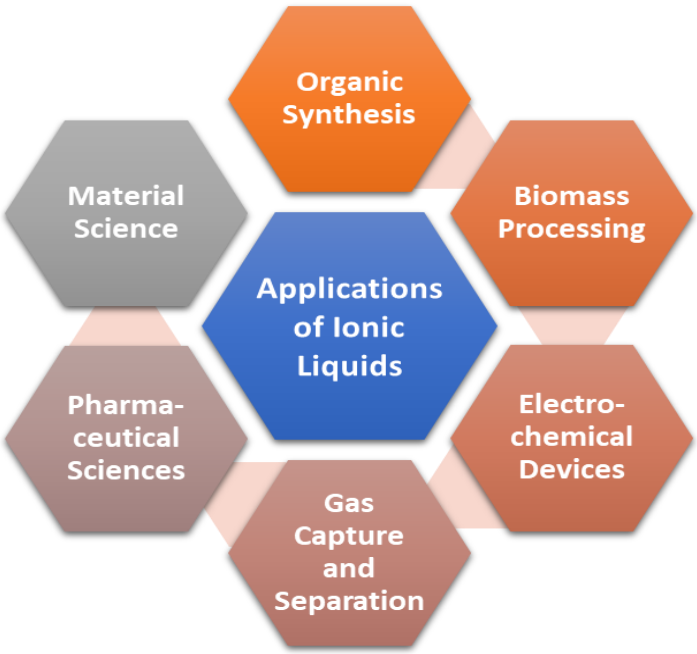
10. Biginelli Reaction

    The multi-component Biginelli reaction to synthesize dihydropyrimidinones proceeds efficiently in ILs like [bmim]Cl, combining good solubility and catalyst support, leading to higher yields and simpler purification [27].



Applications

Ionic liquids (ILs) find wide-ranging and significant applications across multiple scientific fields.



1. **Organic Synthesis**: ILs serve as green solvents and catalysts in numerous organic reactions such as condensations, coupling reactions, hydrogenations, and multi-component processes. They enhance reaction rates, improve selectivity, and facilitate catalyst recovery, contributing to more sustainable chemical processes [4].

2. **Biomass processing:** ILs efficiently dissolve lignocellulosic materials, overcoming challenges associated with biomass recalcitrance. Imidazolium-based ILs, for example, disrupt cellulose crystalline structures, enabling efficient enzymatic hydrolysis and conversion to biofuels and chemicals. This application supports sustainable energy and chemical production from renewable resources, aligning with green chemistry principles [28].
3. **Electrochemical devices**: ILs serve as advanced electrolytes in electrochemical devices including lithium-ion batteries, supercapacitors, and fuel cells. Their wide electrochemical windows, high ionic conductivity, and thermal stability improve device safety, enhance charge storage capacity, and prolong lifetimes compared to traditional electrolytes. These properties promote the development of safer and more efficient energy storage and conversion technologies [29].
4. **Gas capture and separation**: ILs exhibit exceptional CO2 solubility and selectivity, enabling efficient carbon capture and sequestration efforts vital for environmental protection. Their structural tunability allows tailoring to capture specific gases, optimizing industrial gas purification and reducing greenhouse emissions [30].
5. **Pharmaceutical sciences:** Ionic liquids improve drug solubility, stability, and bioavailability. They are employed as solvents and media for drug formulation and synthesis, helping overcome the limitations of poorly soluble drugs. Additionally, ongoing research focuses on IL biocompatibility and toxicity reduction to expand their therapeutic application potential [31].
6. **Material science**: ILs also play critical roles in material science, aiding in the synthesis of nanomaterials and polymers with controlled morphologies and properties. The ionic liquid environment provides unique solvation and templating effects, enabling the fabrication of advanced materials with tailored functionalities for catalysis, electronics, and coatings [32].

These applications demonstrate ILs' broad impact and potential as sustainable, multifunctional tools in modern science and industry.

Recent Advances and Future Scope

Recent advances in ionic liquids (ILs) highlight their expanding role in various scientific fields, driven by their unique physicochemical properties and environmental benefits. Over the past decade, significant progress has been made in synthesizing task-specific ILs tailored for specific applications such as catalysis, energy storage, and environmental remediation. Researchers like Zhao *et al.* (2024) have developed multifunctional ILs with enhanced catalytic activity, broadening their industrial applicability and promoting greener processes [33]. Furthermore,

innovations in designing biodegradable and less toxic ILs are advancing their sustainability profile, addressing environmental and health concerns associated with traditional ILs [34].

The future scope of ILs appears promising due to ongoing research into high-performance IL-based materials, including ionic liquid crystals and nanostructured systems. These materials have potential in advanced electronic devices, sensors, and smart coatings, offering tunable properties and responsiveness [35]. Additionally, integration with emerging technologies like electrochemiluminescence and energy conversion systems could revolutionize battery and supercapacitor industries by providing safer, more efficient, and longer-lasting energy solutions [36].

Furthermore, the development of environmentally benign ILs with enhanced biodegradability and lower toxicity will likely reduce ecological impact, supporting their widespread adoption in sustainable processes [37]. The exploration of novel ILs derived from renewable resources aligns with global efforts towards green chemistry and circular economy principles. Overall, ongoing interdisciplinary research, coupled with technological innovation, will significantly broaden the scope and application of ILs, making them indispensable in future scientific and industrial advancements.

Conclusion:

Ionic liquids have emerged as promising green solvents and catalysts in organic transformations due to their unique physicochemical properties, low volatility, and high thermal stability. Their tunable nature allows for customization to specific reaction requirements, minimizing waste and enhancing efficiency. By replacing traditional volatile organic solvents, ionic liquids contribute to safer and more sustainable chemical processes. Their dual role as both solvent and catalyst simplifies reaction setups, often leading to improved yields and selectivity. Moreover, many ionic liquids can be recycled, further reducing environmental impact. Despite challenges such as cost and biodegradability, ongoing research continues to address these limitations. Overall, the integration of ionic liquids into organic synthesis represents a significant step toward greener and more sustainable chemistry, aligning with global efforts to reduce the ecological footprint of chemical industries.

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HARNESSING SYNERGY: GRAPHITIC CARBON NITRIDE NANOCOMPOSITES AS NEXT-GENERATION PHOTOCATALYSTS

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Abstract:

The photocatalytic transformation of solar energy into chemical fuels and the detoxification of pollutants are two fundamental aspects of sustainable technology. Although many semiconductors have been studied for these purposes, graphitic carbon nitride (g-C_3N_4), an organic, metal-free polymer, has gained a special place due to its stability, ability to absorb visible light, and adjustable electronic properties. Nonetheless, transforming this promising material into an effective photocatalyst requires addressing its inherent limitations, such as quick charge carrier recombination. This chapter argues that constructing g-C_3N_4-based nanocomposites strategically is the most promising way to realize its full potential. We discuss how nanoscale interfacial engineering produces synergistic effects, resulting in superior performance. A new classification system for these composites is proposed, based on their main functions—charge separation, spectral sensitization, or surface engineering. Using this framework, we examine the design, synthesis, and operating mechanisms of various heterostructures, including Z-scheme and S-scheme systems, and assess their effectiveness in applications like hydrogen production, CO_2 utilization, and water purification. The chapter ends with a forward-looking review of the main challenges and emerging directions in this active field.

Introduction:

The global energy and environmental landscape is at a critical juncture. The reliance on fossil fuels has led to an urgent need for renewable energy sources and advanced environmental remediation techniques. Semiconductor photocatalysis, which mimics natural photosynthesis, offers an elegant solution by directly converting abundant solar energy into storable chemical energy or by degrading harmful pollutants. For decades, the field was dominated by metal-oxide semiconductors like titanium dioxide (TiO_2). However, their wide bandgaps restrict activity to the ultraviolet region, which constitutes a mere 4% of the solar spectrum [1].

The discovery in 2009 that a simple, metal-free polymer—graphitic carbon nitride (g-C_3N_4)—could catalyze hydrogen production from water under visible light was a paradigm shift. This

material, which can be synthesized from earth-abundant precursors like urea and melamine, possesses an ideal combination of properties: visible-light responsiveness, remarkable thermal and chemical stability, and an electronic band structure suitable for a range of redox reactions. Despite these advantages, a single-component photocatalyst is seldom optimal. The "Achilles' heel" of pristine g-C_3N_4 is the swift recombination of photogenerated electrons and holes, which dissipates energy as heat before it can be used for chemical reactions [2].

This chapter addresses a central question: How can we engineer g-C_3N_4 to transcend its inherent limitations? The answer lies not in seeking a replacement, but in creating synergistic alliances. By forming nanocomposites with other functional materials, we can design integrated systems where each component performs a specialized task—harvesting light, transporting charge, or facilitating reactions—in a concerted manner. This chapter will dissect the science behind these powerful synergies, providing a roadmap for the rational design of high-performance g-C_3N_4-based photocatalytic systems.

2. The g-C_3N_4 Platform: Structure Defines Function

To appreciate the design of nanocomposites, one must first understand the foundational properties of g-C_3N_4.

2.1 A Two-Dimensional Polymer Network

Unlike its carbonaceous analogue graphene, g-C_3N_4 is a nitrogen-rich semiconductor. Its most stable form consists of two-dimensional layers built from tri-s-triazine (heptazine) ring units, interconnected by tertiary amino groups. This extended π-conjugated system is responsible for its semiconductor behavior and forms a two-dimensional plane ideal for charge migration and interfacial contact with other nanomaterials [3].

2.2 An Electronic Structure Tailored for Redox Catalysis

The electronic band structure of g-C_3N_4 directly results from its chemical bonding. Its valence band, mainly made of nitrogen 2p orbitals, is located around +1.6 V vs. NHE, enabling water oxidation and hydroxyl radical production. Meanwhile, the conduction band, from carbon 2p orbitals, is at about -1.1 V vs. NHE, allowing the reduction of protons to hydrogen. This inherent band alignment makes g-C_3N_4 an effective photocatalyst "straight out of the box," though its ~2.7 eV bandgap and high charge recombination rate restrict its overall efficiency. [4].

3. A Functional Classification of g-C_3N_4 Nanocomposites

Rather than a simple material-based classification, we propose a framework based on the primary role of the co-catalyst in enhancing the photocatalytic cycle.

3.1 Charge Separation Facilitators

The primary bottleneck in photocatalysis is charge recombination. This class of composites is engineered to physically separate electrons and holes.

- **Schottky Junctions with Metals**: Depositing noble (Pt, Au) or non-noble (Ni, Cu) metal nanoparticles on g-C_3N_4 creates a Schottky barrier at the interface. This barrier acts as a efficient electron trap, directing photogenerated electrons from the g-C_3N_4 conduction band into the metal, where they accumulate for reduction reactions (e.g., H_2 evolution). The metal acts as a nano-electrode, while the holes remain on the polymer for oxidation [5].
- **Heterojunction Engineering Coupling**: g-C_3N_4 with another semiconductor with matched band energies is a more sophisticated strategy [6].
- **Conventional Type-II:** Here, band alignment causes electrons to flow to one semiconductor and holes to the other. While this effectively separates charges, it can sometimes sacrifice redox potential.
- **Direct Z-Scheme/S-Scheme:** This emerging paradigm represents a significant advancement. In these systems, the internal electric field at the interface promotes the recombination of the less useful charge carriers (e.g., the electron in the less-reductive CB and the hole in the less-oxidative VB). This leaves the most useful electrons and holes on different components with maximal redox power, combining efficient separation with high reaction driving force. Systems like g-C_3N_4/$BiVO_4$ often operate via this mechanism [7].

3.2 Spectral Sensitizers

These composites aim to capture a broader range of the solar spectrum.

- **Plasmonic Antennas:** Nanoparticles of metals like silver and gold exhibit Localized Surface Plasmon Resonance (LSPR). When coupled with g-C_3N_4, they act as nano-antennas, concentrating light energy and either injecting "hot electrons" into g-C_3N_4 or intensifying the local electromagnetic field to enhance carrier generation in the polymer [8].
- **Up-conversion Partners:** Materials like carbon quantum dots (CQDs) can absorb low-energy photons (e.g., green or red light) and emit higher-energy photons (blue light), which g-C_3N_4 can then absorb. This effectively "up-converts" wasted portions of the solar spectrum into usable energy [9].

3.3 Surface and Stability Enhancers

This category focuses on improving the reaction environment and catalyst longevity.

- **Conductive Scaffolds:** Materials like reduced graphene oxide (rGO) serve a dual purpose. They act as a superior electron acceptor and highway, shuttling charges away from the

recombination zone, while also providing a high-surface-area support that increases the dispersion of g-C_3N_4 and the availability of active sites [10].

- **Molecular-Scale Reactors:** Metal-Organic Frameworks (MOFs) represent the pinnacle of surface engineering. Forming composites with MOFs creates a hierarchical structure where g-C_3N_4 acts as the light harvester, and the ultra-porous MOF framework provides confined pores that concentrate reactants, facilitate mass transfer, and can offer additional catalytic sites [11].

4. Applications in Energy and Environment

The tailored design of g-C_3N_4 nanocomposites has led to dramatic improvements in key photocatalytic applications.

- Hydrogen Evolution: Composites like g-C_3N_4/NiS or g-C_3N_4/MoS_2 demonstrate that non-precious metals can rival the performance of Pt cocatalysts by providing optimal proton adsorption sites for H_2 generation [12].
- CO_2 Reduction: The challenge of activating the inert CO_2 molecule is addressed by composites such as g-C_3N_4/Cu. The copper sites not only accept electrons from g-C_3N_4 but also bind and activate CO_2 molecules, steering the reaction towards specific, valuable products like methane or methanol [13].
- Pollutant Degradation: The generation of powerful oxidizing species is enhanced in composites. For instance, a g-C_3N_4/TiO_2 S-scheme heterojunction can simultaneously preserve the strong oxidation potential of g-C_3N_4's holes while leveraging TiO_2's properties, leading to the rapid destruction of persistent pharmaceuticals and industrial dyes [14].

5. Challenges and Future Trajectories

While the progress is remarkable, the path to commercialization is fraught with challenges that define the future research agenda:

- **From Macro to Nano**: The Interface Problem: Bulk synthesis often leads to poorly defined interfaces. Future work must focus on atomic-level precision in creating heterojunctions to minimize charge transfer resistance.
- **The Scalability Dilemma:** Many high-performing composites are synthesized via complex, multi-step, or energy-intensive methods. Developing one-pot, scalable, and green synthesis routes is a critical, albeit often overlooked, research frontier.
- **Seeing is Believing:** The Need for Operando Analysis: Hypotheses about charge transfer mechanisms (e.g., S-scheme vs. Type-II) are often based on indirect evidence. Widespread

adoption of *operando* spectroscopy and microscopic techniques is essential to visualize charge dynamics in real-time under working conditions.

- **Beyond the Usual Suspects: Novel Applications:** The future will see g-C_3N_4 nanocomposites venturing into more complex and valuable reactions, such as photocatalytic organic synthesis, selective alcohol oxidation, and ammonia synthesis (nitrogen fixation) under ambient conditions.

Conclusion:

Graphitic carbon nitride has transitioned from an initial curiosity to a key platform for designing photocatalysts. Its full potential is realized not on its own, but through deliberate integration into nanocomposites. By combining g-C_3N_4 with metals, semiconductors, and carbon-based materials, we can develop artificial photosynthetic systems that precisely control light, electron, and molecule flow. The classification here—dividing composites as charge separators, spectral sensitizers, or surface enhancers—serves as a straightforward guide for construction. As we pursue more intricate, multifunctional structures, g-C_3N_4 nanocomposites are expected to stay at the cutting edge of sustainable chemical and environmental innovations.

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EXPANDING FRW DARK ENERGY COSMOLOGICAL MODELS WITH BULK VISCOSITY

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Abstract:
This chapter looks at FRW Dark Energy cosmological models that include bulk viscous fluid and a changing cosmological term $\Lambda(t)$ in the general theory of gravity. We find the exact solution to Einstein's field equations by assuming the scale factor $S(t) = (e^{\alpha kt} - t)^{\frac{1}{k}}$, where α and k are both positive constants. The matter in the homogeneous and isotropic cosmological background is represented by a bulk viscous fluid with a viscosity coefficient $\xi(t) = \frac{1}{\xi_0 + S(t)}$, where ξ_0 is a positive numerical constant.

Keywords: FRW Cosmology, Bulk Viscosity, Variable Cosmological Constant, Scale Covariant Theory, Cosmological Parameters

1. Introduction:

Observational evidence for an accelerating Universe has increased interest in FRW cosmologies that include bulk viscous stresses and modified gravity. The development of these models started with research by Ruban and Finkelstein, Barker, and Banerjee and Santos. They found that viscosity can change homogeneous dynamics within general relativity (Ruban and Finkelstein, 1972; Barker, 1978; Banerjee and Santos, 1981a, 1981b).

Subsequent research by Barrow (1988) studied String-driven inflationary and deflationary cosmological models. Shanthi (1989) obtained a conformally flat static space-time in the general scalar-tensor theory of gravitation. Shanthi and Rao (1990) investigated the Bianchi type-VI_0 cosmological model in the general scalar-tensor theory of gravitation. Carvalho also proposed a unified framework based on gamma. Studies in scalar-tensor and string-based theories have highlighted the importance of viscosity in higher-dimensional or scale-covariant settings (Carvalho, 1996; Rao and Kumari, 2012; Rao, Kumari, and Rao, 2012; Rao, Sireesha, and Neelima, 2013; Rao and Neelima, 2014; Rao, Neelima, Vinutha, and Suryanaraya, 2014).

Recent work builds on these ideas beyond the context of General Relativity. This includes new research from Rana and Sahoo (2024), Mete and Dudhe (2025), Thakre, Dhankar, Pourhassan,

and Islam (2025), Barman (2025), Chokyi and Chattopadhyay (2025), and Khatri and Singh (2025). Inspired by this body of work, we developed a scale-covariant FRW model that includes bulk viscosity and a variable cosmological term. We provide clear symbolic expressions for all observables, along with graphical visualizations to support our findings.

2. Metric and Field Equations

We consider homogeneous and isotropic spatially flat Robertson-Walker line elements of the form

$$ds^2 = -dt^2 + S^2(t)(dx^2 + dy^2 + dz^2) \quad (1)$$

where $S(t)$ is the scale factor.

The energy-momentum tensor with a bulk viscous fluid is taken as

$$T_{ij} = (\rho + p)V_iV_j + \overline{p}g_{ij} \quad (2)$$

where ρ is proper energy density and $\overline{p}$ is the effective pressure given by

$$\overline{p} = p - \xi V^i_{;i} \quad (3)$$

satisfying the equation of state

$$p = (\omega - 1)\rho \quad (4)$$

In the above equation, p is the isotropic pressure and v^i is the four-velocity vector satisfying $v^i v_i = -1$.

In gravitational units $8\pi G = c = 1$, the Einstein field equations in the presence of a cosmological constant take the form

$$G_{ij} + \Lambda g_{ij} = T_{ij}, \quad (5)$$

where G_{ij} is the Einstein tensor built from the Robertson–Walker metric and T_{ij} is the bulk-viscous energy-momentum tensor defined above.

The Einstein field equations (in gravitational units 8 π G = C = 1) and varying cosmological constant Λ(t), in the comoving system of coordinates leads to

$$\overline{p} - \Lambda = (2q - 1)H^2 \quad (6)$$

$$\rho + \Lambda = 3H^2 \quad (7)$$

In the above equation, H is the Hubble parameter, and q is the deceleration parameter defined as

$$H = \frac{\dot{S}}{S} \quad (8)$$

$$q = -\frac{S\ddot{S}}{\dot{S}^2} \quad (9)$$

where an overhead dot (.) represents an ordinary derivative with respect to t. The vanishing divergence of the Einstein tensor gives rise to

$$\dot{\rho} + (3\rho + \overline{p})H + \dot{\Lambda} = 0 \quad (10)$$

3. Solution of Field Equations

The bulk viscosity coefficient is taken in the form

$$\xi = \frac{1}{\xi_0 + S(t)} \tag{11}$$

where $S(t)$ is the scale factor.

We assume scale factor as $S(t) = (-t + e^{\alpha kt})^{\frac{1}{k}}$ (12)

where α, k are constants.

The solutions of cosmological parameters are as follows.

$$H(t) = \frac{-\alpha k e^{\alpha kt} + 1}{k(t - e^{\alpha kt})} \tag{13}$$

$$q(t) = \frac{\alpha^2 k^3 t e^{\alpha kt} - \alpha^2 k^2 e^{2\alpha kt} - 2\alpha k^2 e^{\alpha kt} + 2\alpha k e^{\alpha kt} + k - 1}{\alpha^2 k^2 e^{2\alpha kt} - 2\alpha k e^{\alpha kt} + 1} \tag{14}$$

$$\theta(t) = \frac{3(-\alpha k e^{\alpha kt} + 1)}{k(t - e^{\alpha kt})} \tag{15}$$

$$\xi(t) = \frac{1}{\xi_0 + (-t + e^{\alpha kt})^{\frac{1}{k}}} \tag{16}$$

$$\rho(t) = \left[2\alpha^2 k^2 (t - e^{\alpha kt}) \left(\xi_0 + (-t + e^{\alpha kt})^{\frac{1}{k}}\right) e^{\alpha kt} - 3(t - e^{\alpha kt})(\alpha k e^{\alpha kt} - 1) + 2\left(\xi_0 + (-t + e^{\alpha kt})^{\frac{1}{k}}\right)(\alpha k e^{\alpha kt} - 1)^2\right] / [k\omega (t - e^{\alpha kt})^2 \left(\xi_0 + (-t + e^{\alpha kt})^{\frac{1}{k}}\right)] \tag{17}$$

$$\Lambda(t) = \left[-k\left(2\alpha^2 k^2 (t - e^{\alpha kt}) \left(\xi_0 + (-t + e^{\alpha kt})^{\frac{1}{k}}\right) e^{\alpha kt} + 3(t - e^{\alpha kt})(-\alpha k e^{\alpha kt} + 1) + 2\left(\xi_0 + (-t + e^{\alpha kt})^{\frac{1}{k}}\right)(\alpha k e^{\alpha kt} - 1)^2\right) + 3\omega\left(\xi_0 + (-t + e^{\alpha kt})^{\frac{1}{k}}\right)(\alpha k e^{\alpha kt} - 1)^2\right] / [k^2 \omega (t - e^{\alpha kt})^2 \left(\xi_0 + (-t + e^{\alpha kt})^{\frac{1}{k}}\right)] \tag{18}$$

$$p(t) = \left[(\omega - 1)\left(2\alpha^2 k^2 (t - e^{\alpha kt}) \left(\xi_0 + (-t + e^{\alpha kt})^{\frac{1}{k}}\right) e^{\alpha kt} - 3(t - e^{\alpha kt})(\alpha k e^{\alpha kt} - 1) + 2\left(\xi_0 + (-t + e^{\alpha kt})^{\frac{1}{k}}\right)(\alpha k e^{\alpha kt} - 1)^2\right)\right] / [\omega (t - e^{\alpha kt})^2 \left(\xi_0 + (-t + e^{\alpha kt})^{\frac{1}{k}}\right)] \tag{19}$$

$$\overline{p}(t) = \left[3\omega (t - e^{\alpha kt})(\alpha k e^{\alpha kt} - 1) + (\omega - 1)\left(2\alpha^2 k^2 (t - e^{\alpha kt}) \left(\xi_0 + (-t + e^{\alpha kt})^{\frac{1}{k}}\right) e^{\alpha kt} + 3(t - e^{\alpha kt})(-\alpha k e^{\alpha kt} + 1) + 2\left(\xi_0 + (-t + e^{\alpha kt})^{\frac{1}{k}}\right)(\alpha k e^{\alpha kt} - 1)^2\right)\right] / [k\omega (t - e^{\alpha kt})^2 \left(\xi_0 + (-t + e^{\alpha kt})^{\frac{1}{k}}\right)] \tag{20}$$

4. Graphical Results

We now study the Graphical behavior of all observables for $k \in \{0.5, 1.0, 1.5, 2.0\}$ with $\alpha = 1.0$, $\xi_0 = 1.0$, and $\omega = \{-0.5, 0.0, -1.0\}$.

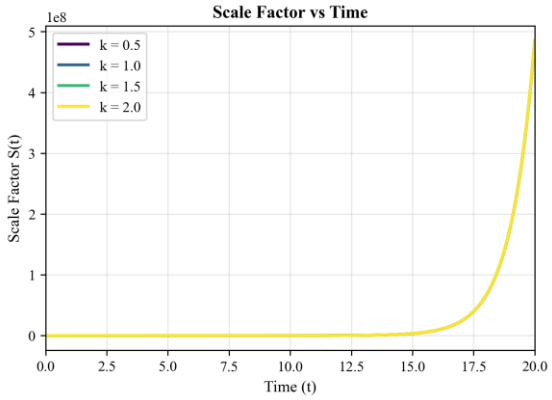

Figure 1: Scale Factor vs time.

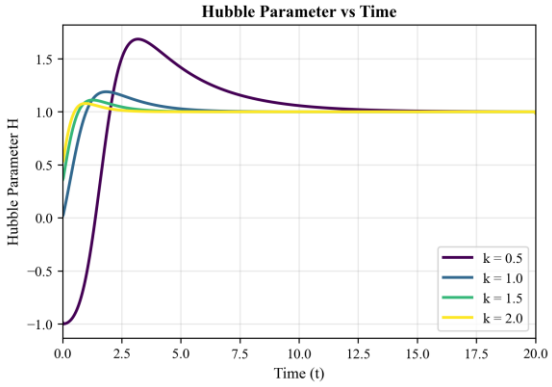

Figure 2: Hubble parameter vs time.

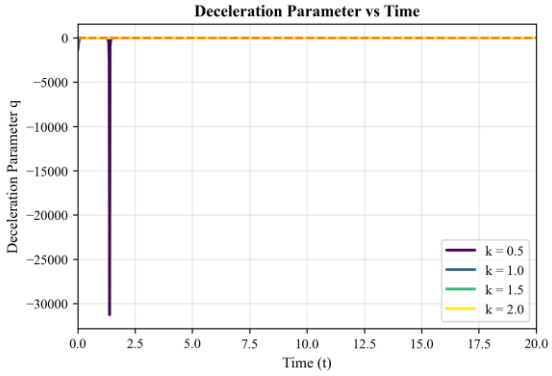

Figure 3: Deceleration parameter vs time

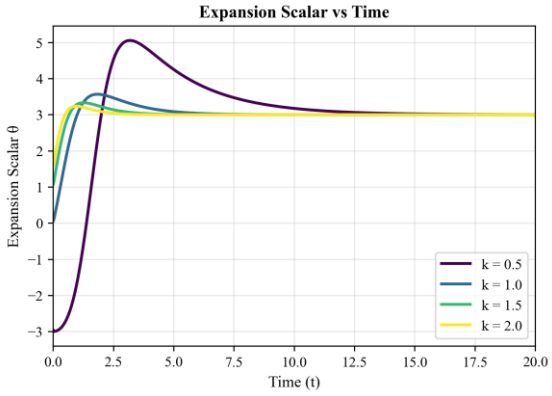

Figure 4: Expansion scalar vs time.

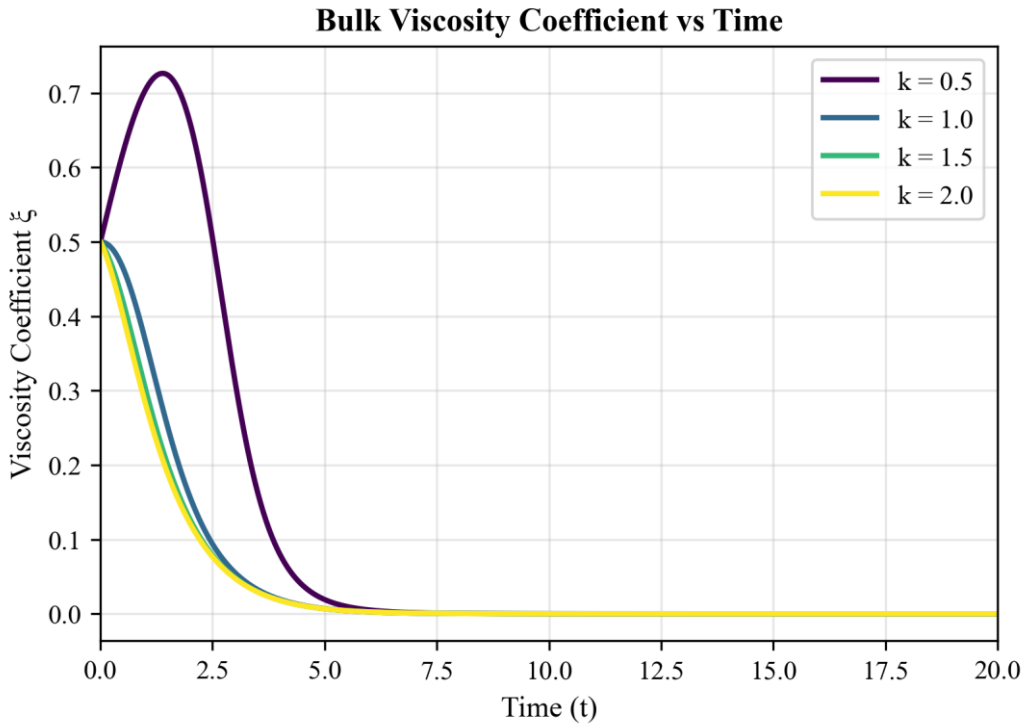

Figure 5: Viscosity coefficient vs time.

Case I : $\omega = -0.5$

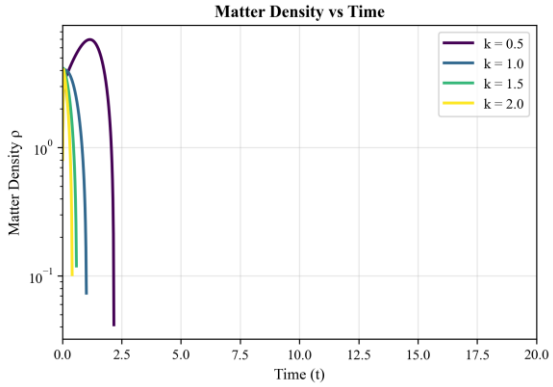

Figure 6: Matter Density vs time.

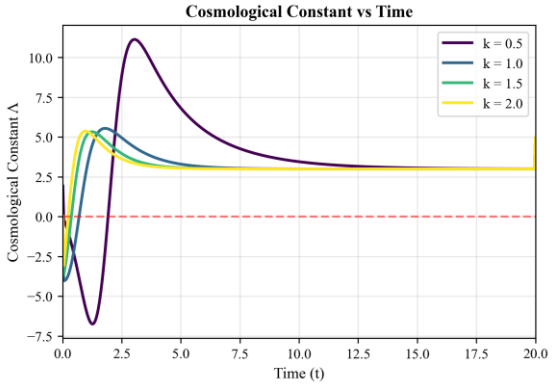

Figure 7: Cosmological constant vs time.

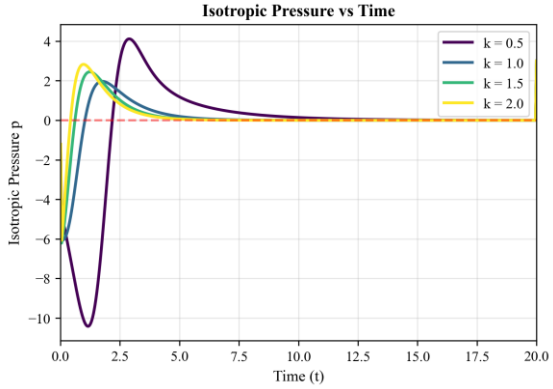

Figure 8: Isotropic pressure vs time.

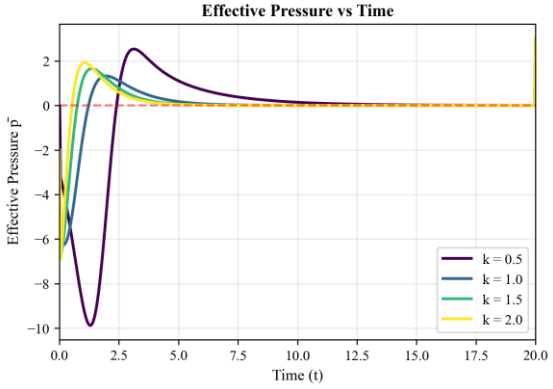

Figure 9: Effective pressure vs time.

Case II : $\omega = 0.0$

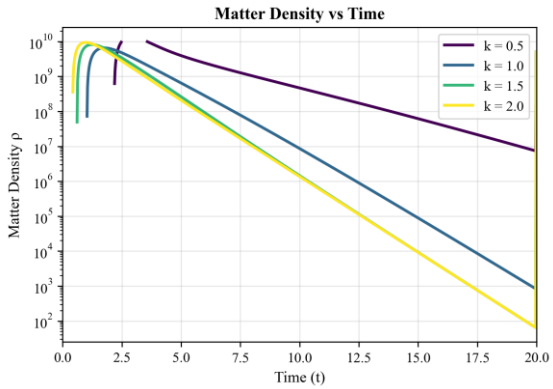

Figure 10: Matter Density vs time.

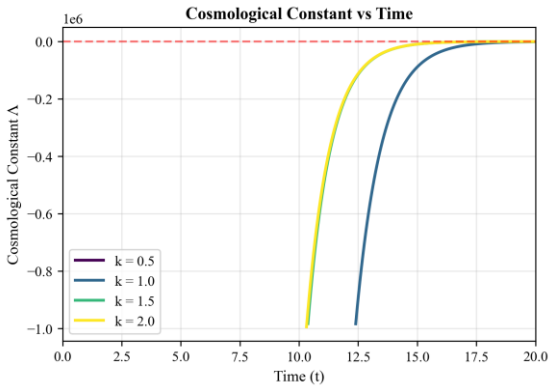

Figure 11: Cosmological constant vs time.

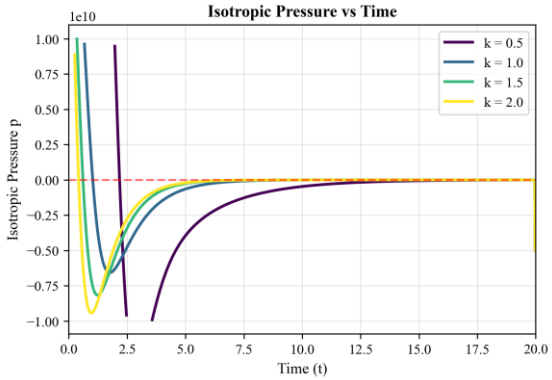

Figure 12: Isotropic pressure vs time.

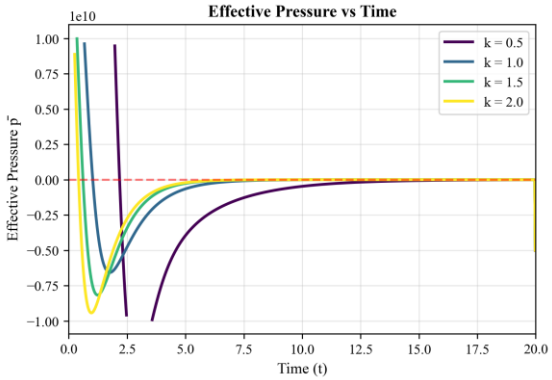

Figure 13: Effective pressure vs time.

Case III : $\omega = -1.0$

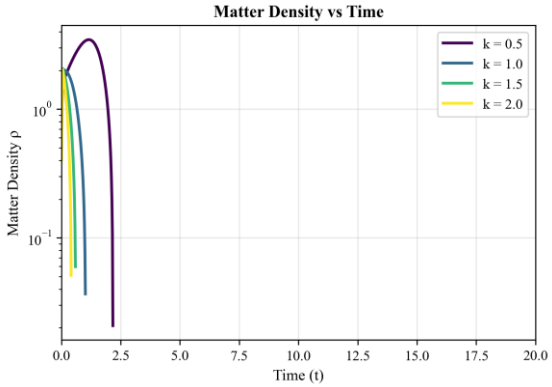

Figure 14: Matter Density vs time.

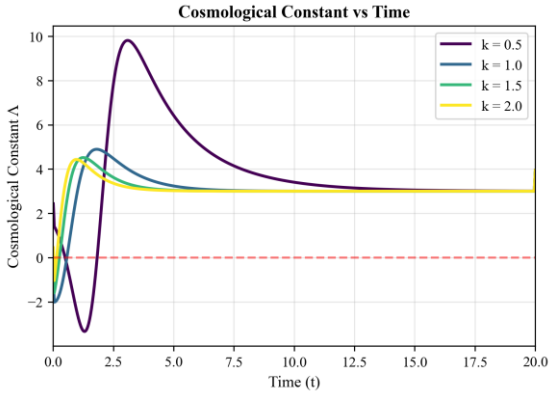

Figure 15: Cosmological constant vs time.

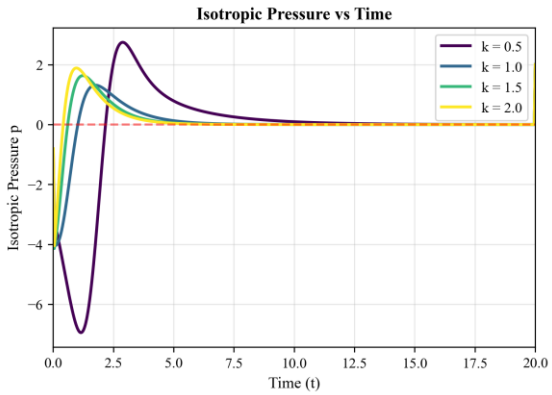

Figure 16: Isotropic pressure vs time.

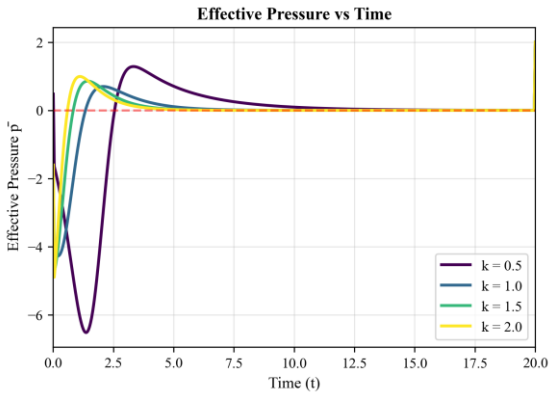

Figure 17: Effective pressure vs time.

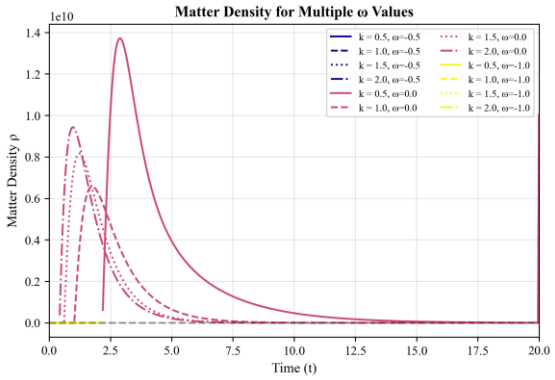

Figure 18: Matter Density (Multiple ω)

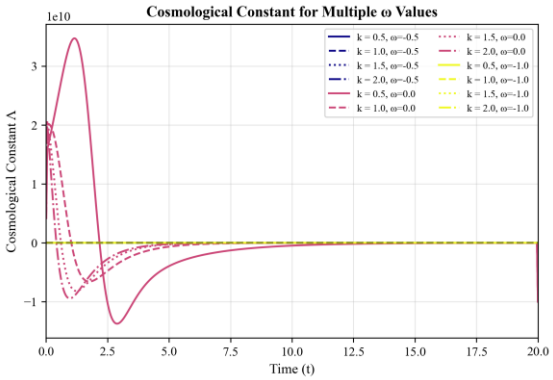

Figure 19: Cosmological Constant (Multiple ω)

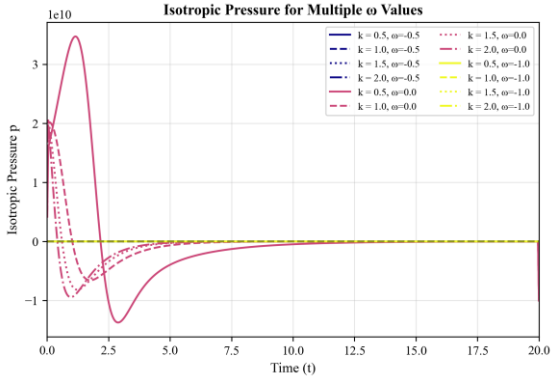

Figure 20: Isotropic Pressure (Multiple ω)

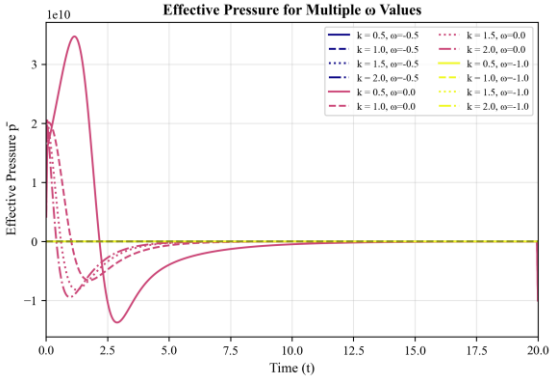

Figure 21: Effective Pressure (Multiple ω)

Conclusion:

We study an FRW model with bulk viscosity and a changing cosmological term in general relativity. We solve the field equations and present a graphical analysis. This analysis leads to three main conclusions

- Figures 1-5 show that S, H, q, θ, and ξ change steadily. The universe expands indefinitely, the deceleration parameter becomes negative, and the viscous coefficient decreases, making dissipation insignificant in the later stages.
- Figures 6, 7, 8, and 9 indicate that ρ, Λ, p, and $\overline{p}$ approach small asymptotic values, regardless of the chosen $\omega = -0.5$. This shows that the viscous fluid gives a strong but ultimately weakening contribution to the energy allocation.
- Figures 10 to 13 and Figures 14 to 17 show the variations of ρ, Λ, p, and $\overline{p}$ for $\omega = 0, -1$.
- Figures 18 to 21 reveal that more negative ω values support higher densities and more negative pressures for longer times, which intensifies late-time acceleration.

Together, these findings demonstrate that bulk viscosity offers a consistent way to mimic dark-energy-like behavior across many types of FRW space-times and gravity extensions.

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ENZYMES AS BIOSENSORS

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Abstract:

Enzymes play a pivotal role in the development of biosensors due to their high specificity, catalytic efficiency, and ability to function under mild conditions. A biosensor is a selfcontained analytical device that integrates a biological recognition element with a physicochemical transducer to produce a measurable signal. Enzyme-based biosensors have evolved significantly since the introduction of the first glucose biosensor in 1962 and are now integral in clinical diagnostics, food analysis, and environmental monitoring. Clinically, enzyme biosensors are employed in the detection of blood glucose, urea, uric acid, and lactic acid, offering rapid, sensitive, and cost-effective tools for disease monitoring. In the food industry, enzymatic biosensors facilitate real-time and on-site analysis of sugars, acids, amino acids, alcohols, pesticides, and heavy metals, ensuring product safety and quality. The advent of allosteric enzyme-based biosensors and recombinant enzyme engineering has further expanded their analytical potential, enabling more selective and responsive detection systems. Additionally, enzyme-linked immunosorbent assays (ELISA) have become standard in diagnostic laboratories for detecting a wide range of antigens and antibodies. Despite their laboratory success, challenges such as enzyme instability, interference, and limited commercial scalability persist. Ongoing advances in enzyme immobilization, nanomaterials, and lab-onchip technologies are expected to enhance biosensor performance and broaden their diagnostic applications. Overall, enzyme-based biosensors represent a cornerstone in modern bioanalytical technology, combining biological precision with engineering innovation to address clinical and industrial needs.

Keywords: Biosensors, Clinical, Allosteric, Food, ELISA

Introduction:

The term ‘biosensor’ was introduced by Cammann (1977). A stricter definition of the term was set later by the International Union of Pure and Applied Chemistry (IUPAC). A biosensor is defined as a self-contained integrated device that is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor) which is in direct spatial contact with a transduction element.’ Therefore, a biosensor

consists of two main components: a bioreceptor and a transducer. The bioreceptor is composed of a biomolecule recognition element (an enzyme, an antibody, a protein receptor, DNA, or whole cells) that recognizes the target analyte, whereas the transducer converts the recognition event into a measurable signal (electrical, optical, thermal, and so on). A typical biosensor construct also normally incorporates signal-processing elements (amplification, filtering, data processing, and storage) and a display of the final result (1). A schematic diagram of a typical biosensor is illustrated in Figure 1. (2)

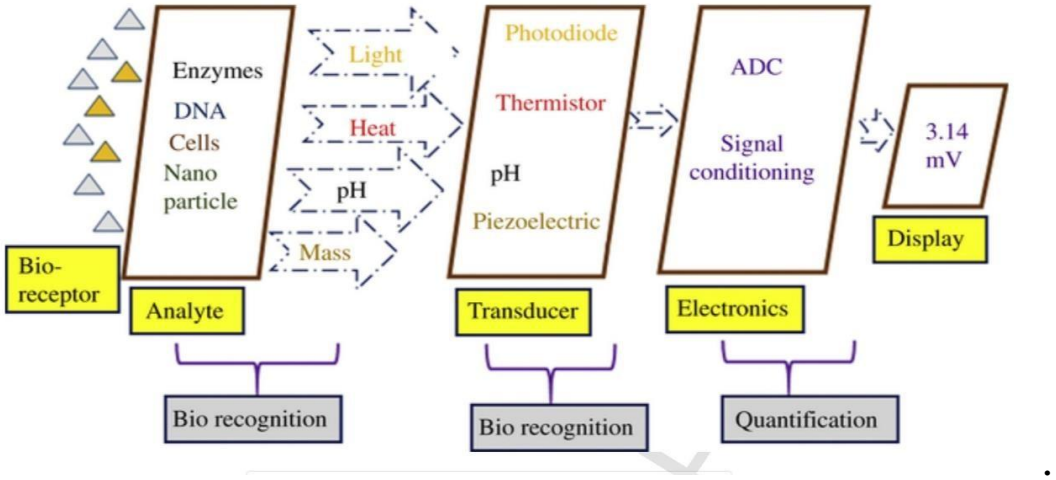

Figure 1: Schematic representation of a biosensor (Source: Kaur *et al.*, 2019)

Chemical biosensors work by detecting the presence of a biological element that is specific for the analyte and stable under typical use and storage settings. Biosensors have used a variety of recognition elements, including receptors, nucleic acids, entire cells, antibodies, and various enzyme groups (3). In 1962, the first biosensor for glucose detection was developed using immobilized glucose oxidase and an oxygen electrode as a transducer. Since then, the enzyme electrode concept has gained popularity and has been used in a range of other enzyme-based biosensors for detecting or sensing a specific analyte. A potentiometric urea biosensor was later developed for detecting urea in clinical samples. The biosensor was created by using urease as a transducer on an ammonium electrode. A thermistor (a heat-detecting device) was also used in the design of a biosensor based on a thermal enzyme probe. Lubbers and Opitz coined the term "optode" to describe a fiber optic sensor with an immobilized indicator that monitors carbon dioxide or oxygen (4).

Complex bimolecular-based sensors require research, development, and commercialization to meet a variety of healthcare needs. Biosensors that are wearable on human skin and pleasantly utilized within the body are widely employed for numerous health applications and daily health monitoring. It's also critical to enhance this technology and find appropriate materials to connect and attach sensors to surfaces (5).

Enzyme-Based Biosensors in Clinical Diagnosis

1) Biosensors for Blood Glucose

Diabetes is an incurable disease characterized by a lack of insulin in the body, which results in either high blood glucose levels (hyperglycemia) or low glucose levels (hypoglycemia) (hypoglycemia). Insulin is produced and secreted by the pancreas, and it regulates glucose metabolism. Diabetes has been labeled as the "hidden pandemic," and considerable efforts have been made to improve diagnosis, monitoring, and treatment since its discovery (4). Glucose levels are often determined by interactions with one of three enzymes: hexokinase, glucose oxidase (GOx), or glucose-1-dehydrogenase (GDH). In many clinical laboratories, the hexokinase assay is used to measure glucose using spectrophotometry. The glucose biosensor works on the principle that immobilized GOx catalyzes the oxidation of -D-glucose by molecular oxygen, resulting in gluconic acid and hydrogen peroxide. To work as a catalyst, GOx requires a redox cofactor—flavin adenine dinucleotide (FAD). FAD works as the initial electron acceptor and is reduced to FADH2 (6).

Biosensors for Urea in Blood and Urine

Urea is a non-toxic, nitrogenous organic end product of protein metabolism that allows the human body to eliminate 80-90% of nitrogen. The normal concentration of urea in blood serum/plasma is 3.3-6.7 mM, while a concentration of > 30 mM indicates the need for dialysis, as well as other probable reasons such as salinity, water depletion, and gastrointestinal tract degradation. The urease enzyme catalyzes the hydrolysis of urea to produce ammonium and bicarbonate ions, which are used in urea biosensors. These organisms have an impact on the pH of the surrounding environment, which is proportional to the amount of urea present. The ammonium ion (NH4 +) in particular can be easily detected in traces by using a specific transducer, namely an NH4 + specific electrode (Ammonium ion-specific electrode, AISE), which is in accordance with urea concentration (7).

Urease is extensively employed in urea detection enzymatic biosensors. Guilbault *et al.* invented the first urea biosensor. They used a cation-selective glass electrode containing urease to get a signal for urea-based on the formation of ammonium ions during hydrolysis. Since then, the use of urease as a biocatalyst in the building of urea biosensors has sparked the interest of many researchers, and numerous types of urea biosensors have been produced (4, 7).

2) Biosensors for Uric Acid

Uric acid is a by-product of the purine nucleotide catabolism processes in the human body. It is created in the liver cells through the primary catabolic pathway of purine breakdown. Additionally, uric acid is not processed in the liver and is eliminated through the kidneys and intestine. The standard range for uric acid in human blood plasma is 3.6 mg/dL to 8.3 mg/dL.

Gout is a kind of arthritis caused by an excess of uric acid in the blood. As a result, monitoring uric acid levels is critical for maintaining human health. For uric acid detection in biological samples, a compact and cost-effective biosensor has been created. The designed biosensor was used to analyze human blood samples, and it showed good agreement with the results obtained by the colorimetric method (4, 8).

3) Biosensors for Lactic Acid

Lactic acid (LA) is an organic compound that is widely distributed in the human body. LA levels in the body can mainly be associated with anaerobic metabolism. Under anaerobic conditions, pyruvate converts to lactic acid by lactate dehydrogenase. Lactic acid later gets dissociated into lactate at physiologic pH ranges. Lactate plays significant roles in clinical medicine and sports physiology, neuron-glia metabolic interaction, and fermentation processes. Lactic acid monitoring has lately gained prominence due to its relationship to certain pathological conditions such as shock, respiratory insufficiencies, and heart disease. Lactic acid is a final product of glycolysis that occurs in all tissues. There are two ways to develop a lactic acid biosensor based on biological materials. One is based on the enzyme lactic acid dehydrogenase (LDH) which catalyzes the conversion of pyruvate to the lactic acid and back and the other is based on the enzyme lactate oxidase, which catalyzes the oxidation of lactic acid, which forms pyruvate and hydrogen peroxide. Further, hydrogen peroxide can be determined electrochemically (4, 9).

Enzymatic Biosensors and the Food Industry

Current analytical procedures in the food industry are time-consuming, costly, and require cumbersome apparatus. However, the food sector demands pocket-sized equipment that can measure undiluted samples quickly and on-site during food manufacture or processing, or for quality control purposes. Enzymatic biosensors can easily overcome the majority of these problems. Food constituents (sugars, acids, amino acids, inorganic ions, alcohols, and carbohydrates), pollutants (pesticide and heavy metal residues), food additives (sorbitol, benzoic acid, sulfites), and markers of food 'freshness' are all measured using enzymatic biosensors (such as biogenic amines) (6).

Biosensors for Analyzing Main Food Components

1) Sugars

Sugar detection is usually performed using electrochemical biosensors. Individual or several enzymes are immobilized effectively on varying sorts of electrochemical transducers (carbon, screen-printed, etc.) in these devices employing several ways. Glucose biosensors are used to monitor fermentation processes as well as in the dairy, wine, beer, and sugar industries. The detection of glucose is based on biosensors that utilize glucose oxidase, which catalyzes the

oxidation of glucose into gluconic acid. Usually, the amount of hydrogen peroxide generated is measured:

$$\text{D- Glucose} + O_2 \xrightarrow{\text{Glucose dehydrogenase}} \text{D- Gluconic acid} + H_2O_2$$

The hydrogen peroxide could then be determined using an electrochemiluminescence reaction with luminol or amperometry with a compatible intermediary such as Prussian blue:

H_2O_2 + [Med] red → [Med] ox + H_2O

An electrode was used to determine the glucose content in real samples. Results were in good agreement with the conventional measurement method (6).

2) Acids

They may be determined by optical, electrochemical, and calorimetric biosensors. Lactic acid is an indicator of the fermentative processes and is related to the freshness, stability, and storage quality of several products such as tomato sauces, fruits, juices, wine, and milk. Typically lactate biosensors are based on the following reactions:

$$\text{L lactate} + O_2 \xrightarrow{\text{L-lactate oxidase}} \text{Pyruvate} + H_2O_2$$

$$\text{L lactate} + \text{NAD} \xrightarrow{\text{L-lactate dehydrogenase}} \text{pyruvate} + \text{NADH} + \text{H+}$$

$$\text{NADH} + \text{H} + + \text{[Med] ox} \xrightarrow{\text{Diaphorase}} \text{[Med] red} + \text{NAD} +$$

A biosensor for the selective determination of lactate in wine, based on robust solid composite transducers, was developed by Katrlik *et al.* The results obtained by the biosensors were in good agreement with those obtained by liquid chromatography (6).

3) Amino acids

The presence of D-amino acids in meals has been linked to a decrease in protein digestibility, altering the bioavailability of key amino acids and severely reducing the nutritional value of the food. D-amino acids are often regarded as major indicators of bacterial contamination of food goods. D-amino acid oxidase (DAAO) is a peroxisomal enzyme that uses FAD as a cofactor and is found in a broad variety of organisms, from yeasts to humans, but not in bacteria or plants. Its role is to catalyze the oxidative deamination of amino acid D-isomer to 2-oxoacid and ammonia (6).

4) Alcohols

Ethanol determination and control are critical in the brewing, winemaking, and distilling sectors. Tax regulations need an accurate assessment of the ethanol concentration, particularly in spirits. Ethanol biosensors are based mainly on immobilized alcohol oxidase or dehydrogenase, catalyzing the following reactions:

$$\text{Ethanol} + \text{NAD} + - \xrightarrow{\text{Alcohol dehydrogenase}} \text{acetaldehyde} + \text{NADH} + \text{H+}$$

$$\text{Ethanol} + O_2 + \xrightarrow{\text{Alcohol oxidase}} \text{acetaldehyde} + H_2O_2$$

Biosensors for Contaminants

1) Pesticides

Pesticides are chemicals used in agricultural production to destroy or control weeds, insects, fungi, and other pests. Pesticides are considered to be some of the most dangerous contaminants, because of their ability to accumulate, and their long-term effects on living organisms. The general principle of enzymatic inhibition biosensors is based on the correlation between the toxicity of pesticides and the decrease in enzyme activity. Therefore, the development of these biosensing systems relies on quantitative measurement of the enzyme activity, before and after exposure to a target analyte. The main weakness of the enzymatic approach for pesticide detection is the limited qualitative information acquired since the response is related to the total amount of pesticides in the sample. Also, the interpretation of results is further complicated by the fact that each pesticide exhibits a different inhibitory effect of the enzymatic activity. Finally, the specificity of these biosensors is rather limited, as they are prone to interference by other compounds in the sample (e.g. heavy metals).

2) Heavy Metals

Heavy metals and their ions are ubiquitous and, by definition, are metals having atomic weights between 63.5 and 200.6 g mol−1 and a specific gravity greater than 5 g cm−3. Heavy metals constitute one of the most serious groups of pollutants. Even in small concentrations, they are a threat to the environment and human health, because they are non-biodegradable. The commonest operational strategy in heavy metal biosensors is inhibition of the enzymatic activity, based on the interaction of metal ions with exposed thiol or methyl thiol-groups of the enzyme's amino acids. Different enzymes, such as acetylcholinesterase, alkaline phosphatase, urease, invertase, peroxidase, L-lactate dehydrogenase, tyrosinase, and nitrate reductase, have been used. The inhibition of the immobilized enzyme can be detected via electrochemical (amperometric, potentiometric, and conductometric) or optical measurements. Heavy metal enzymatic biosensors

exhibit the same weaknesses as pesticide biosensors (i.e. limited selectivity) but are useful for fast screening purposes (6).

Allosteric Enzymes as Biosensors

Allosteric enzymes exhibit regulatable catalytic activities upon the binding of an effector molecule, to a receptor site of the enzyme that is different from the active site. In some cases, modulation occurs through binding to distinct, alternative sites, either inhibitory or stimulatory (10). Allosteric enzymes that catalyze the formation of easily detectable products are potential biosensors. The receptor site acts as the recognition element, the active site as the transducer element, and the whole enzyme integrates both parts through its structure and transmits the binding signal via conformational changes. Natural allosteric enzymes, however, cannot be directly used as biosensors, because most of their modulators are devoid of analytical interest. However, it can be possible to incorporate it with modular engineering. Allosteric biosensor prototypes that have been constructed are the result of a trial-and-error approach, rather than of rational design (4).

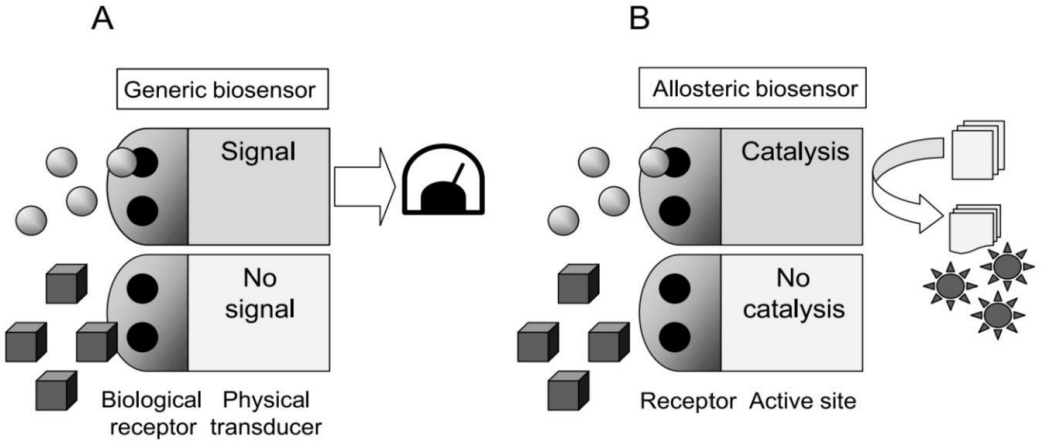

Figure 2: Working principle of an allosteric enzyme-based biosensor

(Source: Villaverde, 2003)

Enzymes Engineered as Allosteric Biosensors

1) Alkaline Phosphatase

Alkaline phosphatase from *E. coli* is a homodimer, non-specific phosphomonoesterase whose activity is highly valued for analytical purposes due to its colorimetric detection. The insertion of an HIV peptide from the structural protein gp120 near the active site resulted in a completely active enzyme, however, the presence of anti-peptide antibodies decreased the catalytic rate by up to 50%. A wireless magnetoelastic (ME) biosensor immobilized with E2 glycoprotein was developed to detect classical swine fever virus (CSFV) E2 antibodies. The detection principle involves a sandwich complex of CSFV E2-rabbit anti-CSFV E2 antibody-alkaline phosphatase-conjugated goat antirabbit IgG formed on the ME sensor surface, with biocatalytic precipitation

used to amplify the mass change of antigen-antibody specific binding reaction, induces a significant change in resonance frequency of the biosensor (4).

2) Green Fluorescent Protein (GFP)

The GFP of *Aequorea victoria* was modified to accommodate the whole sequence of TEM-1 L-lactamase in a predefined, solvent-exposed area near the fluorophore. The binding of the Llactamase inhibitory protein BLIP resulted in a substantial rise in fluorescence. This construct responded to the binding of the L-lactamase inhibitory protein BLIP by a dramatic increase in fluorescence, with no alterations in the emission spectrum. In general, directed evolution procedures have been proven useful to improve the performance of these GFP-derived biosensors (10).

3) Neural Protease

A recent and unusual example of rational design in enzymatic biosensor creation was motivated by the intrasteric control principle that governs several natural enzymes. The *Cereus* neural protease inhibitor has been covalently connected through a short and flexible single-stranded (ss) DNA hinge in a partly synthetic construct, effectively limiting the enzyme activity. The presence of complementary DNA molecules increases the release of the inhibitor and activates the protease by hybridizing with the hinge DNA segment and limiting its flexibility. Its activity, which results to be dependent on the concentration of DNA molecules with a specific sequence, can be detected by fluorescence through the hydrolysis of peptidic substrates containing both a fluorophore and a quencher **(**4, 10).

4) Ribozymes

The nucleic acids exhibit variations in their catalytic activity upon binding to the different ligands, especially in the small organic effector molecules. This allosteric property of the enzyme ribozyme was further improved by protein engineering and realized for the development of biosensors and biochips. However, an exclusively modular rational method seems insufficient for the excellent performance of the biosensor (4).

5) L-lysine-α-Oxidase (LO)

L-lysine-α-oxidase (LO) is an oxidoreductase that catalyzes the oxidation of the well-known essential amino acid L-lysine (Lys) where the produced α-keto-ε-aminocaproate successively dehydrates spontaneously to Δ^1-piperidine-2-carboxylate. The enzyme was firstly isolated from *Trichoderma viride*, but soon was found also in other Trichoderma species and strains and characterized as well. Its allosteric behavior is strongly dependent on pH and Lys concentration thus can be advantageous to control the sensitivity and the dynamic range of LO-based biosensors and when eventually coupled with another enzyme displaying different affinity towards Lys, useful to produce extracorporeal reactor devices, which gates Lys removal at just

above the desired level. Last but not the least, the present study also shows the effectiveness of using an immobilized enzyme and amperometric biosensor not only for substrate analysis but also as a convenient tool for enzyme kinetic studies (11).

Enzyme-Linked Immunosorbent Assay (ELISA) in Diagnostics

ELISA is also known as a solid-phase enzyme immunoassay, and it is used to identify the presence of a certain protein (antigen or antibody) in blood samples. The basic idea behind ELISA is to use an enzyme to detect antigen (Ag) or antibody binding (Ab). ELISA employs enzymes such as alkaline phosphatase, horseradish peroxidase, lactoperoxidase, and βgalactosidase. The three essential concepts involved in ELISA are: I the antigen-antibody reaction, in which the presence of Ag or Ab in a sample is detected; (ii) the enzymatic chemical reaction, in which the rate of creation of Ag-Ab complex is utilized to quantify the amount of Ag or Ab involved in the reaction; (iii) signal detection and quantification, in which the intensity of the colored product generated by the enzyme and substrate is detected and measured (4). The assay used most widely to detect or diagnose virus infection, especially infection of blood-borne viruses e.g. HBV, HCV, HIV, and HTLV is the enzyme-linked immunosorbent assay (ELISA), whose sensitivity and practicability have rendered it the most common primary screening assay. ELISA can be mass screening used automatic or semiautomatic machines (12).

Conclusion:

Due to high specificity, sensitivity, rapid response, ease of self-testing, portability, and other characteristics, enzyme and enzyme-based biosensors are valuable tools for the clinical diagnosis of various diseases. The high specificity of enzymes makes them an excellent choice for medical diagnostics. A review of the literature reveals numerous reports on the use of enzymes in clinical diagnostics. The vast majority of enzyme research in diagnostics is done in the laboratory. So, it is most important to commercialize the applications of enzymes used in the diagnostics of various diseases. Several enzyme-based biosensors have also been developed for disease diagnosis. The glucose biosensor, which is used to detect glucose levels in blood samples, is the most commercialized enzymatic biosensor. There is no doubt that as enzyme technology advances, enzymes and enzyme-based biosensors will become a powerful tool for the detection of other diseases such as cancer, heart failure, epilepsy, and so on. Most enzymatic biosensors perform well in the laboratory, but they present numerous complications when analyzing real-world samples. As a result, novel surface modification strategies for the enzyme electrode are required to eliminate nonspecific adsorption at the enzyme electrode surfaces. Recombinant enzymes and lab-on-chip techniques can help achieve this goal. As a result, enzyme and enzyme-based biosensors can be used to detect very low-level targets promptly.

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A SYMBIONT REVIEW OF ARBUSCULAR MYCORRHIZAL FUNGI (AM FUNGI)

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Abstract:
Arbuscular Mycorrhizal Fungi (AM Fungi) are basically everywhere in soil, a very vital friend they make with like 80% of plants on earth including our main food crops. This old friendship, mostly from the Glomeromycota group, is a proper give-and-take, a two-way exchange it was the fungus takes important sugars and lipids from the plant and in return its hyphal network which is very big helps the plant take up difficult-to-reach things like phosphorus (P), and also nitrogen, and other small nutrients. Not just for food AM fungi do many good things for environment such like they give better tolerance for water lack protect from diseases and make soil structure and fertility better. But these fungi, because they must live with plant always it makes problem for making big amount of them for use in farming as biofertilizer for the sustainable way.

Introduction:
This term mycorrhiza it just means "fungus-root" only It's a symbiotic relationship between fungi and the roots of plant. Among all these types the Arbuscular Mycorrhiza (AM) is the most important one and it is very common very widespread. This AM fungus, it helped the first plants to come live on land may be like 400 to 480 million years before, such a long time!
These fungi are proper obligate biotrophs which means they cannot grow properly or finish their life without a live plant root so host must be there. They do is they goes inside the root cells and makes these tiny and branchy structures called arbuscules which is the main place where the nutrient exchange happens. Also, they make little round storage things, like small balloons, called vesicles inside the root and *of course*, sends many thin threads called hyphae far out into the soil for finding food.

Core Mechanisms and Ecological Roles

1. Bidirectional Nutrient Exchange

The most significant function of AM fungi is the enhancement of host plant nutrition.

- **The Fungal Benefit to the Host Plant:** is predicated upon the vastly superior morphological geometry of the Arbuscular Mycorrhizal (AM) fungal hyphal network; these mycelial strands, exhibiting an acutely elevated surface area-to-volume ratio in comparison

to the plant's own root hairs, thusly effectuates an exponential expansion of the rhizosphere. This sophisticated network are capables of penetrating into edaphic micropores that remain entirely inaccessible to the macroscopic root structure and demonstrates a striking efficiency in the scavenging of oligonutrients, most notably orthophosphate (P), a nutrient ion characteristically hypo-mobile within the soil matrix. The fungus then undertakes the active vectorial transport of these sequestered nutritional species across its hyphal filaments, ultimately delivering them to the intracellular arbuscules.

- **Plant Benefit to Fungus:** For the fungus to continue its industrious subterranean explorations and the efficacious delivery of crucial edaphic sustenance the host plant does not demur, ceding a considerable fraction nigh to a fifth part of its newly synthesized saccharides and lipoidal compounds which are the very carbonaceous dividends accrued through the mechanism of photosynthesis.

2. Enhancing Soil Structure and Health

AM fungi are very important for keeping soil healthy. They make a sticky stuff called glomalin, which is a kind of protein glue. This glomalin together with the fungus's tiny threads, called hyphae, helps stick soil bits together into strong clumps, we call them aggregates. This sticking process makes the soil better for air to get in allows water to soak in properly, and also stop the soil from being washed or blown away this is good.

3. Stress Tolerance and Protection

The presence of AM fungi often provides the host plant with increased resilience against various stresses:

- **Drought and Water Stress:** The long, extraradical hyphae increases soil volume explored and help plant to take up more water specially when soil is very dry.
- **Disease Resistance:** When the AM fungi colonize the plant it makes a special defense system called Mycorrhiza-Induced Resistance (MIR), so the plant get protection against some bad pests and pathogens which lives in the soil.
- **Tolerance to Toxins:** The fungi symbiosis also assisting plants to survive even when there are much heavy metals and salt in the soil, which is very good thing.

Application in Sustainable Agriculture

Given their numerous benefits, AM fungi are increasingly viewed as key components of sustainable agriculture practices and are commercialized as biofertilizers and biostimulants.

- We seeing many good things when we use AM fungi these are very important for us. First, we need less fertilizer using, because the fungi is very good at taking up nutrients like phosphorus. This means the farmer spend less money and the environment don't get spoiled by runoff.

- Crop productivity gets very high. Mycorrhizae help increase how much the crop weigh, the yield, and even the nutritional qualities are better in many vegetables and fruits.
- Soil management, minimum tillage is the right way to go. These methods save the fine hyphal networks from being break up, so the fungi work very efficient.

Conclusion:

Arbuscular Mycorrhizal Fungi is representing one of the most ancient and important partnerships on Earth. They basically link the health of any plant to the soil's quality, so this is very important thing. Their role as natural biofertilizers and for stabilizing soil and also protecting plants against stress from weather or from pests and disease, makes them absolutely necessary for all ecosystems natural jungle or our farms. While it is difficult is there to produce good AM fungal inoculum on a large scale because they must be living with a host plant, research on their genes, ecology, and how they interact with other small microbes promises to show their full power. If we can use the power of these silent symbionts, it is crucial for moving towards farming systems that are more strong more nature-friendly and sustainable for the whole world.

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EMERGING INNOVATIONS IN DIABETES RESEARCH: MECHANISTIC INSIGHTS, TECHNOLOGIES, AND THERAPEUTIC ADVANCEMENTS

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Introduction:

Diabetes mellitus (DM) is a chronic metabolic disorder marked by persistent hyperglycemia due to impaired insulin secretion, action, or both, affecting carbohydrate, protein, and lipid metabolism. Sustained hyperglycemia leads to microvascular and macrovascular complications such as retinopathy, nephropathy, neuropathy, and cardiovascular diseases, often resulting in blindness, kidney failure, amputations, and reduced quality of life. The global prevalence of diabetes continues to rise due to aging populations, sedentary lifestyles, and obesity, making it a major public health concern [1]. Although therapeutic options such as oral antidiabetic drugs and insulin analogs have improved symptom control, many treatments are limited by side effects, variable patient response, and non-curative outcomes. These challenges highlight the need for improved early diagnostics, personalized therapies, and regenerative medicine approaches to prevent disease progression and enhance patient outcomes [2].

Advances in Diabetes Pathophysiology and Molecular Insights

Advances in research have revealed that diabetes involves complex genetic, epigenetic, immune, and metabolic interactions. Genetic and epigenetic regulators influence β-cell function and insulin secretion, while the gut microbiome affects energy metabolism and inflammatory responses. Type 1 diabetes is driven by autoimmune β-cell destruction, whereas type 2 diabetes is associated with insulin resistance and adipokine dysregulation [3]. These insights support precision medicine approaches for improved prevention, targeted therapy, and individualized disease management.

1. Genetic and Epigenetic Regulators of Diabetes

Genetic and epigenetic mechanisms, including DNA methylation, histone modification, and non-coding RNAs, regulate β-cell physiology and glucose metabolism [4]. These changes, influenced

by environmental and metabolic factors, may serve as therapeutic targets or biomarkers. Susceptibility genes such as TCF7L2, INS, and HLA variants interact with epigenetic pathways, offering opportunities for personalized diabetes care and therapeutic innovation [5].

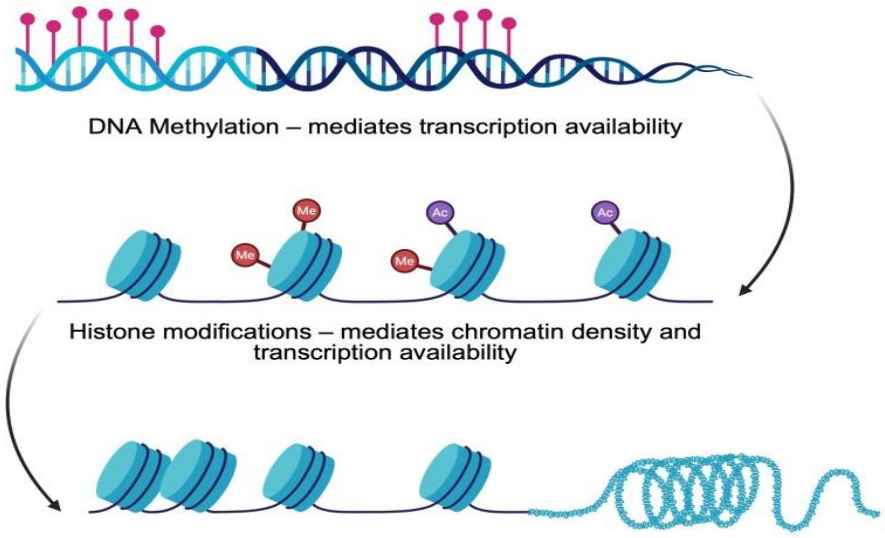

Figure 1: Classic epigenetic mechanisms regulating gene expression [5]

2. Role of the Gut Microbiome and Metabolites

The gut microbiome significantly influences type 2 diabetes (T2DM) through its involvement in metabolic regulation, immune signaling, and gastrointestinal homeostasis. Dysbiosis marked by reduced microbial diversity and altered Firmicutes/Bacteroidetes ratios impairs glucose metabolism, increases insulin resistance, and triggers chronic low-grade inflammation through endotoxin leakage and metabolic endotoxemia [1] [6]. Microbial metabolites such as short-chain fatty acids (SCFAs), bile acids, and lipopolysaccharides act as biochemical mediators affecting intestinal integrity, appetite regulation, gut hormone secretion (GLP-1, PYY), and insulin sensitivity. SCFAs, particularly butyrate, strengthen epithelial barriers and enhance metabolic balance [2][7]. Modulating the microbiome through dietary changes, probiotics, prebiotics, and fecal microbiota transplantation offers emerging therapeutic potential. Understanding microbial pathways supports the development of precision interventions and biomarker-driven diabetes prevention strategies.

3. Immunological Mechanisms and Autoimmune Triggering in Type 1 Diabetes

Type 1 diabetes (T1D) arises from autoimmune destruction of pancreatic β-cells driven by genetic predisposition and environmental triggers such as viral infections and immune dysregulation [8]. Defects in regulatory T cells, heightened inflammatory cytokines (TNF-α, IFN-γ, IL-1β), and antigen-presenting cell activation accelerate immune-mediated β-cell loss [13]. Autoantibodies including GAD65, insulin, and IA-2 serve as predictive diagnostic markers. T1D frequently coexists with autoimmune thyroid disease, celiac disease, and vitiligo, emphasizing the need for early immune modulation and personalized prevention strategies [9].

4. Metabolic Dysfunction and Adipokine Biology in Type 2 Diabetes

In T2DM, adipose tissue dysfunction contributes to insulin resistance through altered adipokine secretion. Increased pro-inflammatory mediators (TNF-α, IL-6, leptin, resistin) and reduced protective adipokines (FGF21, adiponectin) drive metabolic inflammation, oxidative stress, ectopic fat accumulation, and mitochondrial impairment [15,16]. Dysregulated adipokines such as apelin, vaspin, omentin, and visfatin influence systemic metabolism, accelerating progression from prediabetes to advanced T2DM. Targeting adipokine pathways offers promising opportunities for restoring insulin sensitivity, enhancing β-cell survival, and improving cardiometabolic outcomes [10].

Table 1: Various innovative Diagnostic Technologies

Diagnostic Technologies	Key Features	Examples	Clinical/Research Applications
3.1 Biosensor-Based Glucose Monitoring [6]	Electrochemical, optical, enzymatic sensors; wearable/POC devices	GlucoMen, OneTouch Verio, HemoCue Glucose 201	Self-monitoring of blood glucose (SMBG); early detection of hyperglycemia; home use
3.2 Continuous Glucose Monitoring (CGM) [7]	Real-time interstitial glucose measurement; subcutaneous sensors; wireless data	Dexcom G6, Abbott FreeStyle Libre, Medtronic Guardian	Diabetes management; therapy optimization; lifestyle monitoring; T1D & T2D patients
3.3 AI-Enabled Digital Diagnostics & Predictive Analytics [8]	Machine learning algorithms; pattern recognition; integration of multi-parametric data	DiaMonTech, IBM Watson Health, GlucoAI	Early diagnosis, risk stratification, precision medicine; predicting complications; therapy guidance
3.4 Omics-Driven Biomarkers (Genomics, Proteomics, Metabolomics) [9]	High-throughput sequencing; mass spectrometry; metabolite profiling	SNP genotyping for TCF7L2, proteomic panels (insulin signaling proteins), metabolomic profiling for SCFAs	Biomarker discovery, risk prediction, monitoring therapeutic response; personalized medicine; integration with AI for predictive analytics

Novel Pharmacological Interventions

1. Incretin-Based Therapies: GLP-1, DPP-4, and Dual Agonists

Incretin-based therapies play a vital role in type 2 diabetes management by enhancing incretin pathways, particularly GLP-1 and GIP. GLP-1 receptor agonists stimulate insulin secretion, reduce glucagon release, slow gastric emptying, and enhance satiety—thereby improving glycemic control and supporting weight reduction. Meanwhile, DPP-4 inhibitors prevent incretin degradation, offering modest glucose reduction with low hypoglycemia risk. These agents overcome impaired post-prandial incretin responses observed in type 2 diabetes, particularly deficiencies in GIP signaling [11]. Recent advances include dual and multi-agonists targeting GLP-1/GIP or GLP-1/glucagon receptors, yielding enhanced outcomes such as greater HbA1c reduction, β-cell protection, improved cardiometabolic markers, and notable weight loss. Their mechanistic actions span insulin regulation, appetite suppression, lipid metabolism, and inflammation modulation. With flexible pharmacokinetics supporting various dosing intervals, incretin-based therapies establish a precision approach addressing glycemic, cardiovascular, and metabolic needs in diabetes care [12].

2. SGLT2 Inhibitors and Cardiometabolic Benefits

SGLT2 inhibitors function by reducing renal glucose reabsorption, thereby inducing glucosuria independent of insulin pathways. Beyond glycemic improvement, they provide significant cardiometabolic benefits including weight loss, blood pressure reduction, decreased visceral fat, and improved renal outcomes. Substantial clinical data show reduced risks of heart failure, cardiovascular mortality, and kidney disease progression among treated individuals [13]. Mechanistic advantages involve enhanced cardiac energetics, improved ketone utilization, natriuresis, favorable adipokine modulation, and reduced inflammation and oxidative stress [14]. Their dual glucose-lowering and organ-protective effects shift diabetes therapy toward holistic cardiometabolic disease management, especially for patients with established cardiovascular or renal complications [15].

3. Insulin Analogs and Smart/Glucose-Responsive Insulins

Modern insulin analogs—including rapid-, long-acting, and ultra-long-acting types—are engineered to mimic physiologic insulin dynamics, improving glycemic control while reducing hypoglycemia risk. Smart or glucose-responsive insulin innovations represent a next generation approach in which insulin release correlates with circulating glucose levels, enabling more automated glucose regulation [16]. These systems utilize glucose-binding domains, synthetic polymers, or enzymatic triggers to achieve adaptive delivery. Early evidence indicates reduced glycemic variability, lower hypoglycemia risk, and fewer dosing events, potentially improving adherence and patient quality of life. Such innovations represent a promising advancement for

both type 1 and insulin-dependent type 2 diabetes, supporting near-physiological glucose regulation and reduced complication risk [17].

Table 2: Major Antidiabetic Phytochemicals and Their Mechanisms

Phytochemicals	Source Plant (s)	Chemical Class	Primary Antidiabetic Mechanisms
Berberine [18, 41]	*Berberis vulgaris, Tinospora cordifolia*	Isoquinoline alkaloid	AMPK activation, GLUT4 upregulation, inhibition of gluconeogenesis, gut microbiota modulation
Curcumin [18, 41]	*Curcuma longa*	Polyphenolic curcuminoid	Anti-inflammatory (NF-κB inhibition), antioxidant, β-cell protection, improves insulin signaling
Resveratrol [18, 41]	Grapes, berries	Polyphenol stilbene	SIRT-1 activation, mitochondrial regulation, improves insulin signaling
Quercetin [19, 42]	Onion, apples, tea	Flavonoid	Antioxidant, inhibits α-glucosidase, improves insulin sensitivity and pancreatic β-cell function
Gymnemic acids [20, 43]	*Gymnema sylvestre*	Triterpenoid saponins	Reduces intestinal glucose absorption, β-cell regeneration stimulus, sugar taste receptor suppression
Epigallocatechin gallate (EGCG) [21] [22, 44]	Green tea (*Camellia sinensis*)	Catechin	Improves insulin sensitivity, reduces oxidative stress, inhibits carbohydrate digestion enzymes
Rutin [23, 45]	Buckwheat, *Ruta graveolens*	Flavonoid glycoside	β-cell protective, antioxidant, improves glucose uptake, inhibits glycation
Trigonelline [24, 46]	Fenugreek (*Trigonella foenum-graecum*)	Alkaloid	Enhances insulin release, reduces hepatic glucose output, improves lipid metabolism
Ginsenosides [25, 47]	*Panax ginseng*	Triterpenoid saponins	PPARγ modulation, β-cell protection, enhances insulin signaling

4. Targeted Small-Molecule and Peptide Therapeutics

Targeted therapeutics expand diabetes management through precision modulation of metabolic, hormonal, and inflammatory signaling pathways. Small molecules offer advantages such as oral delivery and specificity but may require combination strategies to minimize adverse effects [18]. Peptide-based therapies, including advanced incretin agonists such as semaglutide, tirzepatide, and emerging triple agonists, demonstrate significant improvements in weight control, insulin sensitivity, and β-cell preservation [19]. Novel biologics, including amylin analogs and

monoclonal antibodies, further extend therapeutic coverage into obesity-associated metabolic dysfunction. Artificial intelligence–driven drug design and structural biology advancements continue to accelerate development of multimodal molecules with improved tolerability and durability, supporting individualized and mechanism-based diabetes management approaches [20].

Polyherbal Formulations and Synergistic Approaches

Table 3: Polyherbal formulations and synergistic approaches in diabetes

Polyherbal Formulation / Combination	Key Components	Proposed Mechanisms of Action	Reported Benefits
Triphala-based formulation	*Terminalia chebula, Terminalia bellirica, Emblica officinalis* [26]	Antioxidant effect, modulation of glucose transporters, α-amylase inhibition	Improved glycemic control, reduced oxidative stress
Nishamalaki Yoga	*Curcuma longa* + *Emblica officinalis* [27]	Anti-inflammatory, β-cell protection, insulin sensitization	Reduction in fasting glucose and HbA1c
Diarex® (Ayurvedic polyherbal)	*Momordica charantia, Gymnema sylvestre, Eugenia jambolana, Tinospora cordifolia* [28] [29]	Stimulates insulin secretion, regenerates β-cells, enhances glucose uptake	Hypoglycemic effect with improved lipid profile
Gymnema + Fenugreek combination	*Gymnema sylvestre* + *Trigonella foenum-graecum* [30]	SGLT modulation, insulin secretion, delayed carbohydrate absorption	Enhanced glucose tolerance and postprandial control
Cinnamon + Metformin (synergistic plant-drug)	*Cinnamomum verum* + *Metformin* [31]	AMPK activation, insulin sensitization, reduced hepatic gluconeogenesis	Improved therapeutic response with reduced metformin dose
Green tea + Ginger + Turmeric combination	*Camellia sinensis, Zingiber officinale, Curcuma longa* [32]	Anti-inflammatory, antioxidant, β-cell protection	Reduced insulin resistance and inflammation

Polyherbal formulations and synergistic therapeutic approaches are gaining prominence in diabetes management due to their multi-targeted mechanisms and improved therapeutic potential. Combining multiple plant-derived bioactives enhances glycemic control through complementary mechanisms such as insulin sensitization, β-cell protection, glucose uptake stimulation, oxidative stress reduction, and modulation of gut microbiota. Synergistic effects often lead to improved efficacy, reduced toxicity, and lower required doses compared to single-herb treatments. Many traditional systems like Ayurveda and Traditional Chinese Medicine have long utilized polyherbal combinations for metabolic disorders, and modern research supports their role in regulating key pathways including AMPK activation, α-glucosidase inhibition, and lipid metabolism modulation.

Nano-Phytomedicine for Improved Bioavailability

Nano-phytomedicine combines nanotechnology with plant-derived bioactives to address limitations of traditional herbal antidiabetic therapies. Phytochemicals such as curcumin, quercetin, berberine, and resveratrol possess strong therapeutic potential but are restricted by poor solubility, instability, and low bioavailability. Nanoformulations including nanoparticles, nanoemulsions, liposomes, and solid lipid nanoparticles enhance solubility, stability, targeted delivery, and cellular uptake [33]. These systems also enable sustained release, reduced dosing frequency, and minimized side effects. Organ-targeted nano-phytomedicines improve insulin sensitivity and protect β-cells, with preclinical findings showing superior glycemic control and metabolic regulation compared to conventional extracts [34]. Therefore, nano-phytomedicine offers a promising advancement in diabetes therapy.

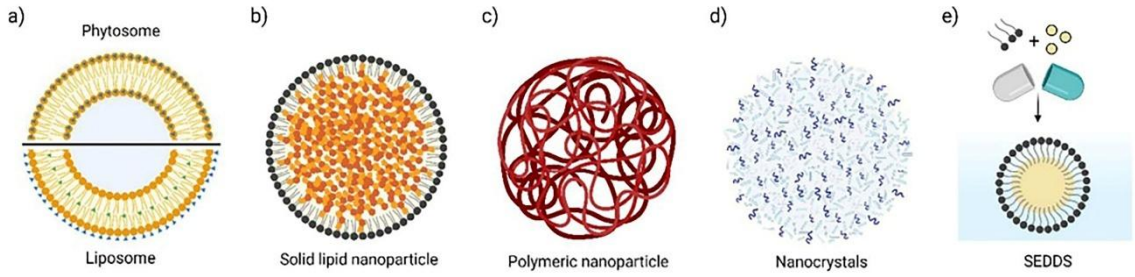

Figure 2: Nanocarrier-Based Delivery Systems for Phytomedicine

Traditional Knowledge Systems in Modern Diabetes Research

Traditional medical systems such as Ayurveda, Traditional Chinese Medicine, and Indigenous practices play a significant role in diabetes care through holistic frameworks incorporating herbal medicines, lifestyle regulation, and detoxification. Plants traditionally used for *Madhumeha* exhibit mechanisms including insulin sensitization, β-cell protection, antioxidant activity, and glucose metabolism modulation [35]. Modern research increasingly validates these mechanisms, leading to standardized herbal extracts, polyherbal formulations, and emerging nano-

phytomedicines. In areas with limited access to modern healthcare, traditional therapies remain preferred due to cultural acceptance, affordability, and availability. Integrating traditional knowledge with evidence-based research strengthens culturally appropriate global diabetes care solutions and supports the development of complementary therapeutic strategies [36].

Lifestyle and Preventive Interventions

Lifestyle-based prevention remains a cornerstone of diabetes management, emphasizing diet, exercise, stress reduction, adequate sleep, and early detection of prediabetes [37]. When combined with pharmacotherapy, lifestyle modification enhances treatment response and long-term metabolic outcomes. Evidence shows lifestyle interventions can reduce diabetes incidence by up to 58%, making them foundational to prevention and disease reversal strategies. Community-centered education and behavioral reinforcement further improve adherence and sustainability [38].

1. Personalized Nutrition and Precision Dietetics

Personalized nutrition integrates genetic markers, metabolic phenotyping, gut microbiome profiling, and behavioral factors to create individualized dietary plans. Precision dietetics optimizes macro- and micronutrient composition and meal timing using tools such as AI-guided analysis and continuous glucose monitoring [39]. Evidence demonstrates superior improvements in HbA1c and inflammatory markers compared with generalized dietary guidelines, making precision nutrition a key component of personalized diabetes care [40].

2. Digital Therapeutics and Behavioural Interventions

Digital platforms including telemedicine, apps, and wearable devices enable real-time monitoring, behavioral reinforcement, and therapy adherence. Behavioral psychology tools such as motivational interviewing and cognitive-behavioral strategies enhance sustainable change [41]. Integrated digital care improves patient engagement, glycemic stability, and remote supervision, demonstrating scalable potential in diabetes management [42].

3. Exercise Physiology and Glycemic Optimization

Exercise enhances insulin sensitivity and metabolic function, with aerobic training, resistance exercise, and HIIT providing complementary benefits. Personalized plans optimize safety and glycemic outcomes, especially when combined with diet and medication [43]. Wearables now support real-time physical activity adjustment, while timing and modality are emerging variables influencing glucose control [44].

Challenges and Ethical Barriers in Translational Research

Despite advancements, translational diabetes research faces ongoing challenges including cost, regulatory constraints, data privacy, and disparities in access. Ethical acceptance of emerging molecular and regenerative therapies depends on cultural alignment and public awareness.

Regulatory frameworks such as RMAT aim to support safe and equitable innovation, requiring global coordination across policy and clinical practice [45–46].

Future Perspectives and Research Directions

Future diabetes care is expected to shift toward predictive, preventive, and personalized medicine using AI, real-time analytics, and precision diagnostics [47]. Integration of genomics, metabolomics, and continuous metabolic monitoring will enable individualized disease prediction and optimized therapies. Research focus is moving toward curative strategies including cell regeneration, gene editing, and targeted molecular therapeutics. The next decade anticipates a convergence of computational and biomedical sciences to reduce disease burden, improve survivorship, and transform diabetes care globally. Multidisciplinary collaboration will be essential for translating innovation into accessible patient-centered healthcare solutions [48].

Conclusion:

Diabetic research is evolving rapidly, driven by advancements in molecular biology, nanotechnology, regenerative medicine, and precision healthcare. Current trends emphasize a shift from conventional symptomatic management to predictive, preventive, and personalized approaches, enabling early detection, risk stratification, and individualized therapeutic interventions. Nutraceuticals, polyherbal formulations, and plant-derived bioactive compounds are gaining attention for their synergistic antidiabetic effects and potential to modulate molecular targets. Nanotechnology-based delivery systems enhance bioavailability, targeting, and efficacy of both conventional drugs and phytotherapeutics, bridging the gap between laboratory discoveries and clinical applications. Integration of artificial intelligence, big data analytics, and omics technologies is transforming disease monitoring, therapy optimization, and outcome prediction. Despite challenges such as regulatory hurdles, ethical considerations, and patient acceptance, translational research continues to progress. Over the next decade, collaborative, interdisciplinary efforts are expected to revolutionize diabetes care, improve disease management, and potentially achieve disease modification, offering hope for better patient outcomes and reduced global burden.

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About Editors

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