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(Registered Under Societies Registration Act 1860)

Aims and Objectives

The Society (SGWSE) has been functioning with following aims and objectives :

- To constitute a forum at international and national level for bringing together individuals and organization involved in agriculture and biological science activities.
- To develop international research/development linkages and disseminate up-to-date technologies in the field of agriculture and biological science.
- To promote and undertake research and development and extension service in the field of agriculture and biological science.
- To explore new areas in agriculture, biological research, biotechnology crop cultivation technologies, development activity and logistics management.
- To propagate utilization on non- conventional and renewable sources in agriculture and biological research.
- To develop purely organic package for growing and cultivation of crops.
- To develop technology of in vivo propagation of important crops.
- To offer recognition and awards to professional groups and individual for attainment of excellence in the field of agriculture, biological science and Biotechnology.
- To interact with government agencies, scientific organization and NGOs to promote and protect interest of agriculture and biological science researcher.

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REVIEW ARTICLE

Innovative Strategies for Strengthening Mycorrhizal Dependency as A Dynamic Phosphatic Bio-Inoculant in Sustainable Farming Practices

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Abstract

Mycorrhizae, or "fungus roots," form mutualistic relationships with plants, dating back to the Ordovician period. Around 40,000–50,000 fungal species associate with 250,000 plant species, primarily as Arbuscular (71%) and Ectomycorrhiza (2%). These fungi enhance nutrient uptake, supplying up to 80% of nitrogen and phosphorus, boosting plant growth, yields, and nutritional value. They also influence secondary metabolite production. Extensive research confirms their role in improving antioxidants, vitamins, and trace elements. Increasingly applied on an industrial scale, mycorrhizal fungi are integral to sustainable agriculture, offering eco-friendly solutions that enhance productivity while minimizing environmental impact through organic and regenerative practices.

Keywords *: Mycorrhizae, Ectomycorrhizae, Orchid mycorrhizae, Eco-friendly, Sustainable Agriculture.*

Introduction

Arbuscular Mycorrhizal Fungal (AMF) inoculants, also referred to as mycorrhizal inoculants or mycorrhizal biofertilizers, are formulations containing AM fungal spores, mycelium, and/or propagules. These inoculants introduce beneficial soil microorganisms, particularly AM fungi, into agricultural and horticultural systems, promoting plant growth, health, and nutrient absorption (Thirkell *et al.*, 2017). AM fungi can establish symbiotic relationships with 70–90% of terrestrial plant species (Shi *et al.*, 2023), presenting significant potential for enhancing sustainability in both agricultural and forest ecosystems.

Recent research highlights the increasing use of AMF inoculants in agriculture (Gianinazzi *et al.*, 2004; Ijdo *et al.*, 2010), with numerous companies striving to enhance AMF inoculum production for practical applications. These inoculants have been widely recognized as effective biofertilizers across various agricultural and farming sectors (Igiehon and Babalola, 2017; Vosatka *et al.*, 2017). Their primary objective is to increase the density of AMF spores in the soil, serving as "biofertilizers" that maximize the utilization of existing soil nutrient reserves to support crop growth (Faye *et al.*, 2013).

The Importance of AM Fungal Symbiosis

The mutualistic association between plants and arbuscular mycorrhizal fungi (AMF) offers significant ecological benefits. AMF contribute to soil structure improvement by promoting aggregation, enhancing stability, and minimizing erosion. Additionally, this symbiotic relationship plays a crucial role in carbon sequestration, helping mitigate climate change by capturing atmospheric carbon dioxide. Notably, recent estimates indicate that global plant communities allocate approximately 3.93 $GtCO_2$ eq. per year to AMF, a substantial figure comparable to a significant share of anthropogenic CO_2 emissions in 2021. This underscores the essential role of AMF in carbon storage and climate regulation (Hawkins *et al.*, 2023).

AMF also play a crucial role in reducing nutrient leaching, particularly of vital elements like phosphorus, thereby enhancing nutrient use efficiency and preserving essential resources within the ecosystem (Ayangbenro, 2022). Their presence fosters interactions and cooperation among diverse soil microbial communities, promoting greater biodiversity and overall productivity (Saleem *et al.*, 2019). By stimulating microbial activity, AMF support

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nutrient cycling and facilitate the decomposition of organic matter. Additionally, they indirectly help mitigate the release of nitrous oxide (N_2O), a potent greenhouse gas, by improving nutrient assimilation in plants and reducing microbial denitrification processes Bhatia and Sindhu (2024d) reported that the use of AMF biotechnology shows great potential for restoring degraded lands by enhancing soil properties and strengthening plant resilience (Dpa, 2022). By forming a common mycorrhizal network (CMN), AMF establish connections among plants within an ecosystem, facilitating nutrient transfer, boosting plant productivity, and further improving soil health (Ullah *et al.*, 2024).

SWOT Analysis for AM Inoculants in Sustainable Agriculture

A diagrammatic representation visually organizes the SWOT analysis of Arbuscular Mycorrhizal (AM) Inoculants, helping to understand key relationships and strategic insights. At the center, label the main node as "AM Inoculants SWOT Analysis." From this central point, create four primary branches: Strengths, Weaknesses, Opportunities, and Threats (SWOT). Use a green branch for Strengths, highlighting phosphorus uptake efficiency, soil health improvement, drought resistance, and reduced chemical fertilizer dependency. Represent these with plant, soil, and microbial icons. The yellow branch for Weaknesses should illustrate slow colonization, host-specific interactions, short shelf-life, and high initial costs. Icons like a clock, microbial interactions, and cost symbols can enhance understanding. For Opportunities, use a blue branch to showcase organic farming growth, biotechnology advancements, climate change mitigation, and integration with precision agriculture. Add visuals like AI, lab equipment, and renewable farming symbols. A red branch for Threats can represent soil disturbances, microbial competition, regulatory barriers, and environmental variability. Use warning signs and competition icons to illustrate risks.

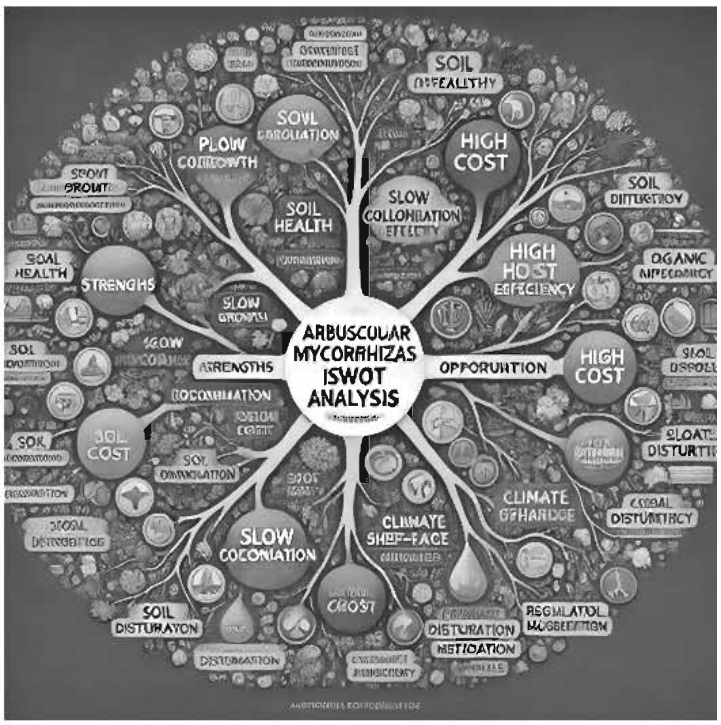

Fig. 1 : Arbuscular Mycorrhiza SWOT Analysis

1. Strengths

1.1 Improved Plant Growth and Yield

Arbuscular mycorrhizal (AM) inoculants play a crucial role in enhancing plant growth and productivity by improving nutrient uptake, particularly phosphorus and nitrogen (Smith and Read, 2008). These fungi establish symbiotic relationships with plant roots, extending their hyphal networks into the soil to access nutrients beyond the root zone, thereby promoting more efficient nutrient absorption (Van der Heijden *et al.*, 2015), also, AM inoculants contribute to increased drought resistance and stress tolerance, leading to improved crop yields even in challenging environmental conditions (Berruti *et al.*, 2016). Studies have shown that plants colonized by AM fungi exhibit greater biomass production and

enhanced root development, further supporting their role in sustainable agriculture (Pellegrino *et al.*, 2015) By fostering plant health and productivity, AM inoculants serve as a valuable biofertilizer alternative, reducing reliance on chemical fertilizers while promoting eco-friendly farming practices (Igiehon and Babalola, 2017).

1.2. Eco-Friendly Alternative

Arbuscular mycorrhizal (AM) inoculants serve as an environmentally friendly alternative to chemical fertilizers, promoting sustainable agriculture by enhancing soil fertility and reducing dependency on synthetic inputs (Aslam *et al.*, 2017). These biofertilizers improve nutrient cycling and soil structure while minimizing environmental pollution associated with excessive fertilizer use (Jansa *et al.*, 2011). AM fungi also enhance carbon sequestration and soil biodiversity, contributing to ecosystem stability (Asmelash *et al.*, 2016). By fostering plant-microbe interactions, AM inoculants support organic farming practices, offering a natural solution for improving crop productivity while preserving soil health and ecological balance (Baral *et al.*, 2020).

1.3. Soil Health Benefits

Arbuscular mycorrhizal (AM) inoculants play a crucial role in enhancing soil health by improving soil structure, increasing organic matter decomposition, and promoting microbial diversity (Rillig *et al.*, 2015). Their extensive hyphal networks facilitate soil aggregation, reducing erosion and enhancing water retention (Lehmann *et al.*, 2017). AM fungi also aid in the bioavailability of essential nutrients like phosphorus and nitrogen, ensuring efficient nutrient cycling (Cavagnaro *et al.*, 2015). Furthermore, their interaction with beneficial soil microbes supports a balanced soil ecosystem, contributing to long-term agricultural sustainability (Van der Heijden *et al.*, 2008).

1.4. Broad compatibility

Arbuscular mycorrhizal (AM) inoculants exhibit broad compatibility, forming symbiotic relationships with nearly 70–90% of terrestrial plant species, including major crops (Brundrett, 2009). This adaptability allows their application across diverse agricultural systems, enhancing plant nutrient uptake and resilience in various soil types and climatic conditions (Brachmann and Parniske, 2006).

1.5. Increased resilience

Arbuscular mycorrhizal (AM) inoculants enhance plant resilience by improving tolerance to drought, salinity, and soil-borne pathogens (Ruiz-Lozano *et al.*, 2016). They boost plant defense mechanisms and support stress adaptation, making them valuable for sustainable agriculture in challenging environments (Begum *et al.*, 2019).

2. Weaknesses

2.1. Inconsistent Performance

The effectiveness of arbuscular mycorrhizal (AM) inoculants varies due to factors such as soil conditions, native microbial communities, and plant species compatibility (Schlaeppi and Bulgarelli, 2015). Environmental variables can impact AM fungal colonization, leading to inconsistent benefits in different agricultural settings (Hart *et al.*, 2018).

2.2. Short shelf life

Arbuscular mycorrhizal (AM) fungal inoculants have a limited shelf life due to their sensitivity to desiccation, temperature fluctuations, and storage conditions (Douds *et al.*, 2006). Maintaining viability requires specialized formulations and controlled environments, posing challenges for large-scale distribution and long-term storage (Singh *et al.*, 2021).

2.3. High Production Costs

The large-scale production of arbuscular mycorrhizal (AM) fungal inoculants is costly due to complex cultivation methods, requiring host plants for propagation and controlled environmental conditions (Ijdo *et al.*, 2011). These factors contribute to high market prices, limiting accessibility for widespread agricultural use (Cavagnaro *et al.*, 2015).

2.4. Limited Awareness

Despite their benefits, the adoption of arbuscular mycorrhizal (AM) fungal inoculants remains low due to limited awareness among farmers and stakeholders (Schüßler and Walker, 2010). Knowledge gaps regarding their application, effectiveness and economic advantages hinder widespread implementation in sustainable agriculture (Jansa *et al.*, 2021).

2.5. Soil Microbial Competition

Arbuscular mycorrhizal (AM) fungal inoculants often face competition from native soil microbes, which can inhibit their establishment and effectiveness (Veresoglou *et al.*, 2012). The presence of antagonistic fungi and bacteria may reduce colonization success, impacting plant-microbe interactions and nutrient uptake efficiency (Xue *et al.*, 2018).

3. Opportunities

3.1. Rising Demand for Sustainable Solutions

The increasing shift towards eco-friendly agricultural practices has amplified interest in arbuscular mycorrhizal (AM) fungal inoculants as sustainable alternatives to chemical fertilizers (Lehmann *et al.*, 2017). Their role in enhancing soil fertility and plant resilience aligns with global sustainability goals (Cozzolino *et al.*, 2021).

3.2. Alignment with Climate Goals

Arbuscular mycorrhizal (AM) fungal inoculants contribute to carbon sequestration and reduced greenhouse gas emissions, supporting climate change mitigation strategies (Gao *et al.*, 2020). Their ability to enhance soil health and resilience aligns with global climate policies promoting sustainable agricultural practices (Chagnon *et al.*, 2013).

3.3. Technological Innovations

Advancements in biotechnology and precision agriculture have improved the mass production, formulation, and application of arbuscular mycorrhizal (AM) fungal inoculants (Hart *et al.*, 2017). Innovations such as encapsulation and microbial consortia enhance inoculant viability and effectiveness, increasing their potential for sustainable farming (Salomon *et al.*, 2022).

4. Threats

4.1. Regulatory Gaps

The commercialization of arbuscular mycorrhizal (AM) fungal inoculants faces challenges due to inconsistent regulatory frameworks across regions (Schlemper *et al.*, 2021). The lack of standardized guidelines for quality control, efficacy assessment, and registration hinders their widespread adoption in sustainable agriculture (Macdonald *et al.*, 2022).

4.2. Economic Constraints

The high production costs and limited financial incentives pose significant challenges for the widespread adoption of arbuscular mycorrhizal (AM) fungal inoculants (Berruti *et al.*, 2016). Additionally, the lack of subsidies and investment in research slows market growth and accessibility for small-scale farmers (Hijri, 2016).

4.3. Environmental Uncertainty

The effectiveness of arbuscular mycorrhizal (AM) fungal inoculants is influenced by environmental factors such as soil conditions, climate variability, and land management practices (Bahram *et al.*, 2020). Unpredictable environmental changes can limit inoculant performance, reducing their reliability in agricultural systems (Koskey *et al.*, 2021b).

4.4. Misuse Risks

Improper application of arbuscular mycorrhizal (AM) fungal inoculants, such as incorrect dosages or unsuitable environmental conditions, can reduce their effectiveness and disrupt native microbial communities (Schwartz *et al.*, 2006). Additionally, low-quality or contaminated inoculants may lead to poor plant responses and reduced agricultural benefits (Jansa *et al.*, 2013).

Arbusclar Mycorrhiza Fungi

Arbuscular mycorrhizal (AM) fungi are essential soil microorganisms that establish mutualistic associations with approximately 70–90% of terrestrial plants, improving nutrient uptake, particularly phosphorus and enhancing plant growth and stress tolerance. These fungi belong to the phylum *Glomeromycota* and form specialized structures, such as arbuscules and vesicles, within plant roots to facilitate nutrient exchange (Parniske, 2008).

AM fungi play a crucial role in soil health by improving soil aggregation, increasing microbial diversity, and aiding in carbon sequestration, which supports sustainable agriculture (Rillig *et al.*, 2019). Their contributions to plant resilience against environmental stressors, including drought and salinity, make them valuable in climate change adaptation strategies (Begum *et al.*, 2019). Advancements in biotechnology are enhancing their application as biofertilizers for improving crop productivity and sustainability (Faye *et al.*, 2013).

Ectomycorrhiza

Ectomycorrhiza (ECM) are mutualistic associations between fungi and the roots of woody plants, including species from the *Pinaceae*, *Fagaceae*, and *Betulaceae* families. Unlike arbuscular mycorrhizae, ECM fungi form a dense sheath around root tips and develop a Hartig net, facilitating nutrient exchange without penetrating root cells (Tedersoo and Smith, 2013). These fungi play a vital role in forest ecosystems by enhancing nitrogen and phosphorus uptake while also providing increased resistance to pathogens and environmental stresses (Corrales *et al.*, 2018).

ECM fungi contribute significantly to soil carbon storage by influencing decomposition rates and stabilizing organic matter (Clemmensen *et al.*, 2013). Their adaptability to nutrient-poor soils makes them essential for afforestation and land rehabilitation efforts (Suz *et al.*, 2021). The ecological and

biotechnological significance of ECM fungi highlights their importance in sustainable forest management and climate change mitigation.

Ericoid Mycorrhizae

Ericoid mycorrhizae (ErM) form symbiotic relationships with members of the Ericaceae family and certain liverworts (Bethlenfalvay and Linderman, 1992). Approximately 2% of all plant species engage in a symbiosis with Ericoid mycorrhizae (Bennett *et al.*, 2009). These fungi primarily belong to the Ascomycota, with some species in the Basidiomycota. Over 150 species of Ericoid mycorrhizae interact with around 3,900 plant species, including those in the Diapensiaceae family (Bonfante and Selosse, 2010).

Molecular and paleontological evidence suggests that the symbiosis between Ericoid mycorrhizae and plants may have originated during the Cretaceous period, around 140 million years ago (Cullings, 1996). Ericoid mycorrhizae are particularly well-adapted to acidic, nutrient-poor soils, though they do not occur in certain Ericaceae subfamilies, such as Monotropoideae, Arbutoideae, and Enkianthoideae. (Gohre and Paszkowski, 2006) reported these fungi are obligate symbionts, meaning they are dependent on living plant roots. Their hyphae penetrate the epidermal cells of the plant, forming characteristic fungal coils. From these coils, the hyphae extend into the cortical cells, creating dense, interwoven networks. The coils are the primary sites where the exchange between the host plant and the fungus occurs.

Orchid Mycorrhiza

Orchid mycorrhiza (OM) represents a unique and essential symbiosis between orchids and specific mycorrhizal fungi, primarily from *Tulasnellaceae*, *Ceratobasidiaceae*, and *Sebacinales* (Dearnaley *et al.*, 2012). Unlike other mycorrhizal associations, orchids rely entirely on fungal partners for germination and early development, as their seeds lack sufficient stored nutrients (Rasmussen and Rasmussen, 2009). These fungi provide carbon and nutrients, enabling orchids to establish in nutrient-poor habitats.

OM also plays a critical role in adult orchid nutrition, particularly in epiphytic and terrestrial species growing in challenging environments (Bidartondo and Read, 2008). The symbiosis influences orchid distribution and survival, making it crucial for conservation strategies (Perotto *et al.*, 2014). Recent advances in molecular techniques have improved the identification of OM fungi, aiding efforts to restore endangered orchid populations (Shefferson *et al.*, 2019).

Other Types of Mycorrhizae

Beyond arbuscular, ectomycorrhizal, and orchid mycorrhizal associations, several other specialized mycorrhizal types exist, including ericoid, arbutoid and monotropoid mycorrhizae, each playing a crucial role in plant adaptation and nutrient acquisition.

Ericoid Mycorrhiza (ERM) primarily associates with plants in the Ericaceae family, enhancing their ability to thrive in acidic, nutrient-poor soils by facilitating organic nitrogen and phosphorus uptake (Hawkins *et al.*, 2023).

Arbutoid Mycorrhiza shares characteristics with both ectomycorrhizal and ericoid mycorrhizal fungi, forming associations with genera like *Arbutus* and *Arctostaphylos* (Cairney and Meharg, 2003).

Monotropic Mycorrhiza is found in non-photosynthetic plants like *Monotropa* species, which rely entirely on fungal partners for carbon, forming tripartite relationships involving trees, ectomycorrhizal fungi, and the mycoheterotrophic plant (Bidartondo, 2005). These unique mycorrhizal types highlight the diversity and ecological significance of fungal-plant symbioses across various ecosystems.

Multiple Advantages of Arbuscular Mycorrhizal Fungal Inoculants

Arbuscular mycorrhizal fungal (AMF) inoculants offer significant benefits in sustainable agriculture by enhancing plant growth, improving nutrient uptake, and increasing stress resilience. AMF form symbiotic associations with plant roots, facilitating phosphorus and nitrogen absorption, which is particularly crucial in nutrient-deficient soils (Smith and Smith, 2011). Additionally, AMF contribute to soil structure by promoting soil aggregation and reducing erosion, leading to improved soil health and fertility (Rillig *et al.*, 2015).

These fungi also enhance plant tolerance to abiotic stresses such as drought and salinity by regulating water uptake and hormonal responses (Ruiz-Lozano *et al.*, 2016). Furthermore, AMF promotes biodiversity by fostering beneficial microbial communities and reducing dependency on chemical fertilizers, thereby lowering environmental pollution (Jansa *et al.*, 2013). Their role in carbon sequestration further supports climate change mitigation, making AMF inoculants a crucial tool in ecological farming practices.

Taxonomy of AM Fungi

Arbuscular mycorrhizal (AM) fungi belong to the phylum Glomeromycota, a distinct lineage of

symbiotic fungi that establish mutualistic relationships with plant roots. This phylum is classified into several orders, including Glomerales, Diversisporales, Paraglomerales and Archaeosporales, which contain multiple genera such as *Glomus, Rhizophagus, Funneliformis,* and *Claroideoglomus* (El-Sawah *et al.*, 2022).

AM fungi are obligate symbionts, meaning they rely on host plants for carbon while facilitating the uptake of essential nutrients like phosphorus. Their unique reproductive strategy involves producing large, multinucleate spores in the soil or within plant roots (Redecker *et al.*, 2013). Molecular phylogenetic studies have refined their classification by utilizing ribosomal RNA (rRNA) and functional gene analyses, revealing extensive diversity within Glomeromycota (Krüger *et al.*, 2012).

Isolation of AM Fungal Spores from Soils

The isolation of arbuscular mycorrhizal (AM) fungal spores from soil is a crucial step in studying their diversity, ecology and potential applications. The most commonly used method is wet sieving and decanting, followed by sucrose or density gradient centrifugation to separate spores from soil particles (Gerdemann and Nicolson, 1963). This technique efficiently concentrates AM spores by exploiting their unique density and size characteristics.

Following isolation, spores are typically examined under a microscope for morphological identification, assessing traits such as size, color, wall structure, and hyphal attachments (Błaszkowski, 2012). Molecular techniques, including PCR-based identification using small subunit ribosomal RNA (SSU rRNA) markers, have further improved the accuracy of AM fungal identification (Öpik *et al.*, 2014). Advances in these methods facilitate large-scale studies on AM fungal communities and their role in soil health and plant productivity.

Morphological Analysis of AM Fungal Spores for Dentification

The identification of arbuscular mycorrhizal (AM) fungi is primarily based on the morphological characteristics of their spores. Key traits used for classification include spore size, shape, color, wall structure, surface ornamentation, and hyphal attachment. AM fungal spores can be single or aggregated in sporocarps, and their walls are composed of multiple layers, which can be smooth, laminated, or ornamented, aiding in species differentiation (Kehri *et al.*, 2018).

Microscopic examination using light and stereo microscopes allows for the initial identification of AM fungal spores, while scanning electron microscopy (SEM) provides more detailed structural analysis (Gai *et al.*, 2006). Additionally, spore staining techniques using Melzer's reagent help distinguish species based on wall reaction patterns. Morphological identification remains essential despite advancements in molecular tools, as it provides a fundamental understanding of AM fungal diversity and taxonomy (Oehl *et al.*, 2011). Combining traditional morphology-based methods with molecular techniques enhances the accuracy of AM fungal classification and ecological studies.

Observation of Intact Spores Under Dissecting Microscope

The examination of intact arbuscular mycorrhizal (AM) fungal spores under a dissecting microscope is a crucial step in their identification and classification. This method allows for the initial assessment of spore size, shape, colour and surface texture without causing structural damage (Li *et al.*, 2022). Dissecting microscopes provide low-magnification views, enabling researchers to sort spores based on their external morphology before conducting detailed microscopic and molecular analyses.

Under a dissecting microscope, spores are typically observed in water or specialized mounting media to maintain their natural integrity. Variations in spore pigmentation, ranging from hyaline to dark brown, can help differentiate genera, as colour changes often correlate with spore maturity and species type (Linderman, 1992). Additionally, spores can be categorized based on their occurrence as single spores, aggregates, or sporocarps, which aids in further taxonomic classification.

This preliminary analysis provides essential data for further identification using light microscopy, scanning electron microscopy (SEM) and molecular techniques (Luginbuehl *et al.*, 2017). By combining morphological and molecular approaches, researchers can achieve more accurate taxonomic identification of AM fungi, contributing to ecological and agricultural studies.

Observation of Spores Mounted on A Glass Slide Under a Compound Microscope

The observation of arbuscular mycorrhizal (AM) fungal spores under a compound microscope allows for detailed examination of their internal and external structures. Spores are typically mounted on glass slides using lactoglycerol, polyvinyl alcohol-lactic acid

(PVLG), or Melzer's reagent to enhance visibility (Mathur *et al.*, 1999). Key characteristics assessed include spore wall layers, hyphal attachment, septation, and cytoplasmic content, which are crucial for taxonomic identification (Oehl *et al.*, 2011).

Staining techniques, such as Melzer's reagent, help differentiate species based on spore wall reactions, particularly amyloid or non-amyloid properties (Muneer *et al.*, 2020). This microscopic analysis, combined with molecular methods, ensures accurate classification of AM fungi.

PVLG (Polyvinyl Lacto-Glycerol) Preparation:

- 1.66 g of polyvinyl alcohol (with a polymerization degree of 1000-1500) is dissolved in 10 ml of deionized water. Complete dissolution may take up to 6 hours at 80°C.
- The dissolved polyvinyl alcohol is then mixed with 10 mL of lactic acid and 1 mL of glycerol.
- The mixture can be used the day after preparation.

Percent Root Colonization of AM Fungi

Pull out plants without damaging fine feeder roots. Wash the roots in running tap water to remove adhering soil particles. Cut the feeder roots into small bits. Place the root sample in a beaker containing 10% KOH and autoclave at 15 lbs pressure for 10 minutes. Rinse with several changes of tap water and wash with distilled water. Acidify in 2% HCl for few minutes and pour of HCl. Stain the root segment with 0.05% Tryphan blue in lactophenol by boiling for 5 minutes. Examine the stained roots under bright-field microscope. Estimation of the percentage of root colonization by using the formula:

$$Percentage\ of\ AM\ colonization = \frac{Number\ of\ VAM\ positive\ segments}{Total\ number\ of\ segments\ scored} \times 100$$

Mass Production of AM Fungi

Pot culture is commonly used for producing AM fungal inoculum with a carrier-based approach. In this method, sterilized soil is typically used, and a wide range of host crops serve as the plants for colonization. However, sterilizing soil can be a labour-intensive process, prompting researchers to explore inert materials as alternatives for AM fungal production. Substances like perlite, montmorillonite clay, and vermiculite have been tested as substrates to replace the need for soil sterilization. Among these, vermiculite has proven to be the most effective substrate for inoculum production.

A trench (1m×1m×0.3m) is formed and lined with black polythene sheet to be used as a plant growth tub.

- Mix 50 kg of vermiculite and 5kg of sterilized soil and pack in the trench up to a height of 20 cm.
- Spread 1kg of AM inoculum (mother culture) 2-5 cm below the surface of vermiculite.
- Maize seeds surface sterilized with 5% sodium hypochlorite for 2 minutes are sown.
- Apply 2 g urea, 2 g super phosphate and 1 g muriate of potash for each trench at the time of sowing seeds. Further 10 g of urea is applied twice on 30 and 45 days after sowing for each trench.
- Quality test on AM colonization in root samples is carried out on 30th and 45th day.
- Stock plants are grown for 60 days (8 weeks). The inoculum is obtained by cutting all the roots of stock plants. The inoculum produced consists of a mixture of vermiculite, spores, pieces of hyphae and infected root pieces.
- Thus within 60 days 55 kg of AM inoculum could be produced from 1 sq meter area. This inoculum will be sufficient to treat 550 m^2 nursery area having 11,000 seedlings.

Preservation and Precautions

Effective preservation of arbuscular mycorrhizal (AM) fungi is crucial for maintaining their viability and functionality. Spores, root fragments, and colonized substrates can be stored at 4°C for short-term use, while cryopreservation at -80°C or in liquid nitrogen ensures long-term viability (Aliasgharzadeh *et al.*, 2001). Desiccation techniques using silica gel also help maintain spore viability.

Precautions include preventing contamination by maintaining sterile conditions, avoiding prolonged exposure to high temperatures, and ensuring proper aeration (Rodriguez *et al.*, 2005). Additionally, periodic viability assessments are necessary to confirm fungal effectiveness.

Method of Application

Nursery Application: 100 kg bulk inoculum is

sufficient for $1m^2$. The inoculum should be applied at 2-3 cm below the soil at the time of sowing. The seeds/cutting should be sown/ planted above the AM fungal inoculum to cause infection.

For Polythene Bag Raised Crops: 5 to 10 g bulk inoculum is sufficient for each packet. Mix 10 kg of inoculum with 1000 kg of sand potting mixture and pack the potting mixture in a polythene bag before sowing.

For Out-Planting: Twenty grams of AM fungal inoculum is required per seedling. Apply inoculum at the time of planting.

For Existing Trees: Two hundred grams of AM fungal inoculum is required for inoculating one tree. Apply inoculum near the root surface at the time of fertilizer application.

AM Fungi and Plant Disease Control

The colonization of plant roots by AM fungi generally helps reduce the severity of diseases caused by plant pathogens. The reduced damage in mycorrhizal plants may result from several factors, including changes in root growth and morphology, histopathological alterations in the host root, physiological and biochemical adjustments within the plant (Jung *et al.*, 2012), improved host nutrition, modifications in microbial populations within the mycorrhizosphere, competition for colonization sites and photosynthates, activation of defence mechanisms, and the potential for AM fungi to parasitize nematodes (Sylvia and Chellemi, 2001). Effective bio protection against plant diseases is typically the cumulative outcome of all these mechanisms, either working independently or in concert. However, challenges to achieving biocontrol through AM fungi include their obligate nature, limited understanding of the specific mechanisms involved, and the influence of environmental factors on these interactions.

Kehri (2018) reported that AM fungi are infrequently present in commercial nurseries, primarily due to the use of composted, soil-free media, heavy fertilizer application, and frequent fungicide treatments. The potential benefits of AM fungi in horticulture, agriculture, and forestry are often not fully recognized by these industries. This lack of recognition may be partly attributed to the challenges associated with large-scale inoculum production and the absence of effective methods for its widespread use.

Cropping systems, fertilization practices, and plant pathogen management strategies significantly influence the presence and effectiveness of AM fungal propagules in soil (Vosátka *et al.*, 2012). To successfully integrate AM fungi into sustainable agricultural practices, it is crucial to understand how factors such as fertilizer application, pesticide use, and soil management affect these fungi (Kankam *et al.*, 2021). Furthermore, identifying and utilizing efficient AM fungal inoculants as biofertilizers, bioprotectants, and bio stimulants is essential for enhancing sustainability in agriculture and forestry.

AM Fungi in Sustainable Agriculture

Arbuscular Mycorrhizal Fungi (AMF) play a crucial role in sustainable agriculture by enhancing plant growth, improving soil health, and reducing dependency on chemical fertilizers. AMF establishes symbiotic relationships with plant roots, aiding in nutrient absorption and increasing stress resistance. This review discusses the potential of AMF in sustainable farming practices (Pozo *et al.*, 2007). AMF are known to improve nutrient uptake, particularly phosphorus, which is essential for plant growth but often limited in agricultural soils. The fungi extend their hyphae into the soil, increasing the root surface area and facilitating nutrient absorption. Studies have shown that AMF associations enhance nitrogen, potassium and micronutrient uptake, reducing the need for synthetic fertilizers (Cameron *et al.*, 2013).

AMF contribute to soil aggregation and stability by producing glomalin, a glycoprotein that binds soil particles. This improves soil porosity, aeration, and water retention, making soils more resilient to erosion and degradation. Additionally, AMF interactions promote microbial diversity, which enhances nutrient cycling and organic matter decomposition, essential for long-term soil fertility. (Eckardt *et al.*, 2022) Plants colonized by AMF exhibit increased tolerance to biotic and abiotic stresses, including drought, salinity, and pathogen attacks. AMF improves water absorption and regulates stress-related gene expression, reducing plant susceptibility to extreme environmental conditions. Furthermore, AMF can suppress soil-

borne pathogens by competing for space and resources, offering a natural alternative to chemical pesticides (Lops, 2023).

Integrating AMF in sustainable agriculture can reduce reliance on chemical inputs while maintaining high crop productivity. Sustainable practices such as reduced tillage, cover cropping, and organic amendments enhance AMF abundance and diversity. Farmers adopting AMF-based biofertilizers benefit from improved crop yield, soil health, and reduced environmental impact. (Putra *et al.*, 2020) AMF plays a vital role in sustainable agriculture by enhancing nutrient uptake, improving soil health and increasing plant resilience to stress. Future research should focus on optimizing AMF applications to maximize benefits across different cropping systems (Koskey *et al.*, 2021c).

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RESEARCH ARTICLE

Mechanisms of Biochemical Basis of Resistance Against Whitefly *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae) in Bhendi Accessions

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Abstract

A study was undertaken to analyze the biochemical basis of resistance in bhendi accessions as influenced by inorganic nutrients against *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae). Two bhendi accessions namely, Salem Local and Arka Anamika were used for this study. Mean nymphal population was minimum in K alone treated plants followed by NPK applied plants of both the accessions. Nymphal populations were higher in Arka Anamika when compared to Salem Local in both preliminary and confirmatory field screenings. Among the biochemical factors, the total phenol concentration was maximum in leaves of Salem Local treated with Potassium. The reducing and non-reducing sugar concentrations were maximum in leaves of Arka Anamika irrespective of the treatments. Total sugars concentration was found to be minimum in Salem Local irrespective of the treatments. Regarding nitrogen content, leaves of Salem Local accession had minimum quantity, irrespective of all the treatments. Phosphorus and potassium content were comparatively higher in the leaves of Salem Local than in Arka Anamika.

Keywords: *Bhendi accessions, Biochemical factors and B.tabaci*

Introduction

Bhendi or Okra, *Abelmoschus esculentus* L. (Moench) (Malvaceae) is an economically important vegetable crop grown in tropical and sub-tropical parts of the world (Thiruveni, 2012). As high as 72 species of insects have been recorded on bhendi (Srinivasa Rao and Rajendran, 2003). The whitefly, *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae), causes severe damage to bhendi plants by feeding on sap, secreting honeydew and transmitting virus diseases (Jose and Usha, 2003). *Bemisia. tabaci* involved in creating multiple problems for the crop by direct sucking the sap from the leaves and indirectly involving in transmission of yellow vein mosaic virus. Both nymphal and adult stages are equally responsible for severe damage. The ideal way to solve the pest problem is by Integrated Pest Management (IPM), Host plant resistance is the main basic component of IPM and the utilization of resistant plants has long been considered as one of the most effective components of insect control. Resistance is a result of one or more mechanisms involving different morphological traits of the host plant and biochemical contents of plant which affect the biology and behaviour of phytophagous insects those feed on the plants. Keeping this in mind, the present study was conducted with an objective, to study the biochemical bases of resistance against shoot and fruit borer *Earias vitella.*

Materials and Methods

Source of bhendi accessions

Based on preliminary and confirmatory field screening of 38 bhendi accessions for resistance against the shoot and fruit borer, *Earias vittella,* a promising accession namely Salem Local was selected (Karthik, 2015) for further studies. For comparison, a susceptible check, Arka Anamika was also evaluated.

Field screening

Two bhendi accessions were sown in plot of 2.8 x 1.2 m size with a spacing of 60 x 45cm. A randomized block design with three replications was adopted. Weekly observations on populations of whitefly nymphs per plant were recorded in five plants per treatment. Three leaves respectively from the top, middle and

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bottom portions of plants in each plant were selected and the nymphs were counted using a hand lens. In comparison a susceptible check Arka Anamika was also evaluated. The influence of inorganic nutrients on inducing resistance in the bhendi accessions against *B.tabaci* was studied. The details on various inorganic inputs used in the present study are furnished hereunder.

Table-1 : Effect of treatments with nutrient combinations, dosage per plot, timing of applications and method of soil application.

S. No	Treatments	Dosage/Plot	Day of application	Method of application
1	N alone (split dose application)	166 g (50%)	ODS	Soil
		83 g (25%)	30 DAS	Soil
		83 g (25%)	60 DAS	Soil
2	P alone	462 g	ODS	Soil
3	K alone	122 g	ODS	Soil
4	**Combination of NP** N (split dose application)	166 g (50%)	ODS	Soil
		83 g (25%)	30 DAS	Soil
		83 g (25%)	60 DAS	Soil
	P	462 g	ODS	Soil
5	**Combination of PK** P	462 g	ODS	Soil
	K	122 g	ODS	Soil
6	**Combination of NK** N (split dose application)	185 g (50%)	ODS	Soil
		92 g (25%)	30 DAS	Soil
		92 g (25%)	60 DAS	Soil
	K	122 g	ODS	Soil
7	**Combination of NPK** N (split dose application)	185 g (50%)	ODS	Soil
		92 g (25%)	30 DAS	Soil
		92 g (25%)	60 DAS	Soil
	P	462 g	ODS	Soil
	K	122 g	ODS	Soil

ODS – On the day of sowing; DAS – Days after sowing

Estimation of total phenols

One ml of the extract was pippeted out in a graduated test tube. To this 1ml of folin-ciocalteu reagent was added, followed by 2ml of sodium carbonate solution. The tubes were shaken and heated in boiling water for exactly 1 minute. The tubes were cooled under running water and solution was diluted to 25ml with distilled water. Then absorbance was measured at 650 nm in a spectrophotometer. The unknown values were read from a standard curve made from different concentrations of catechol. The blank containing all the reagents without leaf extracts was used to adjust the absorbance to zero (Bray and Thorpe, 1954).

Estimation of reducing sugars

Twenty-five parts of reagent A and 1 part of reagent B

were mixed. 1 ml of this mixture was added to 1ml of the extract and heated for 20 minutes in a hot water bath. The tubes were cooled in running water and 1ml of arsenomolybadate reagent was added. The volume was diluted to 25ml and the colour was read in a spectrophotometer at 495nm against a reagent blank. Sterile water with reagent served as control. Standards prepared from glucose were used to get a standard curve the unknowns were calculated. (Nelson-Somogyi, 1944)

Estimation of non-reducing sugars

Non reducing sugars were estimated by the method followed by 1 ml of alcohol extract was pipetted out in a test tube and evaporated the contents to dryness in a water bath. Added 1ml of glass distilled water and 1ml of 1N H_2SO_4. Then the mixture was hydrolysed is heating at 49^0 C for 30 minutes. The acid hydrolysis is effective in splitting the sucrose type linkages. Then the contents were neutralized by adding 1N NaOH drop by drop from a pipette and then the Nelson's method was followed to estimate the sugar content. The absorbance was measured at 610nm in spectrophotometer and the non-reducing sugar were estimated from standard curve (Mahadevan and Sridhar, 1986).

Estimation of total nitrogen

Leaves were chopped and dried in an oven at 60^0C for 3 days one gram of the dried material was transferred to a 50 ml micro- Kjeldahl's flask. A pinch of the digestion mixture (prepared by mixing 10 parts of K_2SO_4, 1 part of $CuSO_4$ and 0.1 part of selection metal power) was added followed by 4 ml of a mixture of concentrated H_2SO_4 and salicylic acid (1g of salicylic acid to 30 ml of concentrated H_2SO_4) and a few crystals of sodium thiosulphate. The contents were digested by heating for 2-3 hours till clear bluish green colour appeared. The flask was removed. Cooled and transferred to micro- Kjeldahl's distillation unit with 3-4 washing. About 25 ml of 40 per cent NaOH was added and ammonia was steam distilled for 10 minutes in 0.1 N H_2SO_4 containing 1 or 2 drops of methyl red indicator. Distillation was continued till all the ammonia evolved the contents were back titrated against 0.1N KOH till the golden yellow colour appeared the volume of H_2SO_4 utilized was calculated by employing the factor 1 ml of 0.1N H_2SO_4 (Bremmer, 1960).

Results and Discussion

The host plant resistant to insect is governed by several biochemical factors. The host-plant may deficient in certain nutritional elements which are required by the insect and hence prove resistant. The nutritionally deficient plant may show antibiotic and antixenoitic effects on the insect. The antibiosis may result from the absence of certain nutritional substances in the host plant and/or an imbalance of available nutrients.

Mean nymphal population was minimum in K alone treated plants followed by NPK applied plants of both the accessions. Nymphal populations were higher in Arka Anamika when compared to Salem Local in both preliminary and confirmatory field screenings. Among the biochemical factors, the total phenol concentration was maximum in leaves of Salem Local treated with Potassium. The reducing and non-reducing sugar concentrations were maximum in leaves of Arka Anamika irrespective of the treatments. Total sugars concentration was found to be minimum in Salem Local irrespective of the treatments. Regarding nitrogen content, leaves of Salem Local accession had minimum quantity, irrespective of all the treatments. Phosphorus and potassium content were comparatively higher in the leaves of Salem Local than in Arka Anamika.

Biochemical contents of host plants have direct impact on the inset attraction and its subsequent infestation (Annathakrishnan, 1996). In the present Study, total phenol content was maximum in accession Salem Local. Reducing sugars, non-reducing sugars and total sugars content were maximum in accession Arka Anamika. Accession Salem Local was least preferred by *Benisia tabaci*. In the present study, phenol content of leaves exerted a negative correlation with nymphal population of *Benisia tabaci*. Phenolics in a fairly large concentration could have direct toxicity to the insects. These findings are in close conformity to fruit borer may be correlated with higher amount phenol content in fruits. Goplakrishnan (2006) stated that phenol content was higher in resistant tomato accession and it exerted a significant negative correlation with larval feeding. Further, Slansky (1990) also reported that reducing and non-reducing sugars had positive correlation with fruit infestation; Taylo and Bernardo (1996) also reported that free sugar may lead to greater attraction and fecundity of hoppers in bhendi.

Conclusion

Bhendi resistant accessions Salem Local show a close relationship of high phenol concentration with low nymphal population, indicating that phenol plays a significant role in the accession resistance to *B. tabaci*.

High phenol content is closely associated to low damage indicating the biochemical protected the plant against *Benisia tabaci*.

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RESEARCH ARTICLE

Efficacy of New Generation Herbicides on Yield and Economics of Direct Seeded Rice

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Abstract

A field experiment was conducted during *kuruvai* season of 2020 to evaluate the efficacy of new generation herbicides on yield and economics of direct seeded rice. The major weeds found were *Echinochloa crus-galli, Echinochloa colonum* among grasses, *Cyperus rotundus, Cyperus difformis* among sedges and *Bergia capensis, Eclipta alba* among broad-leaved weeds. The results revealed that, early post emergence application of bispyribac sodium (200ml/ha) followed by (*fb*) one hand weeding on 40 DAS recorded significantly lower weed population at 30 and 60 DAS, lower weed dry matter production at 30 and 60 DAS, higher weed control index and higher grain yield (5695 kg/ha), net returns (Rs.68568/ha) and higher returns per rupee invested (Rs.2.43).

Keywords: *Direct seeded rice, weed, new generation herbicides, yield and economics*

Introduction :

Rice (*Oryza sativa* L.) is commonly known as Asian rice belongs to family Poaceae. The world's total rice area is 167 million ha and production is about 782 million tonnes with productivity of 4.67 t/ha. Among the various rice growing countries across the globe, India is second largest producer and consumer of rice after China. In India, it is in cultivation since ages and is grown over an area of about 43.37 million ha with annual production of about 115.60 million tonnes and average productivity of 3.96 t/ha (FAOSTAT 2018). Transplanting of rice seedlings in the puddled field is the traditional method of rice cultivation followed in India. Such a rice cultivation requires large quantity of water and labour source to nursery preparation and management, pulling out seedlings, transporting and distribution of seedlings to main field. In this rice production system consumes about 150 hectare centimeter of water and engagement of more workers for transplanting and weeding (Mahajan and Chauhan, 2016). Direct seeded rice is gaining popularity in India due to acute labour shortage during the peak period of transplanting and shortage of water. Direct seeding of rice refers to the process of establishing the crop from seeds sown in the field rather than by transplanting seedlings from the nursery. Weeds are major problem in direct seeded rice due to simultaneous germination of crop and weed, which exerts competition from the beginning of the crop for nutrients and space. It adversely affects the yield of direct seeded rice due to poor establishment of rice seedlings. In India yearly loss of rice grain production is around 15 million tonnes due to heavy weed infestation (Singh *et al.*, 2018). The predominant weed species present under direct seeded rice situation were *Echinochloa colonum* (L.), *Echinochloa crus-galli* (L.) under grasses, *Cyperus rotundus* (L.) and *Cyperus difformis* (L.) under sedges, *Bergia capensis* and *Eclipta alba* (L.) among broad-leaved weeds. Critical period of crop weed competition in rice is influenced by different rice establishment methods *viz.*, transplanted rice (20-40 DAT), wet seeded rice (15-60 DAS), dry seeded rice (15-60 DAS), rainfed direct seeded rice (0-90 DAS), upland direct seeded rice 30 DAS (Arunbabu and Jena 2018). Pre emergence application of penoxsulam + butachlor reduced the density of *Echinochloa crusgalli* and *Ammania baccifera* (Yadav *et al.*, 2019). Application of bispyribac-sodium is a systemic herbicide absorbed by roots and leaves and also inhibits the enzyme acetolactate synthases in susceptible weed plants (Pathak *et al.*, 2011). Fenoxaprop-p-ethyl was most efficient to control *Echinochloa crus-galli* which predominantly inhibits the synthesis of fatty acids in the meristematic tissues of the grassy weeds (Rana *et al.*, 2014). Considering the above facts, field experiment was conducted to study the efficacy of new generation herbicides on yield and economics of direct seeded rice.

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Table-1 : Efficacy of new generation herbicides on total weed population/m^2, weed dry matter production (kg/ha) on 30 and 60 DAS, weed control index in direct seeded rice

Treatments	Total weed population/m^2		weed dry matter production (kg/ha)		weed control index*
	30 DAS	60 DAS	30 DAS	60 DAS	
T_1 - Unweeded check	10.03 (100.71)	12.65 (160.20)	210.31	361.52	----
T_2 - Twice hand weeding on 20 and 40 DAS	4.88 (23.81)	5.68 (32.65)	40.32	58.18	83.90
T_3 - Pre emergence application of penoxsulam + butachlor (0.97% w/w + 38.8% w/w SE) @ 2000 ml/ha on 7 DAS	7.38 (54.54)	9.67 (93.60)	83.78	146.50	55.98
T_4 - Early post emergence application of bispyribac sodium 10% SC @ 200 ml/ha on 15 DAS	4.64 (21.52)	7.99 (63.88)	36.13	137.25	59.47
T_5 – Post emergence application of fenoxaprop-p-ethyl 6.9% EC (6.7% w/w) @ 875 ml/ha on 30 DAS	8.35 (69.77)	10.48 (109.87)	129.25	159.13	62.03
T_6 - Pre emergence application of penoxsulam + butachlor (0.97% w/w + 38.8% w/w SE) @ 2000 ml /ha on 7 DAS fb (followed by) twice hand weeding on 20 and 40 DAS	4.87 (23.71)	5.61 (31.54)	37.13	55.08	84.76
T_7 - Early post emergence application of bispyribac sodium 10% SC @ 200 ml/ha on 15 DAS fb hand weeding on 40 DAS	4.02 (16.34)	4.48 (20.16)	29.44	33.59	90.70
T_8 - Hand weeding on 20 DAS fb post emergence application of fenoxaprop-p-ethyl 6.9% EC (6.7% w/w) @ 875 ml/ha on 30 DAS	5.33 (28.43)	6.56 (43.06)	47.37	69.01	80.91
T_9 - Pre emergence application of penoxsulam + butachlor (0.97% w/w + 38.8% w/w SE) @ 2000 ml/ha on 7 DAS fb early post emergence application bispyribac sodium 10% SC @ 200 ml/ha on 15 DAS	6.84 (46.81)	7.34 (53.99)	65.89	76.13	78.94
T_{10} - Pre emergence application of penoxsulam + butachlor (0.97% w/w + 38.8% w/w SE) @ 2000 ml/ha on 7 DAS fb post emergence application of fenoxaprop-p-ethyl 6.9% EC (6.7% w/w) @ 875 ml/ha on 30 DAS	6.92 (47.89)	7.43 (55.29)	66.48	76.90	78.72
T_{11} - Early post emergence application of bispyribac sodium 10% SC @ 200 ml/ha on 15 DAS fb post emergence application of fenoxaprop-p-ethyl 6.9% EC (6.7% w/w) @ 875 ml/ha on 30 DAS	4.41 (19.50)	4.98 (24.88)	32.81	41.74	88.45
SEm±	0.07	0.11	1.10	1.44	
CD (p=0.05)	0.22	0.33	3.28	4.30	

*Figures in parenthesis are original values, *Not analysed statistically*

Materials and Methods

The experiment was conducted during *Kuruvai* season of 2020 at Pattathikadu village, Karambakudi taluk, Pudukkottai district, Tamilnadu. The soil of the field was sandy clay loam and neutral in pH (6.2), EC (0.08 ds/m), medium in organic carbon content, low in nitrogen, medium in phosphorus and potassium. The experiment consists of eleven treatments comprising of unweeded check, weed control methods *viz.*, twice hand weeding on 20 and 40 DAS, pre emergence application of penoxsulam + butachlor (0.97% w/w + 38.8% w/w SE) @ 2000 ml/ha on 7 DAS, early post emergence application of bispyribac sodium 10% SC @ 200 ml/ha on 15 DAS, post emergence application of fenoxaprop-p-ethyl 6.9% EC (6.7% w/w) @ 875 ml/ha on 30 DAS, pre emergence application of penoxsulam + butachlor (0.97% w/w + 38.8% w/w SE) @ 2000 ml/ha on 7 DAS fb twice hand weeding on 20 and 40 DAS, early post emergence application of bispyribac sodium 10% SC @ 200 ml/ha on 15 DAS fb hand weeding on 40 DAS, Hand weeding on 20 DAS fb post emergence application of fenoxaprop-p-ethyl 6.9% EC (6.7% w/w) @ 875 ml/ha on 30 DAS, pre emergence application of penoxsulam + butachlor (0.97% w/w + 38.8% w/w SE) @ 2000 ml/ha on 7 DAS fb early post emergence application of bispyribac sodium 10% SC @ 200 ml/ha on 15 DAS, pre emergence application of penoxsulam + butachlor (0.97% w/w + 38.8% w/w SE) @ 2000 ml/ha on 7 DAS fb post emergence application of fenoxaprop-p-ethyl 6.9% EC (6.7% w/w) @ 875 ml/ha on 30 DAS, early post emergence application of bispyribac sodium 10% SC @ 200 ml/ha on 15 DAS fb post emergence application of fenoxaprop-p-ethyl 6.9% EC (6.7% w/w) @ 875 ml/ha on 30 DAS. The experiment was laid out in randomized block design with three replications. Pre germinated seeds of short duration rice variety ADT 37 was sown at 15 x 10 cm spacing on well puddled and leveled field with a seed rate of 40 kg/ha. The crop was fertilized with 120:40:40 kg $N:P_2O_5:K_2O$/ha. The entire quantity of phosphorus was applied as basal dose in all the plots. Nitrogen and potassium fertilizers were applied in three equal splits at basal, tillering and panicle initiation stages of crop. Pre emergence herbicides applied at 7 days after sowing, early post emergence applied at 15 days after sowing and post emergence applied at 30 days after sowing as per treatment. Herbicides were sprayed with flat fan nozzle with 500 litres volume of water per hectare using knapsack sprayer. The data on weed density and weed dry weight (at 30 and 60 DAS) were recorded with the help of quadrate (0.5 x 0.5 m). The normality of distribution was not seen in case of observation on weeds hence, the values were subjected to square root transformation ($\sqrt{x}$+0.5) prior to statistical analysis to normalize their distribution. Data on grain yield and straw yield were recorded. The weed control index was worked out on the basis of weed dry matter production using the formula suggested by (Misra and Tosh, 1979). All the data obtained in the study were statistically analyzed using F-test, the procedure given by (Gomez and Gomez, 1984), critical difference values at p=0.05 were used to determine the significance of differences between means.

The net return was calculated by deducting the cost of cultivation from the gross income. The return rupee^{-1} invested was calculated by dividing the gross return to the total cost of cultivation (Rs/ha).

Results and Discussion

Weed population and dry matter production

Among the treatments, lower total weed population (16.34 and 20.16 weeds/m^2 at 30 and 60 DAS respectively) were recorded with early post emergence application of bispyribac sodium 10% SC @ 200 ml/ha on 15 DAS fb hand weeding on 40 DAS. It was followed by early post emergence application of bispyribac sodium 10% SC @ 200 ml/ha on 15 DAS fb post emergence application of fenoxaprop-p-ethyl 6.9% EC (6.7% w/w) @ 875 ml/ha on 30 DAS. The higher weed control index (90.70) was recorded with the early post emergence application of bispyribac sodium 10% SC @ 200 ml/ha on 15 DAS fb hand weeding on 40 DAS (Table 1).

This might be due to the fact that the better placement of herbicides and the better effect of herbicide in controlling the emerging weeds let to suppression of weeds from the beginning. There was no phyto toxicity symptom observed during the observation by using the herbicide bispyribac sodium 10% SC on 15 DAS. This result is also in conformity with the findings of (Ghosh *et al* 2013). Among the weed control treatments, lower weed dry matter production (29.44 and 33.59 kg/ha at 30 and 60 DAS respectively) were recorded with early post emergence application of bispyribac sodium 10% SC @ 200 ml/ha on 15 DAS fb hand weeding on 40 DAS. This might also be due to good aeration of soil and least weed population observed which reduced the crop weed competition for soil moisture, plant nutrients, space and solar radiation during active growth period. This is in line with the findings of (Shah *et al.*, 2020).

Table-2 : Efficacy of new generation herbicides on yield (kg/ha) and economics in rice in direct seeded rice

Treatments	Grain yield (kg/ha)	Straw yield (kg/ha)	Net Income (Rs/ha)	Returns/ rupee invested
T_1 - Unweeded check	1860	2529	-4,088	0.90
T_2 - Twice hand weeding on 20 and 40 DAS	4913	6141	52,090	2.07
T_3 - Pre emergence application of penoxsulam + butachlor (0.97% w/w + 38.8% w/w SE) @ 2000 ml/ha on 7 DAS	2823	3782	13,052	1.28
T_4 - Early post emergence application of bispyribac sodium 10% SC @ 200 ml/ha on 15 DAS	3245	4283	22,250	1.49
T_5 – Post emergence application of fenoxaprop-p-ethyl 6.9% EC (6.7% w/w) @ 875 ml/ha on 30 DAS	2471	3335	5,635	1.12
T_6 - Pre emergence application of penoxsulam + butachlor (0.97% w/w + 38.8% w/w SE) @ 2000 ml/ha on 7 DAS fb twice hand weeding on 20 and 40 DAS	5075	6293	52,610	2.02
T_7 - Early post emergence application of bispyribac sodium 10% SC @ 200 ml/ha on 15 DAS fb hand weeding on 40 DAS	5695	6892	68,568	2.43
T_8 - Hand weeding on 20 DAS fb post emergence application of fenoxaprop-p-ethyl 6.9% EC (6.7% w/w) @ 875 ml/ha on 30 DAS	4545	5772	44,841	1.92
T_9 - Pre emergence application of penoxsulam + butachlor (0.97% w/w + 38.8% w/w SE) @ 2000 ml/ha on 7 DAS fb early post emergence application bispyribac sodium 10% SC @ 200 ml/ha on 15 DAS	3829	4901	31,298	1.65
T_{10} - Pre emergence application of penoxsulam + butachlor (0.97% w/w + 38.8% w/w SE) @ 2000 ml/ha on 7 DAS fb post emergence application of fenoxaprop-p-ethyl 6.9% EC (6.7% w/w) @ 875 ml/ha on 30 DAS	3616	4700	25,975	1.53
T_{11} - Early post emergence application of bispyribac sodium 10% SC @ 200 ml/ha on 15 DAS fb post emergence application of fenoxaprop-p-ethyl 6.9% EC (6.7% w/w) @ 875 ml/ha on 30 DAS	5470	6673	64,193	2.34
SEm±	72.30	107.4		
CD (p=0.05)	214.75	319		

Grain and straw yield

Early post emergence application of bispyribac sodium 10% SC @ 200 ml/ha on 15 DAS fb hand weeding on 40 DAS recorded higher grain and straw yield of 5695 kg/ha and 6892 kg/ha respectively (Table 2). Early post emergence application of bispyribac sodium 10% SC @ 200 ml/ha on 15 DAS fb post emergence application of fenoxaprop-p-ethyl 6.9% EC (6.7% w/w) @ 875 ml/ha on 30 DAS was next in order. The increased grain yield with early post emergence application of bispyribac sodium 10% SC @ 200 ml/ha on 15 DAS fb hand weeding on 40 DAS might be due to timely control of weeds in critical period of crop weed competition has enhanced the availability of nutrients, light and moisture to the crop and also increase the crop yield with timely application of these broad spectrum herbicides combination. The similar results reported by (Rao *et al.*, 2019) and (Pusdekar *et al.*, 2020). The higher straw yield was ascribed to weed management practices provided favorable environment and enhanced the growth of rice crop which in turn was reflected in terms of straw yield. The results are in harmony with the findings of (Parameswari and Srinivas, 2014). The lower grain yield in unweeded check might be due to season long weed competition exerted by the weeds at the critical stages of crop growth. These results are in conformity with that of (Guru *et al.*, 2020).

Economics

Early post emergence application of bispyribac sodium 10% SC @ 200 ml/ha on 15 DAS fb hand weeding on 40 DAS (T_7) registered the higher net income of 68,568 Rs/ha and return/rupee invested of 2.43 (Table 2). It might be due to timely control of weeds, with timely application of herbicides has increased the yield which saves the labour cost and reduce cost of cultivation and higher monitory benefit. These results are in conformity with the findings of (Veeraputhiran and Balasubramanian, 2013). Unweeded check (T_1) recorded the lowest net income and return/rupee invested (0.90). It might be due to more weed population leads to lower gross income, net income and returns/rupee invested. Similar results were reported by (Sunil *et al.*, 2016) and (Das *et al.*, 2017).

Conclusion

From the present study, it can be concluded that weeds are the major constraints in direct seeded rice system which may results in severe losses in terms of yield and economic returns. Hence, the early post emergence application of herbicide is a must for direct seeded rice and the application of bispyribac sodium 10% SC @ 200 ml/ha on 15 DAS fb one hand weeding on 40 DAS was found to be the ideal combination for managing the weeds under direct seeded condition with higher grain yield and economic returns.

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REVIEW ARTICLE

Integrating Biotechnology and Artificial Intelligence in Vegetable Crop Improvement: A Comprehensive Review

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Abstract

The convergence of biotechnology and artificial intelligence (AI) is revolutionizing vegetable crop improvement by addressing the challenges of climate change, population growth and resource scarcity. This review explores the integration of AI-driven biotechnological advancements in genetic enhancement, precision agriculture, disease management and yield optimization. CRISPR-Cas9, coupled with AI-based predictive modelling, enhances genome editing precision, facilitating the development of stress-resilient and nutritionally superior vegetable varieties. Machine learning (ML) techniques, including deep learning models, improve genotype-to-phenotype predictions, accelerate breeding programs and reduce reliance on extensive field trials. AI-powered precision agriculture, encompassing smart irrigation systems, autonomous drones, and robotics, optimizes resource use, minimizes environmental impact, and enhances crop productivity. Computer vision-based disease detection and predictive analytics further contribute to sustainable pest and disease management, reducing chemical dependency and yield losses. Additionally, AI-driven models improve yield forecasting and soil health monitoring, ensuring informed decision-making in agricultural planning. Despite the transformative potential of AI and biotechnology, challenges such as data accessibility, affordability, and ethical considerations hinder widespread adoption. The review highlights the need for affordable AI solutions, enhanced data-sharing frameworks, and multi-stakeholder collaborations to bridge the technological divide between large-scale agribusinesses and smallholder farmers. Addressing these challenges will be crucial for utilizing AI and biotechnology to build a resilient, sustainable, and food-secure agricultural sector. Future research should focus on developing scalable AI-driven solutions tailored to diverse farming systems, ensuring equitable access to technology. By integrating cutting-edge innovations in biotechnology and AI, the agricultural industry can enhance vegetable crop resilience, productivity, and nutritional quality, ultimately contributing to global food security.

Keywords: *Artificial Intelligence, Biotechnology, CRISPR-Cas9, Precision Agriculture, Sustainable vegetable production.*

Introduction

Global challenges such as climate change, population growth, and resource scarcity are significantly impacting agricultural productivity and sustainability. Climate change, characterized by rising temperatures, erratic precipitation patterns, and increased frequency of extreme weather events, poses a serious threat to crop yields and food security (FAO, 2021). Simultaneously, the rapid growth of the global population, projected to reach 9.7 billion by 2050, necessitates a substantial increase in food production to meet rising demands (United Nations, 2019). Additionally, resource scarcity, including the depletion of arable land and water resources, further constrains agricultural expansion and intensification (Tilman *et al.*, 2017).

Vegetable crops play a crucial role in human nutrition by providing essential vitamins, minerals, and

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antioxidants necessary for health and well-being (Bouis and Saltzman, 2017). However, the increasing environmental and demographic pressures demand continuous improvement in vegetable crop resilience, productivity, and nutritional value. Traditional breeding methods, while effective, are often time-consuming and limited by genetic variability. In contrast, modern biotechnological advancements, including genetic engineering, genome editing, and molecular marker-assisted selection, have revolutionized crop improvement by enabling precise trait modifications for enhanced stress tolerance, yield potential, and nutritional enhancement (Gao, 2021).

Furthermore, artificial intelligence (AI) has emerged as a transformative tool in agriculture, offering data-driven insights and automation for optimizing crop management. AI applications, such as machine learning, image recognition, and predictive analytics, facilitate real-time monitoring of crop health, early detection of diseases, and precision agriculture practices to maximize resource efficiency (Kamilaris and Prenafeta-Boldú, 2018). The integration of biotechnology and AI in vegetable crop research and production presents a promising avenue for addressing contemporary agricultural challenges, ensuring sustainable food systems, and enhancing global food security.

Genetic Enhancement through AI-Assisted Biotechnology

CRISPR and AI in Genome Editing

CRISPR-Cas9 technology has revolutionized genome editing by enabling precise modifications in plant genomes, facilitating the development of crops with enhanced traits such as disease resistance, drought tolerance, and improved nutritional value (Zhu *et al.*, 2020). This technology allows targeted gene knock outs, insertions, and modifications, making it a powerful tool for genetic improvement. However, optimizing CRISPR-based editing requires precise target site selection to minimize off-target effects and maximize efficiency.

Artificial intelligence (AI) significantly enhances the CRISPR-Cas9 process by predicting gene functions, identifying optimal target sites, and improving editing outcomes through advanced computational models (Liu *et al.*, 2021). AI-driven tools, such as deep learning models and neural networks, analyze vast genomic datasets to identify functional gene regions and predict the likelihood of successful edits (Xu *et al.*, 2022). These AI algorithms can also evaluate potential off-target mutations, thereby improving the precision and reliability of genome editing in vegetable crops.

For instance, AI-powered CRISPR design tools, such as Deep-CRISPR and CRISPR-Net, have been employed to enhance target specificity and efficiency (Kim *et al.*, 2020). These tools integrate machine learning to analyze genomic sequences, predict guide RNA efficiency, and suggest optimal editing strategies for improving vegetable crop traits. By combining AI with CRISPR-based genome editing, researchers can accelerate the development of resilient, high-yielding, and nutritionally superior vegetable varieties, addressing global agricultural challenges more effectively.

Machine Learning in Genotype-to-Phenotype Predictions

Understanding the complex relationship between genotype and phenotype is crucial for crop improvement, as it allows for the prediction and selection of desirable traits such as yield, disease resistance, and stress tolerance (Huang *et al.*, 2021). Traditionally, this process has relied on quantitative genetics and statistical models, which, although effective, often struggle to capture the intricate interactions between genes and environmental factors.

Machine learning (ML) models, particularly deep learning techniques, have emerged as powerful tools in predicting phenotypic traits from genotypic data with high accuracy. ML algorithms, such as convolutional neural networks (CNNs), long short-term memory (LSTM) networks, and autoencoders, can analyze vast genomic datasets, detect patterns, and make predictions that aid in crop breeding programs (López *et al.*, 2021). These models help in identifying complex gene interactions, epistatic effects, and genotype-environment interactions,

thereby enhancing precision breeding strategies.

For instance, a study by Khaki *et al.*, (2020) demonstrated the application of an LSTM autoencoder-based model in predicting flowering time and grain yield in barley, highlighting its potential for vegetable crop improvement. The model effectively processed high-dimensional genomic data, learning meaningful representations that improved prediction accuracy. Such approaches can be extended to vegetable crops to enhance breeding programs aimed at increasing resilience, nutritional value, and productivity.

By utilizing ML-driven genotype-to-phenotype predictions, researchers can expedite the breeding process, reducing dependency on lengthy field trials while ensuring the selection of superior vegetable crop varieties tailored to diverse climatic conditions and consumer demands.

Precision Agriculture and Crop Management

AI-Driven Smart Irrigation Systems

Efficient water management is essential for sustainable vegetable production, especially in the face of increasing water scarcity and climate variability. Traditional irrigation methods often lead to water wastage or insufficient watering, affecting crop health and yield. AI-powered smart irrigation systems provide a solution by optimizing water usage through real-time analysis of soil moisture levels, weather forecasts, and plant water requirements (Dutta *et al.*, 2021).

These systems integrate Internet of Things (IoT) sensors, machine learning (ML) algorithms, and cloud computing to make data-driven irrigation decisions. For instance, ML models can analyze historical and real-time environmental data to predict optimal watering schedules, ensuring efficient water distribution while minimizing losses (Aydin *et al.*, 2022). AI-driven irrigation systems have been successfully implemented in vegetable farming, leading to significant reductions in water consumption while maintaining or even improving crop yields (Sharma *et al.*, 2021).

Furthermore, predictive analytics enables proactive water management by forecasting drought conditions and adjusting irrigation accordingly. Such advancements contribute to resource-efficient agriculture, reducing environmental impact while enhancing productivity and sustainability.

Autonomous Drones and Robotics

The integration of AI-driven drones and robotics has transformed precision agriculture, improving efficiency in various farming operations such as planting, monitoring, and harvesting. AI-powered drones equipped with high-resolution cameras and multispectral sensors can capture detailed images of crop fields, enabling early disease detection, plant health assessment, and targeted interventions (Zhang *et al.*, 2022).

In cashew farming, AI-driven drones have been used for automated pest and disease detection, facilitating targeted pesticide application and reducing chemical usage (Sarker *et al.*, 2021). Similar applications can be adapted for vegetable crops, where AI-powered drones can monitor plant stress, detect nutrient deficiencies, and ensure precise input application. Autonomous robots, such as robotic harvesters and weed control machines, further enhance efficiency by reducing labor dependency and minimizing crop losses during harvesting (Bac *et al.*, 2021).

By utilizing AI and robotics, vegetable farming can achieve higher precision, lower production costs, and improved environmental sustainability, addressing labor shortages and enhancing food security.

Disease Detection and Management

Computer Vision for Disease Identification

Early detection of plant diseases is essential for minimizing crop losses and ensuring food security. Traditional disease detection methods rely on manual scouting, which can be time-consuming, labor-intensive, and prone to human error. AI-driven computer vision systems offer an efficient alternative by utilizing deep learning models to identify disease symptoms from images captured by cameras, smartphones, or drones (Ferentinos, 2018).

Deep learning-based mobile applications have been

developed to assist farmers in diagnosing plant diseases. For example, a convolutional neural network (CNN)-powered mobile app was designed to detect tomato diseases with high accuracy, providing real-time identification and suggesting potential remedies (Mohanty *et al.*, 2016). Such AI-powered tools make disease diagnostics more accessible, particularly for smallholder farmers who may lack access to agricultural extension services.

Furthermore, hyperspectral and multispectral imaging, combined with AI algorithms, can detect plant diseases at an early stage—before visible symptoms appear—allowing for timely intervention (Singh *et al.*, 2021). This technology is particularly useful in vegetable crop management, where early disease control can significantly enhance yield and quality.

Predictive Analytics for Pest and Disease Outbreaks

Machine learning (ML) models play a crucial role in forecasting pest and disease outbreaks by analyzing historical data, weather conditions, and other environmental factors. These predictive models enable farmers to implement proactive disease management strategies, reducing crop damage and reliance on chemical pesticides (Jeong *et al.*, 2020).

For instance, AI-driven predictive analytics has been applied to forecast late blight outbreaks in potatoes and tomatoes, using climatic variables such as humidity and temperature (Shahhosseini *et al.*, 2019). By integrating satellite imagery and real-time sensor data, these models can detect early warning signs of potential disease outbreaks, allowing for targeted preventive actions.

By reducing dependency on chemical treatments, predictive analytics supports sustainable agriculture, minimizing environmental impact while ensuring crop health. The application of such models in vegetable crops can lead to significant improvements in disease management efficiency, reducing economic losses for farmers.

Yield Optimization and Resource Management

AI in Yield Prediction

Accurate yield prediction is essential for optimizing agricultural planning, resource allocation, and food security. Traditional yield forecasting methods rely on historical data and empirical models, which often fail to capture complex interactions between environmental conditions, soil properties, and crop genetics. AI-driven approaches, particularly machine learning (ML) and deep learning models, have significantly improved yield prediction accuracy by integrating diverse datasets, including remote sensing imagery, climate data, and soil parameters (Liakos *et al.*, 2018).

Remote sensing data collected from satellites, drones, and ground-based sensors are processed using ML algorithms to analyze vegetation indices, crop growth patterns, and environmental stress factors (Sharma *et al.*, 2022). A critical review by Kamilaris and Prenafeta-Boldú (2018) emphasized the role of AI and the Internet of Things (IoT) in agricultural decision-making, highlighting how predictive analytics enhances crop yield forecasts and supports precision farming strategies.

For instance, deep learning models such as recurrent neural networks (RNNs) and convolutional neural networks (CNNs) have been used to predict wheat and maize yields with high accuracy by integrating multispectral images and weather datasets (Jiang *et al.*, 2020). These AI-driven methods can be effectively applied to vegetable crops, enabling farmers to make data-driven decisions that optimize productivity and reduce risks.

Soil Health Monitoring

Soil health is a critical factor in sustainable agriculture, influencing crop yield, nutrient availability, and long-term land productivity. Conventional soil testing methods are often labor-intensive and time-consuming, limiting their scalability. AI-based soil health monitoring systems have emerged as a transformative solution by utilizing data from soil sensors, genomic analysis, and remote sensing to provide real-time insights into soil fertility and microbiome composition (Basu *et al.*, 2021).

AI-powered platforms such as Trace Genomics use advanced machine learning techniques to analyze soil microbiomes, detect nutrient deficiencies, and predict

disease risks, helping farmers make informed decisions regarding fertilization and soil amendments (Bhattacharyya *et al.*, 2022). Additionally, AI models trained on soil quality datasets can predict soil degradation trends and recommend sustainable land management practices to enhance soil resilience (Lal, 2020).

By integrating AI and IoT in soil health monitoring, farmers can adopt precision fertilization strategies, optimize input use, and maintain soil fertility, ultimately improving vegetable crop yields while minimizing environmental impact.

Challenges and Future Perspectives

The integration of biotechnology and artificial intelligence (AI) in vegetable crop improvement presents transformative opportunities for enhancing yield, resilience, and sustainability. However, several challenges must be addressed to ensure widespread adoption and equitable benefits.

Challenges

One of the primary challenges is data privacy and security. AI-driven agricultural solutions rely on large-scale data collection from farms, including soil health parameters, crop performance metrics, and climate data. Ensuring that this data is securely stored and ethically managed is crucial to gaining the trust of farmers and stakeholders (Wolfert *et al.*, 2017). Additionally, proprietary data ownership by large agritech companies raises concerns about accessibility and fair usage rights for smallholder farmers (Carbonell, 2016).

Another major limitation is the need for extensive and high-quality datasets. AI models require large, diverse, and well-annotated datasets for accurate predictions and decision-making. However, in many regions, agricultural data is either scarce, fragmented, or not standardized, which hinders the effectiveness of AI applications (Kamilaris and Prenafeta-Boldú, 2018). Investments in open-access databases and improved data-sharing frameworks are essential for advancing AI-driven agricultural research.

Furthermore, technological accessibility and affordability for smallholder farmers remain a concern. While developed nations and large agribusinesses have the resources to implement AI-powered solutions, small-scale farmers in developing countries often face financial, infrastructural, and technical barriers (Wossen *et al.*, 2017). Bridging this digital divide requires scalable, cost-effective AI solutions tailored to resource-constrained agricultural systems.

Future Perspectives

To fully harness the potential of AI and biotechnology in vegetable crop improvement, future research and policy efforts should focus on the following areas:

1. Development of Affordable AI Solutions

Researchers and technology developers should prioritize creating AI tools that are cost-effective, user-friendly, and compatible with low-resource farming environments. Mobile-based AI applications, simplified machine learning models, and cloud-based platforms can enhance accessibility for smallholder farmers (Kritikos, 2020).

2. Enhancing Data-Sharing Frameworks

Establishing open-source agricultural data platforms will enable researchers, policymakers, and farmers to collaborate and share insights effectively. Public-private partnerships can facilitate the development of standardized data-sharing policies while protecting data ownership rights (Wolfert *et al.*, 2017).

3. Fostering Multi-Stakeholder Collaborations

Bridging the gap between research institutions, industry stakeholders, and policymakers is crucial for scaling AI-driven innovations. Governments should support AI research in agriculture through funding initiatives and policy incentives, while private companies can contribute technological expertise and infrastructure (Goggin and Browne, 2020).

4. Ethical and Sustainable AI Implementation

As AI adoption increases, ethical considerations regarding bias in AI models, environmental sustainability, and responsible data usage must be addressed. Developing transparent AI models that prioritize fairness and ecological balance will be key to

long-term success (van der Burg *et al.*, 2019).

By addressing these challenges and fostering innovation, AI and biotechnology can significantly contribute to sustainable vegetable crop production, ensuring global food security in the face of climate change and population growth.

Conclusion

The integration of biotechnology and artificial intelligence (AI) is transforming vegetable crop improvement by offering innovative solutions to agricultural challenges. Advances in genome editing, like CRISPR-Cas9, and AI-driven predictive modelling have accelerated the development of resilient, high-yielding vegetable varieties. AI-powered precision farming techniques, such as smart irrigation, autonomous drones, and computer vision-based disease detection, optimize resource use and enhance crop health. However, fully realizing these technologies requires overcoming challenges like data accessibility, affordability, and ethical considerations. Collaborative efforts among researchers, policymakers, and industry stakeholders are essential to ensure AI-driven biotechnological applications are accessible across diverse agricultural systems. As demand for nutritious, high-quality food rises, embracing AI and biotechnology in vegetable crop improvement will be crucial for building a resilient agricultural sector. Future research should prioritize scalable and inclusive AI-driven solutions benefiting both large agribusinesses and smallholder farmers, advancing agriculture toward a sustainable, food-secure future.

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REVIEW ARTICLE

Impact of Heavy Metals Contamination in the Environment and the Importance of Bioremediation Approach

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Abstract

Over the past decade, human activities have led to a notable surge in the concentration of heavy metals in the environment. These highly electronegative and toxic substances are notorious for causing a range of issues, including habitat loss, neurological problems, and cancer. Therefore, it is imperative to conduct a thorough evaluation of the impact of heavy metals on the environment. While traditional methods have proven effective in remediating heavy metal-contaminated soils, they are not without limitations. Chemical and physical approaches can often produce by-products such as toxic sludge or pollutants and are not economically efficient and are not cost-effective, while biological processes is beneficial economically as it not produce secondary sludge and are characterized by slow, cost effective and time-consuming remediation. This article delves into the potential of all three major bioremediation approaches and underscores the capacity of biological bioremediation to potentially revolutionize the mitigation of heavy metal toxicity in the environment.

Keywords: *Heavy metals; Bioremediation; Environmental toxicity; Pollutants*

Introduction

Heavy metals are naturally present in the Earth's crust since its beginning. However, the significant increase in the utilization of heavy metals has led to a notable rise in metallic substances in both terrestrial and aquatic environments (Tchounwou *et al.*, 2012). Anthropogenic activities are the primary contributors to heavy metal pollution, stemming mainly from metal mining, smelting, foundries, and other metal-based industries. Additionally, metals leach into the environment from various sources like landfills, waste dumps, animal excrement, and construction activities. (Liu *et al.*, 2021).Agricultural practices also contribute to secondary heavy metal pollution through the use of pesticides, insecticides, fertilizers, and other chemicals. Natural phenomena such as volcanic eruptions, metal corrosion, evaporation of metals from soil and water, sediment resuspension, soil erosion, and geological weathering can further exacerbate heavy metal contamination (Briffa *et al.*, 2020). These activities have led to ecological imbalance and a continuous increase in soil pollution over the years. Consequently, land degradation has become a growing concern, with larger areas of land being affected and deteriorating over time (Zhang and Wang, 2020). The environmental contamination has been intensified by the industrial revolution and human activities. Significant discharge of pollutants into the oceans poses immense threats to coastal ecosystems. Heavy metals (HMs), due to their chronic toxicity, non-biodegradability, and ability to bioaccumulate in the environment, are particularly harmful pollutants (Mishra *et al.*, 2019). Heavy metals can be transferred and biomagnified through food chains, posing serious risks to human health. Elevated levels of heavy metals in marine ecosystems are directly linked to environmental contamination. Additionally, the growth and development of fish can be negatively impacted by a diet rich in heavy metals. Growth inhibition in fish is one of the most noticeable signs of metal toxicity (Aziz *et al.*, 2023).

Effect of Heavy Metals on Environment

A heavy metal is characterized by its high density and potential toxicity, particularly in environmental contexts. Heavy metals are significant environmental pollutants, and their increasing toxicity poses ecological, evolutionary, nutritional, and environmental concerns. These metals and metalloids have an atomic density greater than 4 g/cm^3, or are at least five times denser than water (Tchounwou *et al.*, 2012). While plants require certain heavy metals for growth and maintenance, excessive amounts can be toxic. The ability of plants to accumulate essential metals also enables them to take up nonessential

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metals. Since metals are non-degradable, elevated concentrations within plants can adversely affect them both directly and indirectly (Das *et al.*, 2023). Direct toxic effects of high metal concentrations include the inhibition of cytoplasmic enzymes and damage to cell structures due to oxidative stress. The detrimental impact of heavy metals on soil microorganisms can indirectly impede plant growth and development. Elevated concentrations of heavy metals can reduce the population of beneficial soil microorganisms, leading to a decrease in the decomposition of organic matter (Briffa *et al.*, 2020). This reduction in organic matter decomposition can result in soil fertility decline. Both direct and indirect toxic effects of heavy metals can hinder plant growth, ultimately leading to plant death. Thus, the presence of high levels of heavy metals in soil can have cascading negative effects on soil health, microbial activity, and plant growth (Asati *et al.*, 2016). Toxic sediments laden with heavy metals can also kill benthic organisms, reducing food availability for larger organisms (Tchounwou *et al.*, 2012). Prolonged exposure to high concentrations of heavy metals can deplete energy and adversely affect vital organs such as the brain, lungs, kidneys, liver, and blood. Long-term exposure to heavy metals can result in degenerative physical, tissue, and neurological processes, mimicking diseases like Alzheimer's, Parkinson's, muscular dystrophy, and multiple sclerosis (Zaynab *et al.*, 2022). Non-essential metals can exert toxic effects even at low concentrations. Unlike essential metals, these non-essential metals are not metabolized into intermediate compounds and do not break down in the environment. Heavy metals in ecosystems create a contamination chain that operates cyclically through various stages: industry, atmosphere, soil, water, food, and ultimately, humans. Humans can be exposed to heavy metals through multiple routes, including contaminated food and water, skin contact, and inhalation (Khalef *et al.*, 2022). In soil, elevated concentrations of heavy metals can negatively impact various aspects of plant systems, such as seed germination, plant growth, production, and physiological, biochemical, and genetic elements (Bharti and Sharma, 2022). When heavy metals infiltrate the soil, they enhance the mineralization of organic matter, leading to adverse alterations in the soil's absorption complex by displacing calcium and magnesium ions. These results in decreased enzymatic activity and a reduction in the population of beneficial microorganisms (Lubal, 2024).Concurrently, fungal populations may proliferate, while the activity of various enzymes, such as catalase, is inhibited. These changes degrade soil fertility and compromise its self-purification capabilities. Lead is a significant environmental pollutant and a well-known toxic substance. Among the multitude of modern toxicants, lead stands out due to its pronounced toxicity (Swain, 2024). Chronic lead poisoning is characterized by symptoms such as anemia, intestinal colic, and a dark line along the gums known as a "lead line." Initial symptoms of lead poisoning may include increased excitability and insomnia, which can progress to fatigue and depression. In medical practice, lead poisoning is sometimes misdiagnosed and may be treated as a mental disorder (Luo, 2024). More than 95% of atmospheric lead emissions come from vehicle exhaust. Approximately 90-95% of the lead present in the human body accumulates in the bones, posing a significant risk of chronic intoxication. Lead can also be transferred to infants through breast milk. Individuals at higher risk of lead exposure and its associated health effects include newborns, pregnant women, children, individuals with kidney disease, and those with anemia (Nachona'a, 2019) .

Lead primarily affects the hematopoietic, nervous, digestive and renal systems. It contributes to the development of atherosclerosis and impairs motor coordination, while also causing abnormalities in red blood cell formation. Cadmium plays a role in regulating blood sugar metabolism (El Ati-Hellal and Hellal, 2021). However, excessive intake of cadmium, due to its high chemical reactivity, can replace calcium in bone tissue, resulting in weakened and brittle bones. Elevated cadmium levels in food can lead to widespread dental problems in children. Thus, the presence of heavy metals in the environment poses serious ecological and public health concerns (Baibotayeva *et al.*, 2019). Mercury, an extremely hazardous heavy metal, is found in the biosphere and has become a widespread contaminant due to human activities, leading to increased atmospheric levels. Mercury transforms into the highly toxic methylmercury upon contact with aquatic sediments. Methylmercury enters the human body through the food chain, primarily via fish, seafood, and wildlife that have ingested toxic microorganisms (Mitra *et al.*, 2022). It then enters the bloodstream and causes various neurological problems. Manganese, the most abundant toxic heavy metal, exists in various oxidation states in nature. During the combustion of methylcyclopentadienyl manganese tricarbonyl

Table-1 : Various biomolecules involved into the bioremediations of heavy metals.

Sr.No.	Enzyme	Mode of action	Heavy metals	References
1	Cytochrome P450	• Performs electron transfer reactions and catalysis by reduction or oxidation of heme iron. Utilizes pyridine nucleotides as electron donors producing carbon substrates and oxidized products. • $NAD(P)H + O_2 + R \longrightarrow NAD(P)^+ + RO + H_2O$	Cr	(Bhandari *et al.*, 2021). (Mousavi *et al.*, 2021).
2	Laccase	• Reduction of the O_2 molecule, including the oxidation of one electron with a wide range of aromatic compounds. • Oxidation, decarboxylation and demethylation of substrate.	Cd and Cu	(Karigar and Rao, 2011). (Bhandari *et al.*, 2021).
3	Dehalogenase	Mainly occurred through three mechanisms: (1) Hydrolytic mechanism: water molecule serves as a cofactor; halogen substituent is replaced in SN reaction by the hydroxyl group (2) Oxygenlytic mechanism: catalyzed by mono/dioxygenase incorporating one/two atoms of molecular oxygen into the substrate (3) Reductive mechanism: it is related to the carbamide family; in this course, halogen is substituted by hydrogen under aerobic conditions, where organohalides are used as the terminal electron acceptors.	Pb, Cr, As and Zn	(Bhandari *et al.*, 2021). (Saravanan *et al.*, 2021).
4	Dehydrogenase	Catalyze the reactions with coenzymes such as $NAD^+/NADP^+$ or flavin such as FAD and FMN as an electron acceptor. It transfers two hydrogen atoms from organic compounds to electron acceptors.	Cd	(Jaworska and Lemanowicz, 2019). (Bhandari *et al.*, 2021). (Ayilara and Babalola, 2023).
5	Hydrolase	In triglyceride hydrolysis, one-mole triglyceride (T) reacts with three moles of water (W) to produce one-mole glycerol (G), and three-mole fatty acids (P) peptide bond of protein is broken down by hydrolyzing.	Cd	(Bhandari *et al.*, 2021). (Balali-Mood *et al.*, 2021).
6	Protease	• Catalyze the breakdown of peptide bonds of proteins. • Enzymes that hydrolyze peptide bonds in aqueous environment.	Hg and Zn	(Karigar and Rao, 2011). (Bhandari *et al.*, 2021). (Mousavi *et al.*, 2021).
7	Lipase	• The transfer of a proton between the aspartate, the histidine, and the serine residues of the lipase followed by hydroxyl residue of the serine attacks the carbonyl group of the substrate. In the deacylation step, nucleophile attacks the enzyme regenerating the enzyme and releasing the product. • The hydrolysis of triacylglycerols to glycerols and free-fatty acids.	Fe	(Karigar and Rao, 2011). (Jaworska and Lemanowicz, 2019). (Bhandari *et al.*, 2021).
8	Monooxygenase	Incorporation of oxygen atom to substrate and utilize substrate as reducing agent. Desulfurization, dehalogenation, denitrification, ammonification, and hydroxylation of substrate	Co, Cu, Cr, Fe andMg	(Karigar and Rao, 2011). (Engwa *et al.*, 2019).

9	Dioxygenase	Introduction of two oxygen atom to the substrate results in intradiol cleaving and extradiol cleaving with the formation of aliphatic product	As, Pb, Cd, Hg andNi	(Karigar and Rao, 2011). (Ayilaraand Babalola, 2023).
10	Lignin peroxidase	Oxidation of substrate in the presence of cosubstrate H_2O_2 and mediator like veratryl alcohol.	Fe, Cu, Cd, Pb, Ni and Cr	(Jaworska and Lemanowicz, 2019). (Karigar and Rao, 2011).
11	Manganese peroxidase	In the presence of Mn^{2+} and H_2O_2 the co-substrate catalyses oxidation of Mn^{2+} to Mn^{3+} which results in an Mn^{3+} chelateoxalate, which in turn oxidizes the phenolic substrates.	Fe and Mn	(Karigar and Rao, 2011). (Mousavi *et al.*, 2021).
12	Versatile peroxidase	The enzyme catalyzes the electron transfer from an oxidizable substrate, with the formation and reduction of compound I and compound II intermediates.	Mn	(Karigar and Rao, 2011). (Verma and Kuila 2019).
13	Cellulase	Hydrolyses the substrate to simple carbohydrates.	Pb, Cd, Cu	(Karigar and Rao, 2011). (Tayang and Songachan, 2021).

(MMT), an additive in gasoline, manganese oxides are released into the air. Although manganese is essential for various physiological functions, excessive intake can lead to significant toxicity (Rashid *et al.*, 2023). Cobalt, abundant in the environment and used to make alloys, can be both beneficial and harmful to humans. While small amounts typically have no adverse effects, massive environmental discharges can be fatal. Nickel, a naturally abundant element with extensive industrial applications, is emitted into the atmosphere from both natural and anthropogenic sources. Inhalation of nickel-contaminated air can lead to allergies, nasal and lung cancer, and kidney and cardiovascular diseases (Nyiramigisha, 2021). Antimony poisoning can result in physiological deficiencies, including pancreatitis, cardiotoxicity, and respiratory problems like pleural adhesions, chronic emphysema, chronic bronchitis, respiratory irritation, and inactive tuberculosis. It is also carcinogenic and affects reproduction. Industrial emissions significantly contribute to the increase in atmospheric thallium levels. Thallium exposure is extremely harmful to humans, causing severe health issues (Mitra *et al.*, 2022). Excessive exposure to copper has been associated with cellular damage, leading to Wilson disease in humans. In biological systems, heavy metals have been found to impact cellular organelles and components such as the cell membrane, mitochondria, lysosomes, endoplasmic reticulum, nuclei, and various enzymes involved in metabolism, detoxification, and damage repair (Ali *et al.*, 2019).

Effect of Heavy Metals on Marine

Marine ecosystems are intricate and dynamic, characterized by numerous internal and external interactions that can change over time. Pollutants entering coastal waters and estuaries pose significant challenges, causing extensive harm to aquatic life and potentially leading to mass mortality events. Among these pollutants, the accumulation of heavy metals in marine ecosystems is a global concern (Ansari *et al.*, 2004). Once in the aquatic environment, these metal contaminants usually exist in soluble or suspended forms and eventually settle at the bottom or are absorbed by organisms (Aziz *et al.*, 2023). The gradual and irreversible accumulation of these metals in the organs of marine organisms can lead to metal-related diseases over time due to their toxicity, posing a threat to aquatic biota and other organisms. The bioaccumulation of trace elements in living organisms and their biomagnification within food chains describe the processes and pathways of these potential pollutants from one trophic level to another. This highlights the organisms' ability to accumulate higher concentrations of these substances, further amplifying the risks associated with heavy metal contamination in marine ecosystems (Baby *et al.*, 2010). Under optimal conditions, with appropriate temperature and sufficient food availability, fish tend to increase in both

body length and mass. However, in water contaminated with toxicants, such as heavy metals, fish growth may be stunted. Growth inhibition is a prominent symptom of the toxic effects of metals on fish larvae. Therefore, the body length and mass of fish can serve as indicators of environmental conditions (Lakshmanna *et al.*, 2022). The highest concentrations of heavy metals are typically found in the kidneys and livers of various fish species. Contaminated sediments can pose a threat to benthic organisms, exposing worms, crustaceans, and insects to harmful levels of toxic chemicals. Some toxic sediments can kill benthic organisms, reducing food availability for larger animals like fish (Bandara and Manage *et al.*, 2022). Additionally, when water pH decreases due to acidic rainfall or other acidic episodes, heavy metals can be mobilized and released into the water column, becoming toxic to aquatic biota. Low concentrations of heavy metals can induce chronic stress in fish. While this may not necessarily kill individual fish, it can lead to reduced body weight and smaller size, thereby diminishing their ability to compete for food and habitat (Shah, 2021). Heavy metals in water pose a significant threat to fish juveniles and can substantially reduce fish populations or even lead to the extinction of entire fish populations in polluted reservoirs. Laboratory studies on common carp larvae exposed to water containing lead or copper revealed slowed development and growth rates, as well as reduced survival rates. Exposure to copper inhibited skeletal ossification, while lead exposure resulted in scoliosis. When common carp were exposed to heavy metals, concentrations of red blood cells, blood glucose, and total cholesterol in the fish significantly increased. Serum iron and copper levels also rose. Exposure of three main carp species (Catlacatla, Labeorohita, and Cirrhinamrigala) to sub-lethal concentrations of manganese for 30 days resulted in negative growth in terms of weight. In fish, the toxic effects of heavy metals can impact physiological functions, individual growth, reproduction and mortality (Khayatzadeh and Abbasi 2010).

Effect of Heavy Metals on Agriculture

Prolonged and excessive use of fertilizers leads to the accumulation of heavy metals in agricultural soils, which diminishes soil fertility and subsequently hampers plant growth and productivity. The health of humans is closely intertwined with the health of agricultural ecosystems, making the pollution of these systems by heavy metals a global concern (Oladoye *et al.*, 2022). For most people, dietary intake is the primary route of exposure to heavy metals, aside from occupational exposures in related industries. There have been notable incidents of heavy metal poisoning through the food chain, such as the "Itai-Itai" disease in Japan during the 1930s and the "minamata disease" in Japan during the 1950s (Qin *et al.*, 2021). It's widely recognized that agricultural soils contaminated with heavy metals, as well as atmospheric deposition of metals, can lead to elevated levels of these metals in crops. Industrial effluents, if left untreated, are often discharged into open water bodies like lakes, canals, and rivers, eventually making their way into the oceans. Unfortunately, this polluted water is frequently used by farmers for irrigation purposes, either intentionally or unintentionally, leading to soil contamination (Srivastava *et al.*, 2017). Pollution of agricultural ecosystems by heavy metals often stems from sources like wastewater irrigation, solid waste disposal, vehicle emissions, fertilization, and industrial activities (Liu *et al.*, 2014). The transportation of metal ions across cellular membranes in plant roots allows metals to enter plant tissues. Initially, metals are absorbed into the apoplast, which is a free intercellular space that leads to the xylem in the roots. Heavy metals are then translocated apoplastically into the plant tissue through the continuous pathway of the root epidermis and cortex (Rashid *et al.*, 2023). Vegetables can become contaminated with heavy metals through both absorption from the soil and deposition from polluted air. Heavy metal pollution not only negatively impacts various parameters related to plant quality and yield but also alters the size, composition, and activity of the microbial community in the soil. Soil properties such as organic matter content, clay content, and pH play crucial roles in determining the extent of the effects of metals on biological and biochemical properties. Heavy metals indirectly influence soil enzymatic activities by changing the microbial community responsible for enzyme synthesis (Mohanty and Das 2023). They exhibit toxic effects on soil biota by disrupting key microbial processes and reducing the number and activity of soil microorganisms. Cadmium (Cd) is more detrimental to enzymes than lead (Pb) due to its higher mobility and lower affinity for soil colloids. Copper (Cu) inhibits β-glucosidase activity more than cellulase activity. Lead significantly decreases the activities of enzymes like urease, catalase, invertase, and acid phosphatase (Sandeep *et al.*, 2019). Since metals cannot be broken down, elevated concentrations within plants can have

detrimental effects both directly and indirectly. Direct toxic effects of high metal concentrations include the inhibition of cytoplasmic enzymes and damage to cell structures due to oxidative stress. Indirect toxic effects involve the displacement of essential nutrients at cation exchange sites within plants (Khan *et al.*, 2013). These toxic effects, both direct and indirect, result in decreased plant growth and, ultimately, can lead to the death of the plant (Asati *et al.*, 2016). Heavy metal pollution in agricultural soils tends to be more severe and complex in peri-urban areas due to their exposure to multiple emission sources. Different cropping systems can also influence the uptake of heavy metals from the soil into plants, leading to varying health risks for residents through the food chain (Tóth *et al.*, 2016).In peri-urban areas with diverse cropping systems, traditional ecological evaluation methods, such as Nemerow's synthetic pollution index (Pn) and the Potential Ecological Risk Index (RI), which are solely based on heavy metal concentrations in soils, may not provide a reliable comprehensive assessment of heavy metal pollution. It is essential to quantify and compare the health risks to residents associated with the consumption of food from different cropping systems. This will enable the development of effective management strategies to maintain food safety and protect human health in these areas (Huang *et al.*, 2018). Some heavy metals, such as As, Cd, Hg, Pb, and Se, are not essential for plant growth as they do not serve any known physiological function in plants. On the other hand, elements like Co, Cu, Fe, Mn, Mo, Ni, and Zn are essential for the normal growth and metabolism of plants. However, excessive concentrations of these essential elements can lead to poisoning. The uptake of heavy metals by plants and their subsequent accumulation along the food chain pose potential threats to animal and human health. For instance, in Beta vulgaris (spinach), the uptake and accumulation of Cd, Zn, Cr, and Mn were found to be higher during the summer season, whereas Cu, Ni, and Pb accumulated more during the winter season (Srivastava *et al.*, 2017). During the summer, the relatively high decomposition rate of organic matter may release heavy metals into the soil solution, making them more available for plant uptake. The increased uptake of heavy metals like Cd, Zn, Cr, and Mn during the summer may also be attributed to higher transpiration rates due to elevated temperatures and lower humidity compared to the winter season. Heavy metals can be toxic to plants, leading to phytotoxicity, which manifests as chlorosis, weak plant growth, yield reduction, and may even result in reduced nutrient uptake, disruptions in plant metabolism, and decreased ability to fix molecular nitrogen in leguminous plants (Singh and Kalamdhad, 2011).

Overview of remediation approaches -

The remediation of heavy metal-contaminated sites can be conducted in-situ or ex-situ, either on-site or off-site, and may involve biological, physical, and chemical methods. Additionally, these techniques are often combined to achieve more cost-effective and efficient remediation of ecosystems contaminated with heavy metals (Madhuppriya *et al.*, 2020). Various physical methods have been reported to remove heavy metals from polluted systems by utilizing the physicochemical properties of the metals. These methods include adsorption, electrokinetic techniques, membrane filtration, granular activated carbon, photocatalysis, and soil washing (Kumar *et al.*, 2021).

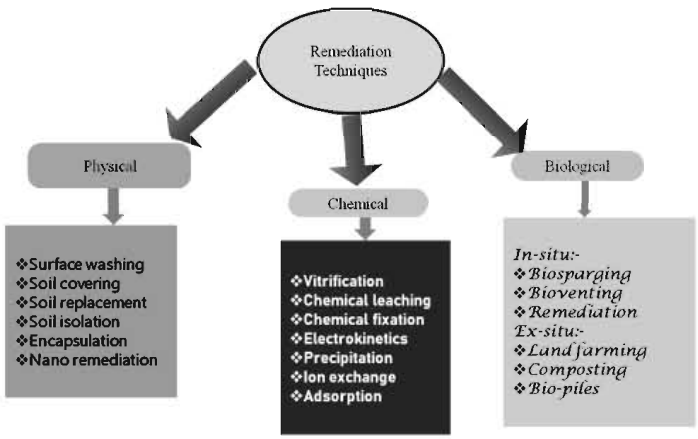

Fig. 1 : Overview of various remediation techniques

The chemical processes for removing heavy metals include chemical precipitation, flotation, ion exchange, coagulation, and flocculation. While these techniques are effective, the extensive use of chemicals can lead to challenges in sludge disposal and the potential for secondary pollution problems (Yadav *et al.*, 2023). Bioremediation is a promising technology that uses microbes or their enzymes to detoxify harmful metals into less harmful forms, thereby purifying contaminated environments. This approach is environmentally friendly and cost-effective, as it leverages natural processes to restore the environment (Akhtar *et al.*, 2020).

A. Physical approach

i. Coagulation

Coagulation and flocculation can be employed to treat wastewater contaminated with heavy metals (Vardhan *et al.*, 2019). By adding coagulants such as aluminum or ferrous sulfate, colloids are destabilized and form aggregates. Flocculation employs polyelectrolytes to combine particles into larger clusters. Commonly used flocculants include polyferric sulfate, aluminum sulfate, and polyacrylamide. These aggregates can then be removed through sedimentation and filtration processes (Vareda, *et al.*, 2019). Coagulation involves reducing the overall surface charge of colloidal particles to stabilize them through the process of electrostatic repulsion; essentially, it refers to the neutralization of particle charges. This process requires the introduction of coagulants and/or dissolved organic matter to form larger aggregates. Common coagulants used include aluminum sulfate (alum), polyaluminium chloride (PACL), magnesium chloride ($MgCl_2$), aluminum hydroxide oxides, polyethyleneimine (PEI), among others (Tang *et al.*, 2016). Flocculation, on the other hand, involves gently mixing the destabilized particles to promote collisions and interactions, leading to an increase in particle size. This is facilitated by inorganic and dissolved organic polymers. Once the smaller particles have aggregated into larger ones, they can be easily separated through filtration, flotation, or straining. Chitosan, a biopolymer, serves as an environmentally friendly option for coagulation and flocculation due to its biodegradability (Yadav *et al.*, 2021).

ii. Filtration

Membrane filtration methods have demonstrated outstanding efficacy in removing heavy metals from wastewater. Membranes consist of intricate structures with dynamic components at the nanoscale. In modern reverse osmosis systems, the membranes typically consist of uniform polymer thin films supported by a permeable backing (Qasem *et al.*, 2021). The permeability of water through the membrane and the rejection of heavy metal ions largely depend on the chemical and physical characteristics of the membrane. The primary advantages of this method are its high removal efficiency, minimal space requirements, and ease of operation (Vardhan *et al.*, 2019). Ultrafiltration (UF) membranes possess pores that are larger than hydrated cations and low molecular weight solutes. To effectively retain dissolved heavy metals, surfactant micelles that bind to cations or polymers that form complexes with these metals are introduced into the effluent. This results in structures that are captured by the membrane. Nanofiltration (NF) can serve as an alternative for certain cations such as nickel, chromium, and arsenic. The membranes utilized in NF are charged, and their unique steric (size exclusion) and electrical (Donnan exclusion) properties enable them to reject charged solutes that are smaller than the membrane's pores (Vareda *et al.*, 2019).This method offers several advantages, such as high efficiency in removing contaminants, achieving a high flux rate, and requiring low energy consumption. However, a notable drawback is the relatively high operating expenses associated with it (Yadav *et al.*, 2021).

iii. Ion exchange

The ion-exchange process is widely used for removing heavy metals from wastewater due to its high removal efficiency, substantial treatment capacity, and fast kinetics. In this method, ions (either cations or anions) in the solution are exchanged with ions of the same type on an insoluble material, known as an ion-exchange resin (Qasem *et al.*, 2021). The heavy metal-contaminated wastewater enters the ion-exchange column at one end and passes through the bed, which effectively removes the heavy metals. When the column becomes saturated with heavy metals, it is backwashed to eliminate the accumulated contaminants, after which the column is regenerated for reuse (Vardhan *et al.*, 2019). The ion exchange process is employed to replace cations or anions present in contaminants. In this approach, undesirable heavy metal ions are substituted with other cations that are typically non-polluting. Co-contaminated soil can also be remediated using an appropriate substrate (soil) solution and a cation exchange matrix. The heavy metal cations in the soil are exchanged with the cations in the matrices, ensuring a balanced charge transfer (Sharma *et al.*, 2018). Ion exchange employs resins to reversibly swap ions between the resin (solid phase) and the effluent being treated (liquid phase). To eliminate heavy metals, cation-exchange resins are required. There are selective resins available that make this process suitable for recovering valuable metals (Vareda *et al.*, 2019).Ion exchange is a cost-effective technique utilizing inexpensive materials and simple operation procedures. This method attracts soluble ions from the liquid phase to the solid phase. It has demonstrated efficiency in removing heavy metals

from aqueous solutions, particularly when dealing with low concentrations of metals. Due to the sensitivity of the matrix, this method is not suitable for removing high concentrations of metal ions (Yadav *et al.*, 2021).

B. Chemical approach

Chemical remediation involves using chemical reagents, reactions, and principles to eliminate contaminants. Major remediation technologies encompass solidification/stabilization, vitrification, soil flushing, soil washing, and electrokinetic. Solidification/stabilization involves mixing contaminated soils with reagents or materials to reduce the mobility of heavy-metal contaminants (Yadav *et al.*, 2021). Vitrification, or the conversion to molten glass, is a form of solidification/stabilization that requires high thermal energy (1400–2000°C). This is achieved by blending the contaminated soil with glass-forming precursors, heating the mixture until it becomes liquid, and then cooling to produce a homogeneous amorphous glass. Soil flushing and washing are effective remediation techniques that utilize water or a suitable washing solution to extract contaminants from the soil (Li *et al.*, 2019). Soil replacement involves using uncontaminated soil to replace or partially replace the contaminated soil. The objective is to dilute the heavy metal concentrations in the soil, increase the soil's environmental capacity, and thereby remediate the soil. Vitrification is a high-temperature process where organic matter is incinerated and mineral matter is melted, resulting in the encapsulation of metals/metalloids in a small volume of vitreous material (Yadav *et al.*, 2021). The electro kinetics technique uses an electric field gradient of appropriate intensity across an electrolytic tank filled with saturated soil. Electrodes are typically placed in constructed wells filled with an electrolytic solution. Under the influence of the electric field, target metal ions migrate towards the oppositely charged electrodes. The contaminants gathered at the electrodes can then be treated using various physical-chemical methods, such as electroplating, precipitation/co-precipitation, pump-and-treat near the electrodes, or sorption with ion-exchange resins (Qasem *et al.*, 2021). Soil solidification entails encapsulating waste materials in a monolithic solid with high structural integrity. Conversely, soil stabilization involves amending contaminated soil with chemical reagents to transform leachable chemicals into physically and chemically more stable forms. This often entails chemical interactions between the target heavy metals and the binding agents. Soil washing refers to the leaching of heavy metals from the soil matrix using various reagents or extractants, including water, inorganic acids, organic acids, chelating agents, and surfactants (Gong *et al.*, 2018). Soil replacement entails the partial or total removal of soil contaminated with metals, followed by the introduction of clean soil. This method is often referred to as the "dig-and-haul" approach. The soil washing process can be conducted *in-situ* by pushing a washing solution through the soil matrix or *ex-situ* by physically digging up the soil and washing it in reactors. Vitrification technology involves subjecting the soil to high temperatures (1400-2000°C) to decompose or volatilize organic matter and produce vitreous materials like solid oxides (Roy Chowdhury *et al.*, 2018). Soil replacement can be executed through two methods : (i) Soil spading: In this method, the contaminated site is excavated deeply, and the heavy metals or metalloids are dispersed into these deeper layers. This approach aims to dilute the metal concentrations. (ii) New soil importing: This involves adding uncontaminated soil to the polluted area. The added soil can either be layered on the surface or mixed into the existing soil to reduce the metal concentration (Waris *et al.*, 2018). In *in-situ* vitrification, an electric current is applied to the soil by inserting a series of electrodes vertically into the contaminated area. However, dry soil might not offer sufficient conductivity for effective vitrification. *Ex-situ* vitrification involves several stages, including excavation, mixing, pretreatment, melting, feeding, and casting of the molten product. Soil electrokinetic remediation operates based on the principle of establishing an electric field gradient of appropriate intensity across an electrolytic tank filled with saturated, contaminated soil (Berdimurodov *et al.*, 2023). Heavy metals or metalloids in the soil are separated through processes like electrophoresis, electric seepage, or electro-migration, thereby reducing contamination levels. Soil washing refers to the process of removing heavy metals or metalloids from the soil using various reagents and extractants (Khalid *et al.*, 2017).

i. Precipitation

Chemical precipitation is a commonly employed method for removing heavy metals from wastewater due to its cost-effectiveness and ease of operation. In this process, the pH of the wastewater is initially adjusted to basic conditions, following which a precipitating agent is introduced. This agent reacts

with the heavy metal ions present in the wastewater, leading to the formation of insoluble precipitates. These precipitates can then be separated from the water through sedimentation or filtration processes (Vardhan *et al.*, 2019). Chemical precipitation typically takes place at a basic pH, generally ranging from 9 to 11. During this treatment process, any associated organic contaminants also undergo alkaline hydrolysis (Sharma *et al.*, 2018). Chemical precipitation is a commonly used and straightforward method for treating wastewater. By introducing a precipitation agent to the effluent, cations in the solution react to form insoluble species that then precipitate out. Typically, this precipitation occurs through hydroxide precipitation, where agents like lime are used to elevate the pH of the effluent. Alternatively, sulfides can be employed, as metal sulfides generally have lower solubility compared to their corresponding metal hydroxides (Lewis, 2017). However, chemical precipitation results in the generation of a significant volume of sludge, which necessitates expensive treatment and disposal methods. Additionally, this approach can increase the concentration of salts in the wastewater, making it unsuitable for disposal according to regulatory standards (Vareda *et al.*, 2019). Primarily valued for their efficiency, cost-effectiveness, precise process control, and suitability across a broad temperature range, these methods come with certain drawbacks. These include the requirement for significant lime and peroxide dosages, as well as the associated expenses of managing sludge (Yadav *et al.*, 2021).

ii. Extraction

This method involves the addition of chemical agents to increase the solubility of metals, thereby bringing them into the aqueous phase to facilitate their extraction or removal from the system. Various types of inorganic elements, chelating agents, and surfactants are commonly employed in this approach. Chelating agents have the capability to form bonds with a variety of metals, making them widely utilized in physical, chemical, and biological remediation processes (Rauret, 1998). Synthetic chelating agents like EDTA, diethylenetriaminepentaacetic acid (DTPA), N-2-hydroxyethyl-ethylenediaminetriacetic acid (HEDTA), nitrilotriacetate (NTA), as well as natural chelating agents such as ethylenediamine-N,N'-disuccinic acid (EDDS), have been effectively utilized to enhance the extraction of lead (Pb) from soil (RoyChowdhury *et al.*, 2018). Chelating agents like EDTA (ethylenediaminetetraacetic acid), NTA (nitrilotriacetic acid), and a chelating resin named CR11 produced by Diaion, have been employed in the treatment of heavy metal-contaminated sludge. EDTA exhibits a strong affinity for metals and can form metal-EDTA complexes (Sharma *et al.*, 2018).

C. Biological approach

Bioremediation harnesses the natural biological mechanisms of plants and microorganisms to remove, or immobilize hazardous contaminants from polluted environments. Compared to conventional chemical and physical methods, which can be costly, inefficient, and often result in the production of significant amounts of toxic sludge, bioremediation offers an eco-friendly and cost-effective alternative for heavy metal removal (Jacob *et al.*, 2018).

These methods are cost-effective, practical, and importantly, they do not generate any additional pollution. There's no need for further treatment of the contaminants, and the natural plants and animals in the polluted area can be restored to their original state. Organisms employ various strategies to survive exposure to toxic metals:

1. Extrusion system: Organisms expel metals from their cells using methods like chromosomal or plasmid-mediated processes (Zabochnicka-Świątek and Krzywonos, 2014).

2. Biotransformation: Organisms transform the harmful metals into harmless forms.

3. Utilizing enzymes such as oxidases and reductases: Organisms produce these enzymes to convert pollutants into manageable by-products (Nascimento *et al.*, 2023).

4. Production of exopolysaccharide (EPS): Microorganisms adapt to polluted environments by releasing EPS, which forms a protective hydrophobic outer membrane containing efflux pumps that counteract cell membrane-disrupting contaminants like solvents.

5. Production of metallothioneins: These are proteins that bind to metals, forming a complex to detoxify those (Sharma *et al.*, 2018).

i. Bacterial bioremediation

Microbial biomass offers a promising alternative for removing heavy metals from various polluted sources. Among these microbes, bacteria possess unique genetic mechanisms and play a crucial role in mitigating environmental pollution. Certain bacterial strains, such as *Bacillus* and *Pseudomonas* sp., are commonly employed for heavy metal removal from wastewater and soil due to their high affinity for

binding metals (Jacob *et al.*, 2018). The most commonly utilized microorganisms for the removal of heavy metals from contaminated soils are bacteria and fungi (Li *et al.*, 2019). Additionally, it aims to enhance soil quality and restore its functionality (Gong *et al.*, 2018).

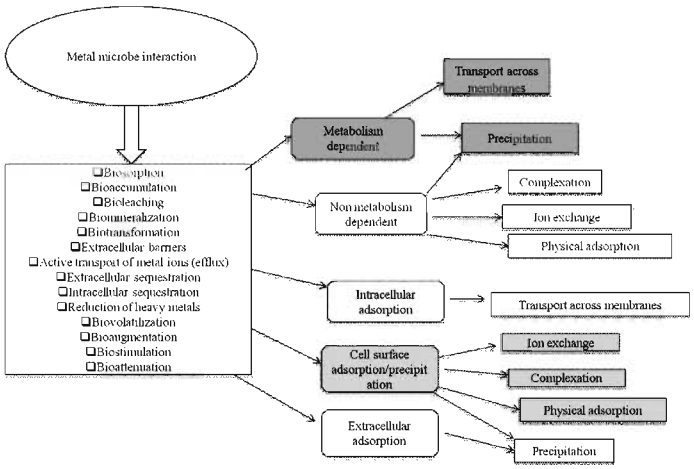

Fig. 2 : Various mechanism involved in the microbial bioremediation

Microbial bioremediation employs several mechanisms to address contamination:

1. Sequestration: Microorganisms can sequester toxic metals through cell wall components or intracellularly by utilizing metal-binding proteins and peptides. Examples include metallothioneins (MT), phytochelatins, and bacterial siderophores. While bacterial siderophores are predominantly catecholates, fungi produce hydroxamate siderophores (Sreedevi *et al.*, 2022).

2. Biochemical Pathway Alteration: Microorganisms can modify biochemical pathways to inhibit or block the uptake of metals, reducing their accumulation within the cell (Sharma *et al.*, 2024).

3. Metal Transformation: Enzymes produced by microorganisms can convert toxic metals into less harmful or inert forms (Kapahi and Sachdeva, 2019).

4. Efflux Systems: Microorganisms possess precise efflux systems that actively pump out and reduce the intracellular concentration of metals, aiding in detoxification.

These mechanisms collectively contribute to the effectiveness of microbial bioremediation in detoxifying and removing heavy metals from contaminated environments (Ojuederie and Babalola, 2017). These biological methods can involve either aerobic (with oxygen) or anaerobic (without oxygen) processes and are effective for eliminating heavy metals (Sayqal and Ahmed, 2021). Bacterial functional groups like hydroxyl, carboxyl, sulfonate, amide and phosphonate are primarily responsible for absorbing metals from aqueous solutions. In control samples without Cr (VI), bacterial cells appeared elongated with smooth surfaces and were distinct from one another (Sharma *et al.*, 2024). In contrast, cells treated with Cr (VI) exhibited irregular surfaces and tended to clump together or adhere. The microbial cell wall plays a significant role in metal binding. The anionic nature of microbial surfaces allows them to bind to metal cations through electrostatic interactions. Gram-positive bacteria have a thicker cell wall composed of peptidoglycan, teichoic acids, and teichuronic acids (Sharma *et al.*, 2024). In contrast, Gram-negative bacteria lack teichoic and teichuronic acids, and their peptidoglycan layer is thinner. Compared to Gram-negative bacteria, Gram-positive bacteria are more effective at capturing metal ions (Jacob *et al.*, 2018). The unique cell wall of bacteria plays a crucial role in the biosorption process due to the variety of functional groups present on its surface. Experimental studies have shown that gram-positive bacteria are superior adsorbents compared to gram-negative bacteria. This superiority is attributed to the presence of glycoproteins responsible for metal binding and uptake on their surface (Pande *et al.*, 2022). *Bacillus subtilis* is a promising candidate for detoxifying and removing metal ions in conventional water treatment processes. *Pseudomonas* aeruginosa is a versatile and resilient bacteria known for its resistance to chemicals, metals, antibiotics, and organic solvents. When biomass is pretreated with immobilized activated carbon, metals such as mercury, copper, nickel, chromium, and zinc can be effectively removed (Kumar *et al.*, 2021). Various bacteria possess resistance genes for different cations and oxyanions of heavy metals within their DNA. To survive in metal-contaminated environments, bacteria employ multiple mechanisms to combat the uptake of heavy metal ions. These strategies encompass biosorption, entrapment, efflux, reduction, precipitation, and complexation (Sayqal and Ahmed, 2021). Bacteria produce siderophores, which can reduce the bioavailability of metals and facilitate their removal from contaminated soil. Studies have shown that bacterial cells can modify their morphology to increase siderophore production, thereby enhancing the intracellular accumulation of metals. For instance, the sulfate-reducing bacterium Desulfovibrio desulfuricans can convert sulfate to hydrogen sulfide, which subsequently reacts with heavy metals like cadmium (Cd) and zinc (Zn) to form insoluble metal

sulfides (Thai *et al.*, 2023).The biomolecules present in microbial cell walls contain negatively charged functional groups such as phosphate, hydroxyl, and carbonyl. These groups bind rapidly to toxic metal ions, aiding in bioremediation efforts. Various bacterial genera have been identified for their bioremediation capabilities, including *Arthrobacter, Enterobacter, Corynebacterium, Stenotrophomonas, Bacillus,* and *Pseudomonas* (Saha *et al.*, 2021).

i. Fungal Bioremediation

Fungi are known for their ability to tolerate and detoxify heavy metal-contaminated effluents. The fungal cell wall is composed of chitin, polysaccharides, proteins, lipids, polyphosphates, and inorganic ions that cement the cell wall together. Due to their larger cell-to-surface ratio, fungi have a higher propensity to interact both physically and enzymatically with their environment (Joshi *et al.*, 2011). Fungal biomass is considered an effective sorption material and can be easily cultured on a large scale using simple fermentation techniques. The high concentration of carboxyl groups in the mannuronic and guluronic acids present in the cell wall polysaccharides, along with the protosufficiency, enhances the biosorption of heavy metals by fungi (Jacob *et al.*, 2018). Fungal hyphae have demonstrated effectiveness in the bioremoval of toxic metals, exposure to which can pose various health risks. One of the key advantages of using fungi as adsorbents is their ability to grow rapidly in large quantities and their amenability to genetic modification. *Rhizopusarrhizu*s, a filamentous fungus, is primarily utilized in the alcohol fermentation industry due to its ability to produce lactic acid and cortisone (Liaquat *et al.*, 2020). Additionally, it serves as an excellent agent for bioremediation purposes. *Saccharomyces cerevisiae*, a species of yeast commonly used in the fermentation, baking, and brewing industries, is also known as top-fermenting yeast. Its widespread availability and cost-effectiveness make it a suitable adsorbent for removing metallic ions such as copper, cadmium, lead, uranium, arsenic, and others (Kumar *et al.*, 2021). Mycorrhizal fungi play a significant role in bioremediation by secreting glomalins, a type of glycoprotein. *Glomalins* help stabilize aluminum in the soil and within the roots of *Gmelina* plants. Various fungal species, including *Aspergillus niger, Aureobasidium pullulans,* Cladosporium resinae, Funaliatrogii, *Ganodermalucidum, Penicillium* spp., *Rhizopusarrhizus* and *Trametesversicolor*, have the ability to extract heavy metals from polluted environments (Goutam *et al.*, 2021). The bioaccumulation potential of *Aspergillus versicolor* for heavy metals was found to be highest at optimal pH values of 6 for 50 mg/L of Cr(VI) and Ni(II), and 5 for Cu(II) ions, resulting in removal yields of 99.89%, 30.05%, and 29.06%, respectively. *Aspergillus fumigatus* has been identified as effective in removing Pb(II) ions from aqueous solutions of electronic waste containing 100 mg/L of Pb, with a maximum adsorption capacity of 85.41% observed during batch sorption experiments (Kumar *et al.*, 2019). Fungi are increasingly being employed as a tool for remediating heavy metal-contaminated areas due to their capacity to accumulate toxic metals. *Coprinopsisatramentaria* has been studied for its bioaccumulation capabilities, demonstrating a capacity to accumulate 76% of Cd^{2+} when the concentration of Cd^{2+} was 1 mg L^{-1}, and 94.7% of Pb^{2+} when the concentration of Pb^{2+} was 800 mg L^{-1} (Sharma *et al.*, 2018). Fungi serve as promising biocatalysts in the bioremediation process, absorbing toxic chemicals into their spores and mycelium. They can thrive in harsh environmental conditions and detoxify metal ions through processes such as accumulation, valence transformation, and both extra- and intracellular precipitation. Several fungi species, including *Aspergillus flavus, Aspergillus awamori, Saccharomyces cerevisiae, Phanerocha-etechry sosporium, Penicillium oxalicum,* and *Trichoderma viride,* have been identified for their potential role in bioremediation due to these capabilities (Saha *et al.*, 2021).

ii. Algal Bioremediation

Algae are photosynthetic organisms capable of converting solar energy into biochemical energy. Based on their size, algae are categorized into microalgae, which are microscopic, single-celled photosynthetic organisms, and macroalgae, which are multicellular and typically found near the seabed. Algae can thrive in various aquatic environments, including freshwater and marine habitats, as well as in moist soil (Mohammadi and Mahmoudnia, 2023). Biosorption is a surface phenomenon that typically involves the sequestration of substances on the cell surface. Therefore, modifications to the algal cell wall can significantly affect the binding of metal ions. Marine algae are rich in biopolymers that have the potential to bind with heavy metals, with brown and red algae being particularly promising for biosorption (Chugh *et al.*, 2022). The polysaccharides present in the cell walls of algae can offer amino and carboxyl groups as nitrogen and oxygen moieties, respectively, which can form coordinate bonds with metal ions. Algal

biomass, which is often used for lipid and biofuel production, can also be effectively utilized to neutralize heavy metal ions in wastewater (Jacob *et al.*, 2018). Different strains of algae exhibit varying adsorption capacities due to the different levels of toxic heavy metal ions they can tolerate. Metal accumulation can either be dependent or independent on cellular metabolism, occurring either on the cell surface or within the cytoplasm, respectively. Various chemical reagents like NaOH, $CaCl_2$, HNO_3, and formaldehyde play a crucial role in physico-chemical modification of biomass (Kanchana *et al.*, 2014).These modifications increase the surface area of the biomass and activate and expose several functional groups on its surface, enhancing its binding capacity with adsorbates. Spirulina, a biomass of cyanobacteria with high protein content, is not only used as a nutrient-rich dietary supplement but also has the ability to adsorb toxic metal ions such as chromium and cadmium under specific pH and temperature conditions (Mahlangu *et al.*, 2024). Utilizing Spirulina for metal remediation offers an eco-friendly, cost-effective, reliable, and efficient method of remediation. *Chlorella vulgaris* is another type of algae cultivated for use as a nutrient supplement (Maurya *et al.*, 2024). It is also employed in biosorption processes, primarily based on its metabolic and surface properties. Surface functional groups like carboxyl and amine can be modified to improve its adsorption efficiency, which plays a significant role in coordinating various metal ions on its surface (Kumar *et al.*, 2021). *Synechocystis* sp. PCC6803, a unicellular blue-green alga, was found to accumulate arsenic at concentrations of 1.0 and 0.9 g/kg dry weight (DW) when exposed to 0.5 mM arsenate and arsenite for 14 days, respectively. When exposed to 2.67 µM arsenite, *Synechocystis* rapidly oxidized the arsenite to arsenate and accumulated arsenic quickly through cellular oxidation (Sattayawat *et al.*, 2021). The green marine alga *Cladophora fascicularis* has been identified as an efficient biosorbent material for the removal of Pb(II) from wastewater. The efficiency of Pb(II) removal varies as a function of time, initial pH, initial Pb(II) concentrations, temperature, and the presence of co-existing ions. Various cyanobacterial species, including *Oscillatoria* sp., *Synechococcus* sp., *Nodularia* sp., *Nostoc* sp., and *Cyanothece* sp., have been found to be suitable for bioremediation, particularly in the biodegradation and biosorption of contaminants like ammonia and nitrate (Refaey *et al.*, 2021).These cyanobacterial species, either individually or in mixtures, demonstrated contaminant removal efficiency (RE) percentages ranging from 69.5% to 99.6% at a concentration of 5 ppm of pollutants. When used as mixed cultures, the RE percentages ranged from 91.6% to 100% (Mani and Kumar, 2014).

Phytobial Bioremediation

Phytobial remediation offers an efficient and environmentally friendly approach to remove heavy metals from soil and water by utilizing both plants and microbes. Various mechanisms are involved in Phytobial remediation, including:

i) Bioprecipitation of metals

ii) Bioaccumulation of metals by metal-binding proteins

iii) Binding of metals on the cell surface

iv) Biotransformation of metals

v) Methylation of metals

vi) Solubilisation of metals

vii) Biosorption of metals

viii) Metal reduction

ix) Siderophore secretion

x) DNA-mediated interaction for heavy metal removal (Asad *et al.*, 2019).

The effectiveness of these mechanisms can be enhanced by integrating suitable bacteria capable of secreting multiple plant growth-promoting substances (PGPS). These substances, which include organic acids, ACC deaminase, siderophores, and biosurfactants, can transform metals into a bioavailable form. Phosphate solubilizing bacteria (PSB) are known to secrete PGPS (Roy *et al.*, 2015). During the immobilization process, the mobility of the contaminant is restricted by altering its physical and chemical properties. Oxidase enzymes present in the microbes oxidize the metals, rendering them less mobile and less toxic (Selvi *et al.*, 2019). Plant growth in metal-contaminated environments can be facilitated by endophytic bacteria, which produce plant growth-promoting chemicals, siderophores, and phytohormones. These bacteria enhance the bioavailability of mineral nutrients and provide protection against plant pathogens. The improved growth of plants can increase their potential for metal removal (Selvi *et al.*, 2019).Furthermore, endophytic bacteria contribute to metal accumulation by increasing mobilization, producing extracellular polymeric substances and biosurfactants, and biotransforming toxic forms of metals into non-toxic forms. Additionally, endophytic bacteria may

influence the antioxidant enzymes in plants and can themselves accumulate metals to reduce toxicity to the plants. Thus, endophytic bacteria play a crucial role in supporting plant growth and aiding in the remediation of metal-contaminated environments (Shukla *et al.*, 2018). Plant-microbe-based bioremediation involves two key aspects. Firstly, microorganisms support the host plant by helping it to withstand harsh environmental conditions through the provision of nutrients. Secondly, the plant itself plays a crucial role in creating favorable environmental conditions. This includes improving soil organic matter and increasing the availability of essential nutrients like phosphorus (P), potassium (K), and nitrogen (N). These improved conditions allow soil microorganisms to thrive and contribute to enhancing the remediation or reclamation process of the contaminated environment (Saha *et al.*, 2021). Plant Growth-Promoting Rhizobacteria (PGPR) can produce ACC deaminase, an enzyme that breaks down ACC, which is a precursor to ethylene in plants. By producing ACC deaminase, PGPR reduce the level of ethylene in the plant. Ethylene is known to inhibit root elongation and overall plant growth, especially under heavy metal (HM)-stressed conditions. Therefore, the production of ACC deaminase by PGPR can improve plant growth in environments contaminated with heavy metals (Alsafran *et al.*, 2023). Mycorrhizae also play a significant role in phytoremediation processes. They contribute by retaining heavy metals on their fungal mycelium, acting as a physical barrier. Additionally, mycorrhizae immobilize heavy metals in the soil through a process called "gloaming," thereby reducing the bioavailability, translocation, and bioaccumulation of these metals in plant tissues. The primary protective mechanism provided to plants by mycorrhizae is likely the immobilization of metals in fungal hyphae through chelation and sequestration (Raklami *et al.*, 2022).

Role of Endophytes in Bioremediation

Endophytes are microorganisms, including bacteria and fungi, that live within plant tissues without causing any harm to the host plant. Some fungal endophytes are capable of producing secondary metabolites (Govarthanan *et al.*, 2016). Additionally, certain bacterial endophytes have been found to exhibit heavy metal tolerance. For instance, Methylobacterium strains isolated from the herb Pterisvittata have been reported to exhibit tolerance to heavy metals (Dixit et al., 2015). This ability of endophytic bacteria to tolerate and potentially detoxify heavy metals can be beneficial for plants growing in contaminated environments and can contribute to the plant's overall health and growth under such stressful conditions (Selvi *et al.*, 2019).

Role of rhizobial microbes in Bioremediation

The rhizosphere is the soil region directly influenced by the roots of plants. In this zone, certain microbes establish symbiotic relationships with plants by secreting various substances such as exudates, secretions, mucilages, mucigel, and lysates (Sahoo *et al.*, 2024). These microbial secretions play a crucial role in promoting plant growth and health. For instance, siderophores produced by these microbes aid in the chelation and solubilization of metals, making them more available for plant uptake (Gupta *et al.*, 2024). This process is particularly beneficial in rhizo-remediation, a plant-based strategy for environmental cleanup. Through rhizo-remediation, plant growth can be stimulated, heavy metals can be immobilized in the soil, and the accumulation of metals in plant tissues can be facilitated, thereby assisting in the remediation of metal-contaminated soils (Selvi *et al.*, 2019). Arbuscular mycorrhizal (AM) fungi establish a direct connection between the soil and plant roots. They are well-known for enhancing the uptake of plant mineral nutrients, including heavy metals. These fungi can boost plant resilience to heavy metal stress, promote plant growth in contaminated environments, or mitigate the adverse effects of metal contamination on plants (Mani and Kumar, 2014).

Nanoparticle based Bioremediation

The use of nano-biosorbents with ultrafine structures and large surface areas offers several advantages. Firstly, they provide superior chemical activity and adsorption capacity compared to conventional materials. Secondly, they increase the surface binding energy, enhancing the efficiency of metal adsorption. Thirdly, they reduce internal diffusion resistance, facilitating faster adsorption kinetics (Ali *et al.*, 2023). In the magnetic modification approach, the target species adsorbs onto the surface of the nano-biosorbent from the solution, allowing for its magnetic separation at low field gradients. When nanotechnology is employed to produce biosorbents, these nano(bio)materials exhibit high surface area, improved adsorption capacity for heavy metals, enhanced adsorption kinetics, and the ability to regenerate and reuse the nanomaterial (Baby *et al.*, 2022). Nanoparticles inherently contain various functional groups such as -COOH, -NH_2, and -OH.

Tailoring these functional groups through physical/chemical activation or surface modification enhances the removal of heavy metals. Graphene-based nanomaterials, for example, are utilized for the reduction of various heavy metals like Hg(II), Cr(VI), Cu(II), Ni(II), and Cd(II) in the environment, increasing the absorption capacity of biosorbents due to the availability of selected functional groups that provide more sites for interaction with metal oxides (Ekrami *et al.*, 2022). Nanomaterials are often used in combination with microorganisms to enhance heavy metal reduction, rendering them more effective than when applied separately. Several parameters influence the synergy between nanomaterials and microorganisms, including the chemical properties, particle size, coating characteristics, and shape of the nanomaterial, as well as the crystalline phase, mode of metabolism, degree of contamination, and tolerance of the nanomaterial to toxic pollutants and environmental conditions (Yogeshwaran and Priya, 2019).To create a nanocomposite, microorganisms can be immobilized or trapped within the nanomaterial. For example, gram-negative Halomonas sp. entrapped within polyvinylpyrrolidone-coated iron oxide nanoparticles has been tested for the removal of Cd(II) and Pb(II). This approach holds promise for efficient heavy metal remediation in various environmental contexts (Verma *et al.*, 2021). Nanoparticles possess a higher surface-to-volume ratio, making them versatile tools in various applications such as water treatment, catalysis, biosensing, and pollutant degradation. When integrated with microbial cells, nanoparticles offer significant potential for cleaning up contaminated environments due to their reactive sites, which can rapidly interact with contaminants, leading to detoxification or immobilization (Prakash, 2023). This integration allows nanoparticles to immobilize microbial cells like Pseudomonas, which can efficiently degrade or biorecover specific chlorinated chemicals. Additionally, biologically synthesized gold and silver nanoparticles have been demonstrated to degrade various dyes like methylene blue and methyl orange (Dhanapal *et al.*, 2024). Nanoremediation offers several advantages, including mitigating the toxic effects of heavy metals on microorganisms, enhancing microbial activity towards specific contaminants, and ultimately reducing the time and overall costs associated with remediation efforts. By leveraging the unique properties of nanoparticles and microbial cells, nanoremediation holds promise for addressing environmental pollution and promoting sustainable remediation practices (Jacob *et al.*, 2018). Indeed, heavy metal removal has been successfully achieved through the utilization of various nanomaterials, each offering unique properties and advantages. Metal oxide nanoparticles, graphene and its derivatives, magnetic nanoparticles (MNPs), and carbon nanotubes (CNTs) are among the nanomaterials that have shown promise in heavy metal remediation (Yang *et al.*, 2019). Nanotechnology provides several advantages over traditional methods for heavy metal analysis and removal from food and water resources. One key advantage is the broad linear range offered by nanomaterial-based detection and removal techniques. This enables the detection and removal of heavy metals across a wide range of concentrations, making the methods versatile and applicable to various scenarios (Sudarman *et al.*, 2023). Additionally, nanotechnology offers low detection and quantification limits, allowing for the detection and removal of heavy metals even at very low concentrations. This high sensitivity is crucial for ensuring the safety and quality of food and water resources, where even trace amounts of heavy metals can pose health risks (Saeed *et al.*, 2023). Furthermore, nanomaterial-based techniques exhibit excellent selectivity, meaning they can specifically target and remove heavy metals without interfering with other components present in the sample. This specificity enhances the efficiency and accuracy of heavy metal removal, minimizing the risk of false positives or negatives (Mathur *et al.*, 2022). Overall, the use of nanotechnology for heavy metal analysis and removal presents numerous benefits that make it a promising approach for addressing heavy metal contamination in food and water resources, contributing to improved environmental and public health outcomes (Mitra *et al.*, 2022). Nanoparticles inherently contain various functional groups such as $-NH_2$, –COOH, and –OH, which contribute to their adsorption capabilities. Tailoring these functional groups through physical or chemical activation, or surface modification, further enhances the elimination of HMs by providing more specific binding sites for the metal ions (Ahmad *et al.*, 2023). Several factors influence the interaction between nanomaterials and microbes in environmental remediation efforts. These include the chemical properties of the nanomaterial, including its particle size, coating characteristics, and shape. The metabolic processes of the microbes, as well as the crystalline phase of the nanomaterial, also play crucial

roles. Additionally, the extent of contamination, the resistance of nanomaterials to hazardous contaminants, and prevailing environmental conditions all influence the efficacy of nanomaterial-microbe interactions in HM removal processes (Pande *et al.*, 2022).

Mechanism of tolerance against Heavy metals

Microbes aid in the mineralization of organic pollutants into end-products like CO_2 and H_2O, or into metabolic intermediates that serve as primary substrates for cell growth during bioremediation (Igiri *et al.*, 2018). Microorganisms maintain a two-way defense system by (i) producing degradative enzymes for target contaminants and (ii) resisting relevant heavy metals. They play a crucial role in environmental restoration through various methods such as binding, immobilization, oxidation, transformation, and volatilization of heavy metals (Pande *et al.*, 2022).

Microorganisms are capable of dissolving metals and facilitating the reduction and oxidation of transition metals. Although cell membranes can be disrupted by contamination from organic solvents, microorganisms sometimes develop defense mechanisms, such as hydrophobic or solvent efflux pumps, which protect the outer cell membrane (Verma and Kuila, 2019). However, plants have developed various physiological and molecular strategies to overcome or mitigate heavy metal (HM) related stress. HMs negatively impact the normal development and productivity of plants. In response, plants activate their defense systems to regulate mineral uptake, sequester HMs, activate metal-binding proteins, and enhance antioxidant metabolism. Additionally, plants can combat HM toxicity by producing specific plant hormones, overexpressing enzymes, activating their antioxidative systems, and regulating HM transport and resistance gene expression, among other mechanisms (Verma *et al.*, 2021). Among microbes, bacteria possess specific genetic mechanisms and play a crucial role in mitigating environmental contamination. Notable bacterial strains such as Bacillus and Pseudomonas species are widely used for removing heavy metals from wastewater and soil due to their high metal-binding affinities. Bacterial functional groups, including hydroxyl, carboxyl, sulfonate, amide, and phosphonate groups, are primarily involved in metal uptake from aqueous solutions (Firincă *et al.*, 2023). The bioremediation of heavy metals by bacteria arises from their basic self-defense mechanisms, which involve cell surface changes and cell agglomeration to counteract the toxic effects of heavy metals. The microbial cell wall plays a key role in metal binding, with its anionic nature allowing the binding of metal cations through electrostatic forces. Gram-positive bacteria, with their thicker cell walls composed of peptidoglycan, teichoic, and teichuronic acids, are more efficient at trapping metal ions compared to Gram-negative bacteria, which lack teichoic and teichuronic acids and have a thinner peptidoglycan layer (Medfu *et al.*, 2020). A comprehensive study on chromium (Cr[VI]) biosorption by a novel haloalkaliphilic bacterium showed that both intracellular and extracellular reducing mechanisms, along with cell surface functional groups such as alkanes, amides, and amines, are involved in chromium biosorption and immobilization on cell surfaces. Fungi are well known for their ability to tolerate and detoxify heavy metal-contaminated effluents (Gonzalez and Ghneim, 2021). The fungal cell wall is composed of chitin and other polysaccharides, along with proteins, lipids, polyphosphates, and inorganic ions that strengthen the cell wall. Due to their higher cell-to-surface ratio, fungi have an increased tendency to come into physical and enzymatic contact with their surroundings. The mechanisms involved in fungal detoxification of heavy metal-contaminated environments include valence transformation, intra- and extracellular precipitations, and active uptake (Mishra and Malik, 2013). The high content of carboxyl groups in the mannuronic and guluronic acids of the cell wall polysaccharides, along with their protosufficiency, significantly enhances heavy metal biosorption (Jacob *et al.*, 2018). The ability of microorganisms to alter the ionic states of heavy metals is a significant process with far-reaching implications for the solubility, bioavailability, and movement of these metals in both soil and aquatic environments. The mobilization and immobilization of heavy metals are critical aspects of microbial remediation, involving a variety of intricate mechanisms such as oxidation-reduction, chelation, modification of metallic complexes, and biomethylation (Hassen *et al.*, 1998). Notably, microbial enzymatic catalysis plays a pivotal role in reducing metals from higher to lower oxidation states, enhancing their solubilization. Additionally, microorganisms employ membrane-linked transport mechanisms to convert heavy metals into non-hazardous forms, which is vital for their survival in metal-polluted environments (Zhou *et al.*, 2023).

Table-2 : Various negative effects caused by various heavy metals

Sr.No.	Disease	Heavy metals	References
1	▪ Carcinogenic, mutagenic, endocrine disruptor, lung damage and fragile bones, affects calcium regulation in biological systems. ▪ Lung and kidney damage, bone-calcium problems, gastrointestinal tract damage, coma.	Cadmium	(Yadav *et al.*, 2017). (Igiri *et al.*, 2018). (Roy *et al.*, 2024).
2	▪ Brain and kidney damage, elevated levels result in liver cirrhosis and chronic, anemia stomach and intestine irritation. ▪ Nose-mouth-eye irritation, headache, stomach upset, nausea, vomiting, diarrhea, liver problem and kidney problem, Wilson's disease, hepatic cirrhosis, brain damage, and kidney disease.	Copper	(Yadav *et al.*, 2017). (Pande *et al.*, 2022). (Jayaram *et al.*, 2022). (Roy *et al.*, 2024).
3	▪ Allergic skin diseases, such as itching, cancer of the lungs, nose, sinuses, throat through continuous inhalation, immunotoxic, neurotoxic, genotoxic, affects fertility, hair loss. ▪ Asthma and chronic bronchitis, birth defects, lung embolism, respiratory failure, allergies, heart conditions, pneumonitis, erythematous, skin ulceration, nose cancer, larynx cancer, prostate cancer, sickness and dizziness.	Nickel	(Yadav *et al.*, 2017). (Igiri *et al.*, 2018). (Roy *et al.*, 2024). (Tang *et al.*, 2024).
4	▪ Excess exposure in children causes impaired development, reduced intelligence, short-term memory loss, disabilities in learning and coordination problems, risk of cardiovascular disease. ▪ Anorexia, headache, blood pressure, abdominal pain, kidney problem, kidney fatigue, insomnia, arthritis, mental illness, birth abnormality, autism, allergies, learning disability, weight loss, paralysis, weakness, brain damage, kidney damage, death.	Lead	(Yadav *et al.*, 2017). (Pande *et al.*, 2022). (Thai *et al.*, 2023). (Roy *et al.*, 2024).
5	▪ Dizziness, fatigue, etc. ▪ Dermal irritation, hyperglycemia, abdominal pain, nausea, vomiting, lethargy, anemia, dizziness, cell death.	Zinc	(Yadav *et al.*, 2017). (Pande *et al.*, 2022). (Roy *et al.*, 2024).
6	▪ Nose ulcers, runny nose, breathing problems, such as asthma, cough, shortness of breath, or wheezing and hair loss. ▪ Ulcers on the nose bone, DNA damage, chromosomal abnormalities, skin inflammation, stomach upset, difficulties in respiration, immunodeficiency, kidney and liver damage, lung carcinoma, and death.	chromium	(Yadav *et al.*, 2017). (Igiri *et al.*, 2018). (Jeyakumar *et al.*, 2023). (Roy *et al.*, 2024).
7	▪ Autoimmune diseases, depression, drowsiness, fatigue, hair loss, insomnia, loss of memory, restlessness disturbance of vision, tremors, temper outbursts, brain damage, lung and kidney failure. ▪ Skin rashes, elevated heart rate or blood pressure, depression, memory issues, tremors, exhaustion, headache, hair loss, lung damage, vomiting, diarrhea, nausea, damage to the brain and kidneys, and harm to the growing fetus.	Mercury	(Yadav *et al.*, 2017). (Pande *et al.*, 2022). (Jayaram *et al.*, 2022). (Roy *et al.*, 2024).

8	▪ Affects essential cellular processes, such as oxidative phosphorylation and ATP synthesis. ▪ Nausea, vomiting, abdominal pain, diarrhea, encephalopathy, peripheral neuropathy, multisystem failure.	Arsenic	(Yadav *et al.,* 2017). (Pande *et al.,* 2022). (Roy *et al.,* 2024). (Tang *et al.,* 2024).
9	▪ Exposure may cause skin and other body tissues to turn gray or blue-gray, breathing problems, lung and throat irritation and stomach pain. ▪ Argyria, allergic dermatitis, dizziness, respiratory irritation, headaches, irritability of the skin, eyes, throat, or lungs, discomfort of the stomach, nausea, vomiting, or diarrhea, and narcosis	Silver	(Yadav *et al.,* 2017). (Igiri *et al.,* 2018). (Thai *et al.,* 2023). (Roy *et al.,* 2024).
10	Cause cardiac arrhythmias, respiratory failure, gastrointestinal dysfunction, muscle twitching and elevated blood pressure.	Barium	(Jaishankar *et al.,* 2014). (Yadav *et al.,* 2017). (Pande *et al.,* 2022). (Abo-Alkasem *et al.,* 2023).
11	▪ Dietary exposure of around 300µg/dayaffects endocrine function, impairment of natural killer cells activity, hepatotoxicity and gastrointestinal disturbances. ▪ Brittle hair and misshapen nails, rashes, heat, swelling of the skin and excruciating pains, burning, itching, and tearing of the eyes, garlic breath, bronchitis, pneumonitis, bronchial asthma, nausea, chills, fever, headache, and sore throat, shortness of breath, conjunctivitis, vomiting, abdominal pain, diarrhea, and enlarged liver, red staining of the teeth, nails.	Selenium	(Yadav *et al.,* 2017). (Igiri *et al.,* 2018). (Jayakumar *et al.,* 2023). (Roy *et al.,* 2024).
12	Kidney damage, respiratory irritation, damage to the respiratory tract, neurobehavioral changes.	Uranium	(Pande *et al.,* 2022). (Jayaram *et al.,* 2022). (Abo-Alkasem *et al.,* 2023). (Roy *et al.,* 2024).
13	Acute nausea, coma, encephalopathy, initial moderate kidney failure progressing to acute tubular necrosis, abnormalities, pulmonary fibrosis, lung disease, respiratory tissue damage, neuropsychological impairment, disruption of memory function, hypocalcaemia.	Tungsten	(Pande *et al.,* 2022). (Abo-Alkasem *et al.,* 2023). (Roy *et al.,* 2024). (Tang *et al.,* 2024).
14	DNA changes, cancer, skin and mucous membrane allergies, organ damage, including to the intestines, kidneys, and bone marrow, and hearing loss.	Platinum	(Igiri *et al.,* 2018). (Abo-Alkasem *et al.,* 2023). (Thai *et al.,* 2023). (Roy *et al.,* 2024).
15	Skin irritation, bone marrow damage, liver and kidney damage.	Palladium	(Jaishankar *et al.,* 2014). (Abo-Alkasem *et al.,* 2023). (Roy *et al.,* 2024).
16	Liver dysfunction, articular deformities, erythema, edema, and joint pain in the knees, hands, and feet.	Molybdenum	(Pande *et al.,* 2022). (Jayakumar *et al.,* 2023). (Abo-Alkasem *et al.,* 2023). (Roy *et al.,* 2024).
17	Leg cramps, paralysis, emotional distress, drowsiness, weakness, obesity, glucose intolerance, blood clotting, skin issues, low cholesterol, skeleton issues, birth defects, hair color changes, and neurological symptoms.	Mn	(Igiri *et al.,* 2018). (Jayaram *et al.,* 2022). (Abo-Alkasem *et al.,* 2023). (Roy *et al.,* 2024).

18	Gastrointestinal problem, nausea and diarrhea, low blood pressure, drowsiness, tachyarrhythmia, hepatic necrosis, lung cancer, DNA damage.	Fe	(Pande *et al.*, 2022). (Roy *et al.*, 2024). (Tang *et al.*, 2024).
19	Skin problem and inflammation.	Au	(Pande *et al.*, 2022). (Abo-Alkasem *et al.*, 2023). (Thai *et al.*, 2023). (Roy *et al.*, 2024).
20	Vomit sickness, vision irritation, heart disease, thyroid problems.	Co	(Jaishankar *et al.*, 2014). (Pande *et al.*, 2022). (Abo-Alkasem *et al.*, 2023). (Roy *et al.*, 2024).
21	Vomiting, diarrhea, irregular and difficulties in respiration, hypothermia, gastrointestinal disorders and death.	Sb	(Pande *et al.*, 2022). (Abo-Alkasem *et al.*, 2023). (Jayakumar *et al.*, 2023). (Roy *et al.*, 2024).

During stress situations caused by heavy metals (HMs), microorganisms either succumb to the toxicity or develop resistance mechanisms to thrive. These resistance mechanisms include extracellular barriers, extracellular and intracellular sequestration, active transport of metal ions, and enzymatic detoxification. Microbial cell surfaces possess various characteristics that prevent metal ions from entering by adsorbing them on their surface and acting as barriers (Mukhi *et al.*, 2023). The biofilms produced by microbes, composed of extracellular polymers, can accumulate metal ions and protect the cells within them. Extracellular sequestration involves the complexation of metal ions into insoluble compounds or their accumulation by cell components in the periplasm. In intracellular sequestration, metal ions are complexed by specific compounds within the cell cytoplasm (Alvarado *et al.*, 2023). Another strategy to combat HM stress is the active transport of HM ions out of the intracellular environment through efflux mechanisms that regulate intracellular HM ion concentrations. Metal-exporting proteins, such as ABC transporters, P-type efflux ATPase, cation diffusion facilitators, and proton-cation antiporters, are widely distributed in the cell membrane to facilitate HM ion efflux (Buaisha *et al.*, 2021).ABC transporters, also known as traffic ATPases, help microorganisms survive HM-induced stress by mediating the membrane translocation of HM ions. Additionally, resistance to HM ions in microbes is supported by enzymes that biologically transform or chemically modify HM ions, converting them from highly hazardous forms to less toxic forms (Pande *et al.*, 2022).

Conclusion

Heavy metals pose significant threats to the environment, marine ecosystems, and agriculture through contamination and toxicity. Effective remediation requires a combination of physical, chemical, and biological methods tailored to specific sites and contamination levels. Ongoing monitoring and maintenance are essential to ensure long-term remediation success and environmental health. Sustainable and innovative approaches, such as phytoremediation and bioremediation, offer promising solutions for mitigating heavy metal pollution. The composite approach which is an integral of all the different types of techniques can be groundbreaking and can become a biggest weapon in the arsenal against heavy metal toxicity. This can pave a way forward for a better future.

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REVIEW ARTICLE

AI-Powered Plant Genomics: Revolutionizing Crop Breeding for the Future

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Abstract

Artificial intelligence (AI) is revolutionizing plant breeding by integrating machine learning (ML) and deep learning (DL) to enhance trait selection, genomic analysis, and crop improvement. AI-driven approaches enable high-throughput phenotyping, automated disease detection, and predictive breeding, improving efficiency and accuracy. Genomic selection (GS) and genome-wide association studies (GWAS) utilize AI to process high-dimensional genomic data, identifying SNP-trait associations and optimizing breeding programs. AI enhances image-based phenotyping through convolutional neural networks (CNNs) and computer vision for plant trait identification, stress analysis, and disease detection. Deep learning models, such as ResNet50, InceptionV2, and EfficientNetV2-B4, have achieved over 90% accuracy in detecting crop diseases in bananas, maize, and wheat. AI-driven molecular breeding incorporates explainable AI (xAI) to improve GWAS model interpretability, addressing non-linear trait interactions and missing heritability. Integrating crop growth models (CGMs) with AI improves genotype-environment interaction predictions for traits like drought tolerance and yield. AI-based phenotyping platforms like CropQuant-Air use deep learning for wheat spike detection and yield classification, achieving over 97% accuracy. Automated machine learning (AutoML) tools, such as AutoKeras, enhance crop trait classification while reducing computational complexity. AI in genomic selection improves predictive accuracy by integrating molecular markers and environmental data, accelerating breeding cycles. AI-powered speed breeding and synthetic biology open new avenues for plant improvement, ensuring sustainable agriculture and food security.

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Introduction

Plant breeding is crucial in enhancing crop yield, and ensuring food security for a growing population (Qaim, 2023). It helps develop resistant varieties, reducing losses caused by pests and pathogens (Ammar *et al.*, 2024). Additionally, breeding improves stress tolerance, enabling crops to withstand drought, salinity, and extreme temperatures (Paniza, 2024). It also enhances the nutritional quality of crops, addressing deficiencies. By developing high-yielding and resilient varieties, plant breeding contributes to sustainable agriculture.

Plant breeding has evolved significantly from its early stages to modern advancements. Initially, humans practiced selection breeding, choosing superior plants for propagation (Acquaah, 2015). Later, hybridization emerged, enabling controlled crossbreeding to develop improved varieties (Posselt, 2010). Mendel's discoveries in genetics established the basis for scientific plant breeding, allowing structured and precise trait selection. The Green Revolution (1960s-70s) introduced high-yielding and disease-resistant crops, drastically improving food security (Barrett, 2021). The advent of marker-assisted selection (MAS) revolutionized breeding by enabling the precise identification of desirable traits along with their genomic regions (Hasan *et al.*, 2021). Genetic engineering allowed the development of transgenic and genetically modified (GM) crops with enhanced resistance to pests, diseases, and environmental stress (Kumar *et al.*, 2020). The discovery of CRISPR-Cas9 gene editing in plants further enabled targeted genetic modifications and overcame the problem of transgenics related issues (Arora and Narula, 2017). Speed breeding techniques have accelerated crop development cycles, producing new varieties faster (Swami *et al.*, 2023).Modern genomic selection and AI-driven breeding utilize big data for predictive breeding, and optimizing desirable trait selection for the development of superior genotypes (Wójcik-Gront *et al.*, 2024). Synthetic biology and epigenetics are

opening new frontiers in plant improvement (Essemine *et al.*, 2024).

Artificial intelligence (AI) is revolutionizing contemporary agriculture through increased efficiency, accuracy and sustainability exclusively in plant science (Gupta *et al.*, 2024). AI-based predictive analytics and tools assist breeders in the selection of high-yielding and stress-resistant crop varieties (Zhang *et al.*, 2024). Machine learning algorithms scan genomic information, speeding up trait selection and enhancing the efficiency of breeding. Computer vision and drones track plant health, diagnosing diseases and nutrient deficiencies early (Gano *et al.*, 2024). Automated phenotyping facilitates the large-scale screening of desirable traits, minimizing manual intervention (Chawade *et al.*, 2019). Artificial intelligence-based climate modeling is used to predict the environmental effects on crops and assist in adaptive breeding strategies (Gryshova *et al.*, 2024). Smart irrigation systems ensure efficient water usage, enhancing drought tolerance (Bwambale *et al.*, 2022). Further, robotic automation makes precision farming, from seeding to harvesting, easier (Mahmud *et al.*, 2020). AI technology in plant science guarantees increased productivity, efficiency in resource usage, and sustainable agriculture.

This review explores the transformative role of AI in plant breeding and its impact on crop improvement. It highlights key AI technologies such as machine learning, deep learning, and genomic selection in trait prediction and breeding efficiency.

Machine Learning and Deep Learning Approaches for Plant Breeding

Machine Learning (ML) is a branch of artificial intelligence (AI) that enables computers to learn from data (Tyagi and Chahal, 2020) and make decisions or predictions without being explicitly programmed. It uses statistical techniques and algorithms to identify patterns, improve performance over time automatically (Jordan and Mitchell, 2015). Supervised and unsupervised learning are two primary types of machine learning techniques used for different purposes.

Supervised learning involves training a model using labeled data, meaning each input has a corresponding output. The algorithm learns by mapping inputs to the correct outputs based on a given dataset (Nasteski, 2017). Common supervised learning tasks include classification and regression (Li, 2019). Popular algorithms in supervised learning include Decision Trees, Support Vector Machines (SVM), Neural Networks, and Random Forests (Osisanwo *et al.*, 2017). Plant trait identification using **machine learning (ML)** involves analyzing phenotypes at various levels, including plant organs (development), whole plants (growth), and fields (production). Key traits such as leaf area, root volume, fruit size, chlorophyll content, photosynthetic activity, biomass, and yield are influenced by environmental factors like temperature, light, humidity, and soil composition. While traditional sensors measure traits like weight and gas concentrations directly, they cannot capture morphological and geometrical features critical for plant phenotyping **Imaging-based ML techniques** overcome this limitation by extracting structural and functional plant traits using deep learning and computer vision. Algorithms analyse spectral, thermal, and 3D imaging data to track growth, detect stress, and optimize breeding. These approaches enable high-throughput, automated, and non-invasive plant data collection and help in precision breeding (van Dijk *et al.*, 2021).

Unsupervised learning, on the other hand, works with unlabelled data. The algorithm finds patterns, structures, or relationships within the data without explicit guidance(Ghahramani, 2003). Common unsupervised learning techniques include clustering and dimensionality reduction (Greene *et al.*, 2008). Density-based clustering effectively detects clusters of arbitrary shapes and is more robust to noisy data compared to partition- or hierarchy-based methods. Dimensionality reduction (DR) helps extract meaningful information from multi-omics data by reducing redundancy and noise. It is categorized into linear methods like PCA, ICA, and MDS and non-linear methods such as kernel-based techniques, manifold learning, and neural networks (Yan and Wang, 2022). The key difference between the two is that supervised learning relies on predefined labels, whereas unsupervised learning discovers patterns independently.

AI-Driven Phenotyping of Crops

AI-driven phenotyping of crops leverages machine learning and computer vision to analyze plant traits, enabling high-throughput and precise assessment of growth, stress, and yield thereby reducing the entire manpower and cost involved in phenotyping. Techniques like deep learning and spectral imaging enhance trait detection, aiding in crop selection and

genetic improvements.

The study on image-based plant phenotyping approach using convolutional neural networks (CNNs) for plant disease detection, aligning with the International Plant Phenotyping Network benchmark focuses on classifying maize and grape diseases in Turkey, utilizing 1,600 annotated images across eight classes. The custom CNN model achieved 97.03% accuracy, outperforming existing models. The findings support its use for trait selection, linking crop resilience to genetic traits (Ensari *et al.*, 2020). Yet another study showcases image-based plant phenotyping, achieving over 97% accuracy in root and shoot features identification and localization. A fully automated deep learning approach was used for trait identification and QTL detection in root architecture datasets. The model successfully identified 12 out of 14 manually detected QTLs, demonstrating its reliability. Deep learning-based feature detection enhances precision in plant phenotyping (Pound *et al.*, 2017). The study on the use of AutoML for image-based plant phenotyping, using wheat lodging assessment with UAV imagery. The performance of AutoKeras was compared to transfer learning with CNN architectures for classification and regression tasks. Transfer learning with Xception and DenseNet-201 achieved the highest classification accuracy of 93.2%, while AutoKeras followed closely of 92.4%. AutoKeras demonstrated up to 40-fold faster inference times, highlighting the potential of AutoML in advancing crop breeding and precision agriculture (Koh *et al.*, 2021).

Pheno-parenting, inspired by plant phenotyping, utilizes advanced tools to support plant growth across different stages. A Deep Neural Network based approach analyzed plant species recognition, growth, health, and yield stage identification in a hydroponic system with Petunia, Pansy, and Calendula. Side-view images were more effective for species recognition and growth tracking, while top-view images captured leaf texture and flower budding. This technique is used to estimate the Growth Development Index regarding the plant growth precisely which initially increases with nutrient input (up to 31 ml) before reaching saturation (Hati and Singh, 2023). CropQuant-Air is an AI-powered software integrating deep learning and image processing for wheat spike detection and phenotypic analysis using low-cost drone imagery. The XGBoost model was applied for yield-based classification using a dataset of 210 records, split into 70% training (147 lines) and 30% testing (63 lines). Cross-validation was performed during Boosting iterations to optimize the model. The trained XGBoost model classified 101 high-yield, 90 medium-yield, and 19 low-yield wheat varieties. Accuracy validation using confusion matrices showed 97.0% for high-yield, 96.4% for medium-yield, and 94.7% for low-yield groups (Chang-Brahim *et al.*, 2024).

AI-powered disease detection uses machine learning and computer vision to identify plant infections early, enabling timely intervention. Deep learning models classify diseases with high accuracy, improving precision agriculture and reducing yield losses with pre-optimized algorithms and softwares. Banana (*Musa spp.*) production is threatened by diseases and pests, requiring efficient detection for timely intervention. A study developed an AI-based banana disease and pest detection system using deep convolutional neural networks (DCNNs) and transfer learning. ResNet50 and InceptionV2 outperformed MobileNetV1, achieving over 90% accuracy in classifying 18 disease classes. The model demonstrated high predictive accuracy, making it a promising tool for early disease detection (Selvaraj *et al.*, 2019). A robust drone-based deep learning approach enhances the plant disease detection, integrating an improved EfficientNetV2-B4 with additional dense layers. The model extracts deep key points and classifies them using an end-to-end training architecture. Performance evaluation was conducted on the PlantVillage Kaggle dataset and drone-captured samples under diverse conditions. The model achieved 99.63% precision, 99.93% recall, and 99.99% accuracy, outperforming recent techniques. These results confirm the approach's effectiveness, demonstrating superior accuracy with reduced computational complexity(Albattah *et al.*, 2022).

AI Integration in Molecular Breeding

Artificial intelligence is revolutionizing crop breeding and plant science by enabling precise image analysis and genomic modeling. Machine learning (ML) and neural networks (NNs) enhance efficiency, accuracy, and scalability in agricultural research. Explainable AI (xAI) further improves transparency, making AI-driven solutions more reliable for smart agriculture. AI is transforming genome-wide association studies (GWAS) in wheat breeding by addressing key challenges such as high-dimensional data, non-linear trait interactions, and missing heritability. Machine learning models enhance GWAS by overcoming the

limitations of traditional Bonferroni correction and linear regression, improving the identification of SNP-trait associations. Integrating crop growth models (CGMs) with AI-driven genomic analysis allows for a more precise simulation of genotype-environment interactions, aiding in the prediction of complex traits like drought tolerance and grain yield. Additionally, explainable AI (xAI) techniques improve the interpretability of GWAS models, enabling breeders to make informed trait selection decisions while refining genetic predictions for sustainable wheat production (Chang-Brahim *et al.*, 2024).

Genomic selection (GS) is an advanced breeding strategy that integrates artificial intelligence (AI) to improve the selection of desirable crop traits. By analyzing dense molecular markers across the genome, GS enables breeders to predict genetic values without relying on extensive phenotypic assessments, thereby expediting the breeding cycle and enhancing efficiency (Goddard and Hayes, 2007) (www.illumina.com). AI techniques, particularly deep learning, are employed to analyze large datasets, integrating both genomic and environmental information to improve the accuracy of predictions regarding quantitative traits(Jubair and Domaratzki, 2023). This method not only reduces costs and time associated with traditional breeding methods but also increases the genetic gain per year, making it a vital tool for addressing global food security challenges (Bhat *et al.*, 2016; Budhlakoti *et al.*, 2022). AutoGP (http://autogp.hzau.edu.cn) is a web-based platform integrating genotype extraction, phenotypic analysis, and genomic selection (GS) models for genotype-to-phenotype prediction. It features an advanced sequencing chip for high-confidence SNP identification and an automated workflow for plant trait extraction from smartphone videos. Users can choose from five machine learning models (e.g., support vector machine, random forest) and four deep learning models for genomic prediction and trait analysis. AutoGP enhances accuracy and efficiency in genomic selection (Wu *et al.*, 2025). Analysis of multi-omics data from 156 maize recombinant inbred lines, including 2,496 SNPs, 46 image traits from 16 developmental stages, and 133 primary metabolites. Machine learning models like Partial Least Squares (PLS), Random Forest (RF), and Gaussian process with Radial Basis Function kernel (GaussprRadial) improved yield prediction. These models effectively ranked biologically relevant traits linked to photosynthesis and kernel development. Integrating multiple omics data with RF increased yield prediction accuracy from 0.32 to 0.43. This study highlights AI-driven approaches for enhancing crop genetic improvement (Wu *et al.*, 2024).

Future Prospects and Conclusion

The integration of artificial intelligence (AI) in plant breeding is expected to drive major advancements in crop improvement, sustainability, and food security. Future developments will enhance genomic selection (GS), genome-wide association studies (GWAS), and phenotyping technologies, improving the efficiency of breeding programs. AI-powered multi-omics analysis will provide deeper insights into complex trait interactions, leading to more precise trait predictions. Additionally, explainable AI (xAI) will improve model transparency, increasing trust in AI-driven breeding decisions. Advancements in automated machine learning (AutoML) will make AI more accessible, reducing computational complexity and enabling non-experts to apply AI in breeding. AI-driven climate-resilient crop development will help mitigate the effects of climate change by optimizing adaptive breeding strategies. Integrating synthetic biology with AI may further revolutionize plant engineering, creating genetically optimized crops for specific agronomic needs. Despite these advancements, challenges such as data standardization, model generalizability, and ethical concerns remain. Addressing these issues will require collaboration between biologists, data scientists, and agronomists. In conclusion, AI is transforming plant breeding by accelerating genetic improvements, optimizing trait selection, and improving breeding efficiency. Its integration with biotechnology and precision agriculture holds immense potential to enhance food security and ensure sustainable crop production for future generations.

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RESEARCH ARTICLE

Micellar Effect on Itaconic Acid Production by Fermentation Process

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Abstract

Due to the increasing demand and focus for sustainable chemicals and fuels that are independent from fossil resources, itaconic acid gained interest and recognized for market position as a potential bio-based platform chemical. Itaconic acid can be produced via a chemical pathway or a biotechnological pathway, the more effective production way is the latter one, which is currently conducted in industrial scale production. Some *Aspergillus* species, like *A. itaconicus* and *A. terreus*, show the ability to synthesize this organic acid and *A. terreus* can secrete significant amounts to the media. Itaconic acid is an unsaturated organic acid with two carboxyls and one methyl group. The presence of these functional groups, along with a conjugated double bond makes itaconic acid a versatile molecule with a vast number of applications. In the present communication the authoress has studied efficacy of some micelles *i.e.* magnesium dodecyl sulfate on biological production of itaconic acid by *Aspergillus terreus* SS-201. It has been found that the micelles under trial *i.e.* magnesium dodecyl sulfate has beneficial impact and has enhanced yield of itaconic acid to an extent of 16.685% higher in comparison to control (4.429g/100ml).

***(Keywords :** Itaconic acid fermentation process, magnesium dodecyl sulfate and Aspergillus terreus SS-201*

Introduction

Itaconic acid (2-methylidenebutanedioic acid) is an unsaturated di-carbonic acid. It has a broad application spectrum in the industrial production of resins and is used as a building block for acrylic plastics, acrylate latexes, super-absorbents and anti-scaling agents (Willke and Vorlop, 2001; Okabe et al., *et al.*, 2009). Since the 1960s the production of itaconic acid is achieved by the fermentation with *Aspergillus terreus* on sugar containing media (Willke and Vorlop, 2001). Although also other microorganisms like *Ustilagozeae U. maydis, Candida* sp. (Tabuchi *et al.*, 1981) and *Rhodotorula* sp. (Kawamura *et al.*, 1981) were found to produce itaconic acid, *A. terreus* is still the dominant production host, because so far only bred strains of this species can reach levels of up to 80–86 g/L (Okabe *et al.*, 2009; Kuenz *et al.*, 2012). Since the 1990s, itaconic acid as a renewable material is attracting a lot of interest. Although the production costs for itaconic acid are declining in the last years ($ 4 per kg in 2001; Willke and Vorlop, 2001), it is still a valuable product with an estimated price of $ 2 per kg. Currently, the worldwide production capacity of itaconic acid is expected to be about 50 kt per year, facing a demand of about 30 kt (Shaw, 2013, Itaconix Corporation, personal communication). Especially, for the production of polymers it is of interest, because in the future it can function as a substitute for acrylic and methacrylic acid used for the production of plastics (Okabe *et al.*, 2009). However, these applications require an even lower price of the starting material. The current knowledge about the biotechnological production of itaconic acid was recently reviewed (Willke and Vorlop, 2001; Okabe, *et al.*, 2009;Tevz, *et al.*, 2010; Jore, *et al.*, 2011, Li, *et al.*, 2012, Blum Hoff, *et al.*, 2013). The latter review covers the industrial production of itaconic acid and the applications of this product. Therefore, we focus in this report on the recent advances with an emphasis on the biochemistry of the process and new genetic engineering targets. For rational strain improvement, it is essential to understand the underlying biological concepts and biochemical pathways leading to the production of this important organic acid in microorganisms. Itaconic acid (methylenesuccinic acid, $C_5H_6O_4$) (**Table 1**) is a white colorless crystalline, hygroscopic powder soluble in water, ethanol and acetone. It is an unsaturated diprotic acid, which derives its unique chemical properties from the conjugation of one of its two carboxylic acid groups with its methylene group.

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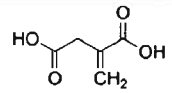
Table 1: Selected properties of itaconic acid

Property	Value
Chemical formula	$C_5H_6O_4$
Chemical structure	
Appearance	White & crystalline
pK_a values	3.84 & 5.55
Molecular weight	130.1 g/mol
Solubility in H_2O at 20°C	83.1 g/L
Boiling point	268°C
Melting point	168°C

Itaconic acid (methylenesuccinic acid, C5H6O4)

Recently Singh reported (Shilpi Singh 2024) Potassium Octyl sulfate very effective for Itaconic acid production and found that the micelles under trial enhances the yield of Itaconic acid to an extent of 16.685% higher in comparison to control.

Micelles are self-assembling amphiphilic molecule consisting of hydrophobic cores and a hydrophilic capsule and can potentially carry hydrophobic drugs encapsulated in the care and enhance their bioavailability. Micelles have been studied as drug delivery carriers for decades.

Many different drug carriers have been developed for controlled drug delivery in recent decades, including micelles, liposomes, polymer or protein-drug conjugates, polymeric nanoparticles and pathogens (Verma G. and Hassan P. (2013), Kamaly, *et al.*, (2012), Service (2010), Zhang, *et al.*; (2008), Service, (2005), Yoo, *et al.*, (2011), Scheinberg, *et al.*, (2010), Petros and DeSimone (2010), Kim, *et al.*, (2009), Guo, *et al.*, (2014), Tong, *et al.*, (2014), Hu, *et al*; (2011). Among these drug carriers, micelles have a number of attractive features. Micelles are self-assembled microstructures formed by surfactants in an aqueous system and are usually < 50 nm in diameter Kulkarni and Shaw (2016).

A tiny particle made of substances that are soluble in water and that come together to form a ball like shape. These particles can carry other substances inside them. Today, science has found various practical applications for micelles. The properties of surfactant substances in aqueous media, been studied by scientists and there are currently several practical applications of the phenomenon of micelles formation.

The process of forming micelles is known as micellisation and forms part of the phase behaviour of many lipids according to their polymorphism (McBain, (1993), Naugdoesht and, Alan, (1997), Hamley (2007), Schurtenberger, *et al.*, (1990), Tung, *et al.*, (2006), Lequeux, *et al.*, (1997). A number of recent review articles have been written on the rheology and applications of wormlike micelle solutions.

Thus, from the above brief review it is evident that micelles are required for exploitation specially for Itaconic acid fermentation and in view of this the authoresses has studied the influence of magnesium dodecyl sulfate on itaconic acid production by *Aspergillus terreus* SS-201

Material and Method

The influence of magnesium dodecyl sulfateo-nitaconic acid production by *Aspergillus terreus* SS-201.

The composition of the production medium for itaconic acid production by *Aspergillus terreus* SS-201 isolate has been prepared as follows :

Glucose: 22.0 g; $MgSO_4.7H_2O$: 0.35 g; NH_4NO_3 :0.90 g; KCl : 0.05 g; NaCl :0.05g; KH_2PO_4: 0.25 g; pH : 2.2.

The pH of the production medium was adjusted to 2.2 by adding requisite amount of NH_4OH buffer solution. The production medium was sterilized in an autoclave maintained at 15 lbs steam pressure for about 15 minutes and cooled to room temperature.

The above composition medium represents volume of a fermentor flask, i.e., 100 ml for itaconic acid production by *Aspergillus terreus* SS-201 isolate.

Now, the same production medium for itaconic acid production by *Aspergillus terreus* SS-201 isolate was prepared for 99-fermentor flask, *i. e;* each contained 100 ml of production medium. The above 99-fermentor flasks were then arranged to 11-sets each comprising of 9-fermentor flasks. Each set was then rearranged in 3-subsets, each consisting of 3-fermentor flasks. The remaining 9-fermentor flasks out of 99-fermentor flasks were kept as control and these were also rearranged in 3-subsets each consisting of 3-fermentor flasks.

After preparing the above sets of fermentor flasks M/1000 solution of magnesium dodecyl sulfatewas prepared and from the above micelle solution 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10 ml was added to the fermentation flasks of above 1st to 10th sets respectively. The control fermentor flasks contained no micelles.

Now, the total volume in each fermentor flasks was made upto 100 ml by adding requisite amount of distilled water. Thus, the molar concentration of magnesium dodecyl sulfate in 1st, 2nd, 3rd, 4th, 5th,

6th, 7th, 8th, 9th and 10th subsets were approximately as given below:

$A \times 10^{-x}$ M, i.e., 1.0×10^{-5}M to 10.0×10^{-5}M

Where A = amount of chemical micelle in ml

x=Molarity of the micelle solution respectively.

The above fermentor flasks were then sterilized, cooled inoculated, incubated at 32°C and analysed after 7, 8 and 9 days for itaconic acid (Bentley and Tniesen, 1957) formed.

Results and Discussion

The data recorded in the table 1 shows that magnesium dodecyl sulfate has stimulatory effect on the itaconic acid production by novel *Aspergillus terreus* SS-201 isolate.

The maximum yield of itaconic acid, i. e. 5.240g/100 ml in the presence of magnesium dodecyl sulfate was observed at 7.0×10^{-5} M molar concentration in 8 days of optimum incubation period which is 18.311% higher in comparison to control fermentor flasks, i.e; 4.429 g/100 ml in the same times course and other same experimental parameters.

The higher molar concentrations of magnesium dodecyl sulfate were not much favourable for the itaconic acid production by *Aspergillus terreus* SS-201 isolate. So the gradual addition of themicelle magnesium dodecyl sulfate after certain concentrations were not beneficial for the itaconic acid fermentation process.

It has been observed that molar concentration of the micelle, i.e.,magnesium dodecyl sulfate from 1.0×10^{-5} M to 7.0×10^{-5} M enhances the yield of itaconic acid to a certain order being 0.338%, 1.264%, 2.912%, 3.680%, 5.960%, 10.363%and 18.311% higher in comparison to control flasks but at 8.0×10^{-5} M to 10.0×10^{-5} M the yield of itaconic acid shifted to be in lower range, i.e., 11532%, 7.044% and 2.072% higher in comparison to previous concentrations of magnesium dodecyl sulfate taken into experimental trials.

It has been observed further that after optimum concentration, i. e., 7.0×10^{-5} M, the addition of the same micelle to the production medium causes fall in the yield of itaconic acid gradually and reaches to 2.032%; However, at all the experimental concentrations of magnesium dodecyl sulfate used for the itaconic acid production by *Aspergillus terreus* SS-201 isolate has been found higher in comparison to control fermentor flasks.

Table-2 : Studies on biotic production of itaconic acid exposed tomagnesium dodecyl sulfate

Concentration of micelle used	*Yield of itaconic acidin g/100mL			% Difference in the yield of I.A after 8 days
	7 days	8 days	9 days	
Control– micelle	2.550	4.429	3.725	-
1.0×10^{-5}M + micelle	2.563	4.444	3.739	0.338
2.0×10^{-5}M + micelle	2.572	4.485	3.755	1.264
3.0×10^{-5}M + micelle	2.626	4.558	3.832	2.912
4.0×10^{-5}M + micelle	2.650	4.592	3.869	3.680
5.0×10^{-5}M + micelle	2.700	4.693	3.942	5.960
6.0×10^{-5}M + micelle	2.799	4.888	4.090	10.363
7.0×10^{-5}M + micelle**	2.957	5.240***	4.310	18.311
8.0×10^{-5}M + micelle	2.856	4.940	4.160	11.537
9.0×10^{-5}M + micelle	2.716	4.741	3.970	7.044
10.0×10^{-5}M + micelle	2.589	4.519	3.789	2.032

** Mean of three observations,*

*** Optimum concentration of micelles*

**** Optimum yield of itaconic acid, (+)ve values indicate % increase in the yield of itaconic acid Experimental deviation (+) 1.5% to 3.5%*

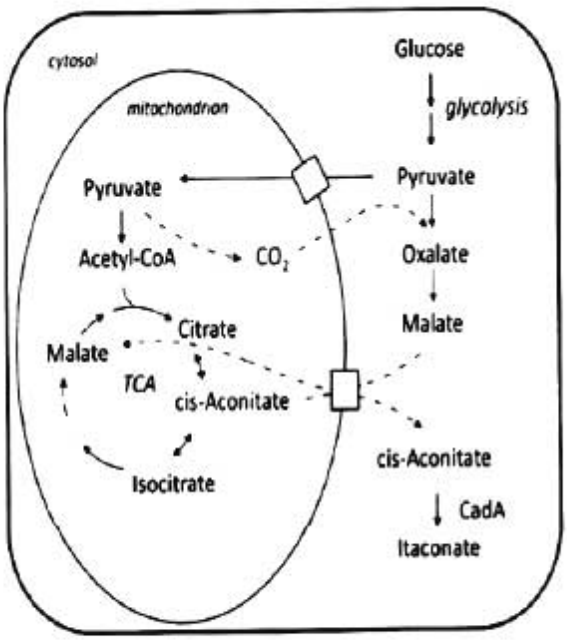


Fig. 1 : Biosynthesis pathway of itaconate

Fig. 2 : Aspergillus terreus

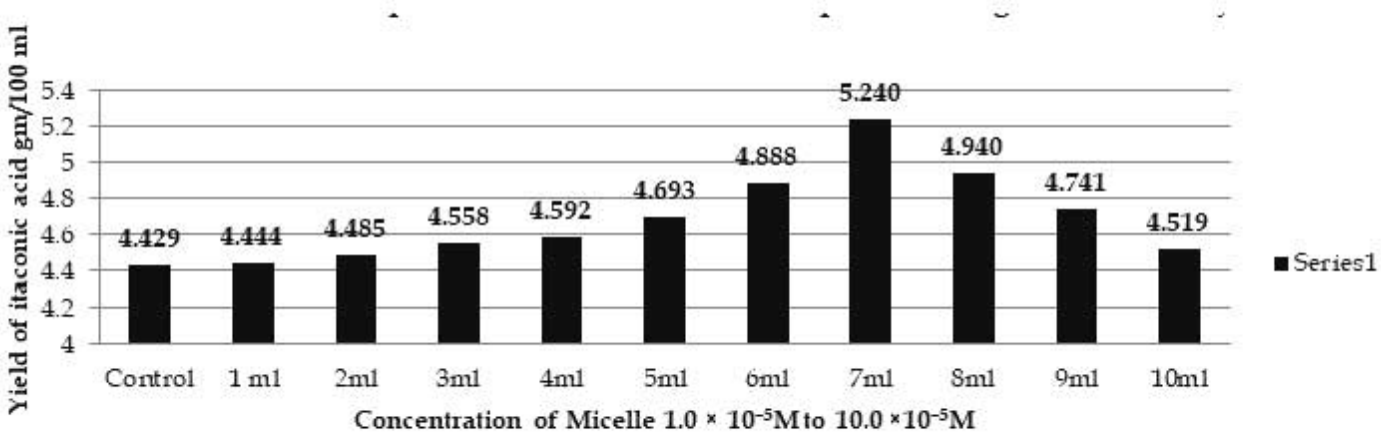


Fig. 3 : Studies on biotic production of itaconic acid exposed to magnesium dodecyl sulfate

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REVIEW ARTICLE

Enhanced Production and Purification of Keratinase from *Paenibacillus koreensis* YC 300

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Abstract

Chicken feather waste is a high-protein byproduct of the poultry industry and a significant environmental concern due to its slow degradation. The current investigation is focused on the production and purification of keratinase from *Paenibacillus koreensis YC 300*. The maximum keratinase activity was observed after culturing *P. koreensis* with 1% inoculum for 5 days in the neutral medium (pH 7) at 45°C. The keratinase was purified with 4% yield, specific activity o 320 U/mg of protein, and a molecular mass of 67 kDa. Keratinase and CFW degradation production were more efficient in submerged fermentation (SmF) than in the solid-state fermentation (SSF) process. The keratinase from fermentation showed optimal activity at pH 8 and 55°C. The structural changes owing to the degradation of CFW feathers were revealed by Scanning Electron Microscopy analysis. This study demonstrates that *P. koreensis* can influence CFW management, thereby offering an eco-friendly approach to the remediation of keratin-rich waste.

Keywords: *Keratinase, SmF, purification, CFW remediation,*

Introduction

A variety of domestic waste is enormously dumped into the environment causing a harsh and serious solid waste disposal problem. The extent of the natural accumulation of domestic wastes reflects a serious cause of toxic compounds and public health threats. Amongst the most complex biological materials is keratin-biomass, which comprises of skin appendages, hair, nails, tortoise shells, horns, beaks, claws, and feathers. The animal meat manufacturing process generates millions of tonnes of keratinous materials and by-products. The quantity of meat produced in 2020 was about 100.5 million tonnes and more than 4.7 million tonnes of poultry feathers around the world. On a yearly basis it is projected that more than 24.8 billion chickens will be produced yearly by 2030 reaching 37.0 billion in 2050 (United Nations of Food and Agriculture Organization.

Keratin is considered as the third most abundant polymer in nature, following cellulose and chitin. Chicken feathers are composed of 91% protein (keratin), 1% lipids, and 8% of water. Keratin is an insoluble scleroprotein highly resistant to physical, and chemical activities owing to the presence of several disulphide (S-S) cross-linkages. The keratinolytic microbes can degrade keratin by secreting keratinase. The biodegradation of keratin involves sulfitolysis and proteolysis. Sulfitolysis is the breakdown of disulfide bridges between the polypeptide chains of keratin which causes the amino acids in the beta-sheet of keratin to shift conformation, resulting in new hydrolytic sites for keratinases. Other methods in practice for keratin breakdown include physical, chemical, and biological processes. Some of these treatments require high temperature and pressure which destroy heat sensitive amino acids (like; tryptophan, lysine, and methionine) and generate significant amount of sulphur and ammonia gases. Therefore, microbial degradation strategy represents a promising alternative eco-friendly technology for recycling keratinous wastes.

Generally, bacteria, fungi, and actinomycetes participate in keratin degradation. However, bacteria represent most abundant keratin-degraders, followed by fungi and actinomycetes. Keratinolytic microbes are widely dispersed in nature and have been isolated from a variety of sources, including decomposing feathers, penguin feathers, poultry waste digester, and slaughter house polluted water. Among potent keratin degrading bacteria are gram-positive and belong to the genus *Bacillus* species including *B. halotolerans, B. cereus, B. licheniformis, B. subtilis; actinobacteria viz., Streptomyces fradiae, Nacardiopsis halotolarns, Amycolatopsi skeratiniphila,* and fungi including *Aspergillus flavus, Trichophyton* sp., *Chrysosporium indicum, Purpureocillium lilacinum* have been reported

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and registered in literature with exceptional keratinolytic activity. Keratinases are mostly extracellular enzymes secreted into the culture medium, but cell-bound and intracellular enzymes have also been discovered.

Although many keratinolytic enzymes have been isolated over the years, the precise mechanism of keratin biodegradation remains to be clearly understood. Mechanistically, keratin biodegradation is thought to involve adsorption of enzyme to the macromolecular surface followed by the catalytic action through the reduction of disulphide bonds (sulfitolysis) and disruption of the peptide chain (proteolysis). This study attempts to characterize a thermotolerant keratinase and optimize its production by fermentation, purification, and biochemical characterization.

Material and Methods

Keratinase from *Paenibacillus koreensis YC 300*

A keratinase-producing strain was isolated from chicken feather waste. The enzyme was extracted from *P. koreensis* cultured in feather meal broth (FMB: g/100ml: 0.05 NaCl, 0.04 KH_2PO_4, 0.03 K_2HPO_4, 1 chicken feather) and incubated at 45°C, 100 rpm for 5 days. After centrifugation (10,000 rpm, 10 min, 4°C), the supernatant was used for keratinase assay. Activity was measured using azocasein as substrate at 55°C and pH 8.0, with absorbance read at 440 nm. One unit of activity corresponded to an absorbance change of 0.01 in 10 minutes. Protein content was determined by the Folin–Ciocalteu method and free amino acids by the ninhydrin method.

Optimization of Keratinase Production

The growth of *P. koreensis* was evaluated under varying conditions. pH levels (5–9), temperatures (35–55°C), and different nitrogen (ammonium sulphate, urea, yeast extract, peptone) and carbon sources (glucose, sucrose, mannose) were tested in 100 mL of FMB. Feather concentrations (0.1–2%) and inoculum sizes (1–5%) were also varied to assess their effects on growth. Finally, keratinase production was monitored at different time intervals (12–120 hours) using optimized conditions.

Production of keratinase in Solid State Fermentation (SSF) and Submerged Fermentation (SmF)

Solid State Fermentation (SSF)

SSF was carried out by inoculating 1 mL of overnight culture into 80 mL of FMB (40% moisture) containing 2 g of chicken feathers. The setup was incubated at 45°C without agitation for 5 days. Keratinase activity, free amino acids, and feather degradation were measured every 5 days.

Moisture = Volume of medium (ml)/ chicken feathers (g)× 100%

Submerged Fermentation (SmF)

In SmF, 1 g of feather was added to FMB, sterilized, and inoculated with 1 mL of overnight culture. The culture was incubated at 45°C, 100 rpm for 15 days. After day 15, fresh FMB was added, and fermentation continued to day 30 with samples taken every 5 days for analysis.

Scanning electron microscopy (SEM) of chicken feathers

The feathers after fermentation by SSF and SmF were collected, washed with distilled water, dried at room temperature, and subjected to SEM to examine alterations in morphological features.

Fourier Transform Infrared Spectroscopy (FTIR) analysis of chicken feathers

The changes in functional groups occurring during degradative fermentations by SmF and SSF were analysed by FTIR analysis.

Purification of Keratinase

The fermentation broth was centrifuged (10,000 rpm, 10 min, 4°C), and the supernatant was used for keratinase purification.

Ammonium sulphate fractionation

Keratinase was precipitated with 0–80% ammonium sulfate under stirring at 4°C for 1 hour, followed by overnight refrigeration. The precipitate was recovered by cold centrifugation and dissolved in 0.1 M Tris buffer (pH 8.0).

Dialysis

The protein solution was dialyzed overnight at 0–4°C against 10 mM Tris buffer (pH 8.0).

Ion exchange chromatography

The dialyzed sample was applied to a DEAE column pre-equilibrated with 0.5 M Tris buffer (pH 8.0). Elution was done with a 0.5 M NaCl gradient at 24 mL/h. Active keratinase fractions were pooled.

Gel filtration chromatography

The pooled enzyme was further purified using a Sephacryl S-300 column, eluted with 0.5 M Tris buffer (pH 8.0) at 10 mL/h. Fractions were analyzed for keratinase activity and protein content.

Biochemical Characterization of Enzyme

Determination of molecular weight of keratinase by SDS-PAGE

Keratinase-active fractions from gel filtration were pooled, lyophilized, and analyzed by SDS-PAGE (12% resolving, 4% stacking gel). Molecular weight was determined by comparing *Rf* values with standard proteins.

Effect of pH and temperature

Studies on the effect of pH and temperature on keratinase were performed as described before using 50 mM Acetate buffer (pH 4.5 – 5.5), 50 mM Phosphate buffer (pH 6.0 – 7.0), 50mM Tris buffer (pH 7.5 – 9.5) and temperature range of 25°C - 70°C. The experiments on the effect of pH and temperature on keratinase stability were carried out by incubating the keratinase at pH (4.5 – 9.5) and temperature (25 - 70°C). The standard enzyme assay then determined the enzyme activities.

Effect of Enzyme Inhibitors

The inhibitor effect was assessed by incubating the keratinase with 1 – 10 mM of protease inhibitor; phenylmethanesulphonyl fluoride (PMSF), ethylenediaminetetraacetate (EDTA), and iodoacetamide (IAA) at 30°C for 10 min.

Determination of K_m and V_{max}

Keratinase kinetic parameters were measured at different substrate concentrations (2 – 10 mg) under optimum assay conditions, Michaelis-Menten constant (K_m) and maximum velocity (V_{max}) values were determined with Lineweaver-Burk plots.

Statistical Analysis

All the experiments were performed in triplicate, and data are presented as mean ± standard deviation (SD) using GraphPad Prism version 10.

Table-1: Properties of the culture broth after 5 days of keratinase production by P. koreensis (mean ± SD, n=3).

Keratinase	Units
Keratinase activity	1.8 U/ml ± 0.2517
Protein	0.096mg/ml ± 0.01930
Free Amino acids	0.013435mg/ml ± 0.0100
Specific activity	18.75U/mg ±1.600

Results

P. koreensis was isolated from CFW and cultured in FMB. After 5 days, keratinase was detected in the culture supernatant. The following results were observed.

Optimization of growth conditions for keratinase production

Keratinase production was optimized under different conditions. The highest enzyme activity (98.29 U/mg ± 1.105) and free amino acid content (8.09 mg/mL± 0.130) were observed at pH 7.0. The optimal temperature was 45°C, with keratinase activity of (74.17 U/mg ± 3.66) and amino acid release of (10.29mg/mL ± 0.9552). Peptone was the most effective nitrogen source, yielding (11.39 U/mg ± 1.631) of keratinase and (0.37 mg/mL ± 0.031) of amino acids. Glucose supported the highest keratinase production (8.18 U ± 0.556). A 1% feather concentration gave maximum enzyme activity (28.05 U/mg ± 2.55), while 2% yielded the most amino acids (0.38 mg/mL ± 0.059). An inoculum size of 5% was ideal, producing (24.75 U/mg ± 0.6502) keratinase and (1.04 mg/mL ± 0.096) amino acids. The best incubation time was five days, resulting in (59.14 U/mg ± 0.2452) of keratinase and (7.702 mg/mL ± 0.5474) of free amino acids.

Comparative study on keratinase production and feather degradation by Solid State Fermentation (SSF) and Submerged Fermentation (SmF)

Keratinase and free amino acid production varied between SSF and SmF. Under SSF, maximum levels were reached by day 5 (9.527U/mg ± 0.4446 keratinase; 0.03 mg/mL ± 0.0010 amino acids), while in SmF, peak production occurred at day 25 (34.21U/mg ± 0.744 keratinase; 0.015 ± 0.0004163 mg/ml amino acids).

SEM analysis

P. koreensis treatment caused noticeable feather degradation. Unlike the intact structure in untreated feathers (Fig. 1), those under SSF and SmF showed partial breakdown of the rachis, barbs and barbules.

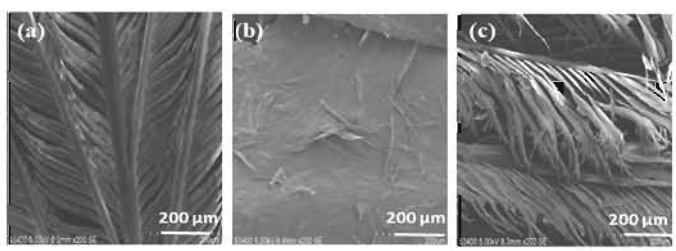

Fig. 1. SEM micrographs: Control a; partially degraded feather by SmF b; and partially degraded feather c by SSF.

FTIR analysis

FTIR analysis (Fig. 2) of feathers treated with *P. koreensis* showed reduced disulfide bonds and changes in protein secondary structure compared to untreated feathers.

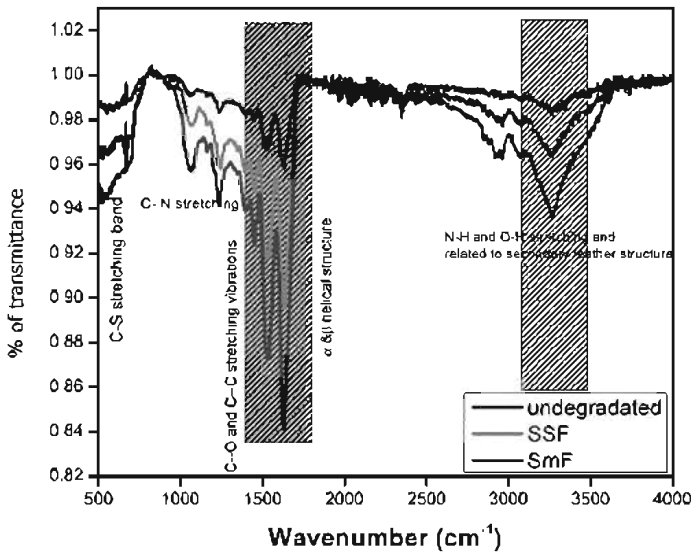

Fig. 2 : FTIR analysis of feather degraded under SSF and SmF.

Purification of Keratinase

Keratinase from *P. koreensis* was purified using 80% ammonium sulfate precipitation, DEAE-Sepharose ion exchange, and Sephacryl S-200 gel filtration. Peak activity was observed in DEAE fractions 23–30 and Sephacryl fractions 11–17 (Figs. 3, 4). The process achieved a 4% yield and 25-fold purification (Table 2).

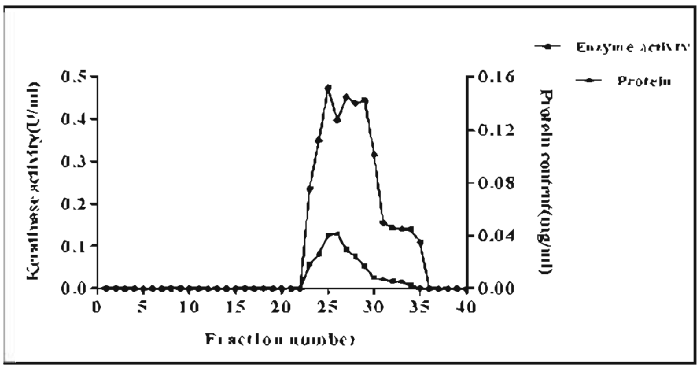

Fig. 3 : Elution profile of keratinase from P. koreensis by DEAE cellulose chromatography.

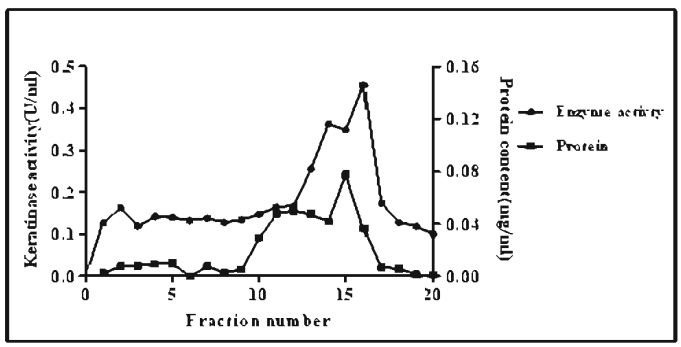

Fig. 4 : Elution profile of keratinase from P. koreensis by Gel filtration column chromatography.

Table-2 : Purification of P. koreensis keratinase.

Purification Steps	Volume (ml)	Total activity (U/ml)	Total protein (mg/ml)	Specific activity (U/mg)	Fold purification	% Yield
Crude	100	96.17	0.987	99.45	1	100
Ammonium sulfate fractionation (0-80%)	15	82.11	0.761	107.05	1.303	76.70
DEAE Ion Exchange Chromatography	28	22.8	0.104	219.23	9.61	10.400
Sephacryl S-300 gelfiltration Chromatography	8	12.8	0.0040	320	25	4

Keratinase biochemical properties

SDS-PAGE revealed the molecular weight of purified keratinase from *P. koreensis* YC300 as 65 kDa (Fig. 7). The enzyme showed optimal activity and stability at 55°C (Fig. 6) and pH 8.0 (Fig. 5), but lost activity at higher temperatures. PMSF strongly inhibited keratinase, while EDTA and IAA caused partial inhibition, indicating it's a serine protease (Tab 3). Kinetic studies were performed at pH 8.0 and 55°C (Fig. 5).

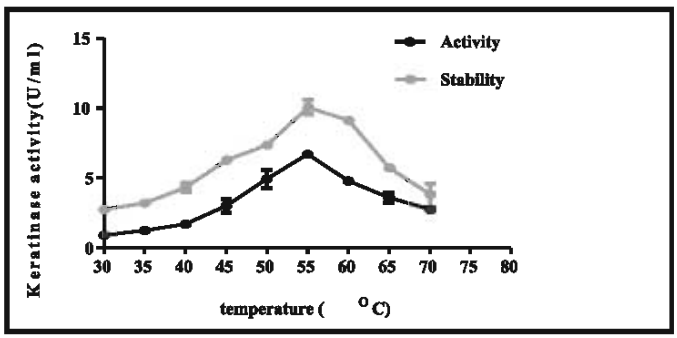

Fig. 6. Effect of temperature on the activity and stability of purified keratinase (Data are mean ± SD; n = 3)

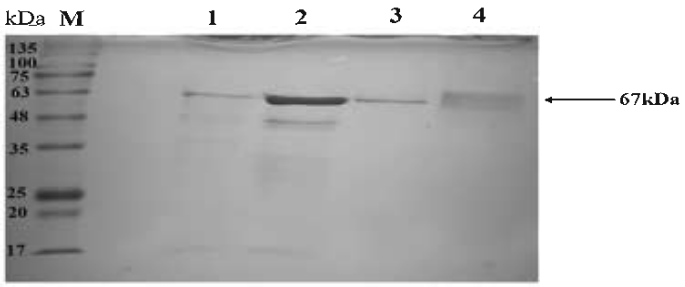

Fig. 7. Determination of molecular weight of P. koreensis keratinase by SDS-PAGE. Lane M : protein ladder, Lane 1: Crude, Lane 2: Ammonium sulphate (80%) fractionation, Lane 3 : DEAE column fraction and Lane 4 : Sephacryl S-300 Gel filtration column fraction

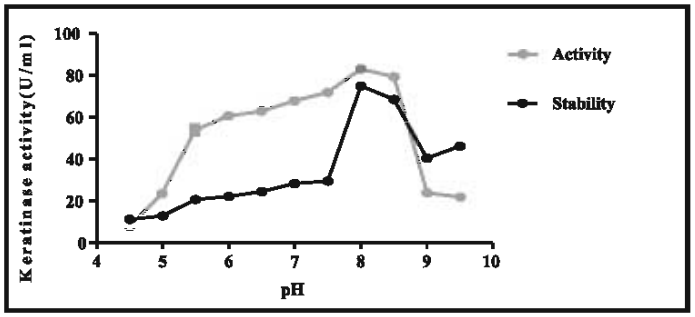

Fig. 5. Effect of pH on the activity and stability of purified keratinase, Data are mean ± SD (n = 3)

Table-3 : Effect of inhibitors on keratinase from P. koreensis

Inhibitors	% inhibition	
	10mM	5mM
PMSF	81	55
EDTA	7	2
IAA	28	18

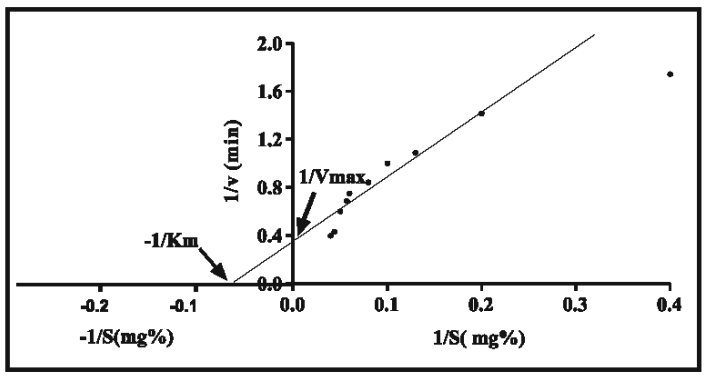

Fig. 8. Lineweaver-Burk plot for keratinase (azocasein as a substrate) from P. koreensis

Discussion

Microorganisms are the resources of variety of degradative enzymes including keratinases. The primary step involved in the commercial enzyme production is to find a suitable microorganism that can produce enzymes in high concentrations. This is followed by optimization of the process parameters (media, pH, temperature, etc.). Screening of the soil from poultry farms resulted in isolation of a feather-degrading bacterium. The bacterium was identified as *Paenibacillus koreensis YC300* based on 16S rRNA gene sequencing and phylogenetic analysis (NCBI Accession number: PP059639). The bacterium belongs to *Paenibacillus* genus which is equivalent to *Bacillus*. The literature survey resulted in a report on the degradation of CFW by *Paenibacillus woosongensis TKB2* degrading 87 % CFW (0.8 %, w/v) in 2 days. This is the first communication reporting the degradation of feathers by *P. koreensis*. The optimum culture conditions for keratinase production have been investigated and established for *P. koreensis*. The maximum activity observed at pH 8 and 55°C indicates that keratinase is alkaliphilic and thermophilic. The pH is known to influence keratinase production by involving in the transfer of various nutrients in and out of the microorganism cell membrane. The bacterium *P. koreensis* showed maximum keratinase activity at pH 7.

Keratinases from most microorganisms are best active in the range of neutral and alkaline pH. The enzyme required for industrial purpose is expected to retain its activity over a wide range of pH. Temperature is another important parameter that influences production by regulating its biosynthesis and energy metabolism. The optimum temperature for keratinase production and amino acids was found to be 45°C. Many keratinolytic bacteria often show profuse activity at higher temperature from 30°C - 55°C. An optimal temperature of 70°C has been reported for *Thermoanaerobacter* and *Fervidobacterium* spp. In our study chicken feather served as the only source of carbon and nitrogen for keratinase production. The experiments showed that incorporating additional simple sugars like glucose into the culture medium lead to decreased keratinase production, in contrast inclusion of complex carbohydrates like starch showed elevated keratinase production. Among the various substrates incorporation of feather concentration of 1.0% has shown the highest keratinase and amino acid production and 2%. The feather can act as substrate and inducer or inhibitor for keratinase production. Inoculum size of 2.0 % showed enhanced keratinase and amino acid production. A study reported the optimum production of keratinase at 2% inoculum for *Bacillus cereus*. Another study found that increasing the inoculum concentration to 4% resulted in an increase in keratinase synthesis by *B. cereus*. The accumulation of keratinase and amino acids in culture broth occurred from 24 h to 120 h.

Comparative analysis showed that *P. koreensis* in SmF led to higher keratinase activity and 68% feather degradation, compared to 33% in SSF. Unlike previous reports favoring SSF for better degradation, this study found SmF more effective, likely due to its aqueous nature supporting enzyme secretion. SEM analysis confirmed distinct morphological changes in feathers under both conditions. The SEM results demonstrated that the bacterium was able to hydrolyse soft structures (outer vane, inner vane, and parallel barbs) completely in SSF whereas in SmF hard structures (feather tip and rachis) were degraded partially. The FTIR spectral observations displayed peaks at 3200-3400 cm^{-1} corresponding to the OH and SH, followed by peaks at 1300,1400, 1500 and 1600 cm^{-1} corresponding indicate C-N, C-O, C=O, and α, β helical structures in proteins. The degraded feathers under SSF and SmF showed peaks of high resolution (red & blue). Among SmF and SSF, SmF showed efficient degradation as indicated by broad and intense peaks at 3400 and 3300 cm^{-1} free N-H and O-H stretching groups. A sharp peak at 1600, and 1560 cm^{-1} reveals the presence of keto and amide groups. The 1350-1400 cm^{-1} spectral peaks correspond to the C-C and C-O bonds. A weak C-S stretching band is observed at the lower wavenumber region (600). Some small peaks correspond to the binding modes of amide groups. The above results suggest that feather degradation under SmF is more than SSF. The results obtained in the current study showed more efficiency of feather degradation.

The keratinase has been purified sequentially by ammonium sulphate precipitation, dialysis, ion exchange and gel filtration procedures (Table 2). Similar purification strategy has been adopted for enzyme purification from *Bacillus subtilis* KD-N2, *Bacillus subtilis*, *Bacillus tequilensis* strain Q7. The molecular weight of keratinase is established to be 67kDa by SDS-PAGE analysis. However, previous reports hint at presence of varying keratinase mass in other organisms; *Bacillus sp. JB 99* (66kDa), *Streptomyces albidoflavus* (18 kDa), *Bacillus sp. SCB3*(134kDa), *B. cereus DCUW* (80kDa), *Bacillus tequilensis* strain *Q7* (28.3 kDa), *Bacillus subtilis* (40kDa) and a keratinase between 26 and 130 kDa. The keratinase from *P. koreensis YC 300* is inhibited by PMSF, hence it belongs to a serine protease family. Several reports have shown that metalloprotease inhibitors influence serine proteases slightly and keratinase from gram-positive bacteria are mostly serine proteases. The kinetic studies on enzymes aim to measure the affinity with which the enzyme binds to substrate and the turnover rate. There is a report that shows a sigmoidal curve for varied concentrations of substrate and also enzyme had a high Km value. Thus, this attempt achieved the successful optimization and purification of keratinase from *P. koreensis* that marks a significant step towards development of a sustainable biotechnological processes.

Conclusion

In conclusion, this study successfully optimized the

physical parameters required for keratinase production by *P. koreensis,* achieving optimal enzyme activity and efficiency under specific pH and temperature conditions. The identification of *P. koreensis* as a novel keratinase producer highlights the vast potential of natural microbial sources for industrial applications enabling the development of sustainable and eco-friendly solutions. This study contributes significantly to the expanding field of keratinase research, identifying *P. koreensis* as a promising resource for industrial applications.

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REVIEW ARTICLE

Microbial Inoculants for Enhanced Nutrient Uptake in Organic Vegetable Production

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Abstract

Plant biostimulants or agricultural biostimulants, include diverse substances and microorganisms that enhance plant growth. These foster plant growth and development throughout the crop life cycle from seed germination to plant maturity in a number of ways, including improving the efficiency of the plant's metabolism to induce yield increases and enhanced crop quality; increasing plant tolerance to and recovery from abiotic stresses; facilitating nutrient assimilation, translocation and use; including sugar content, colour, fruit seeding, etc. enhancing quality attributes of produce, rendering water use more efficient; enhancing certain physicochemical properties of the soil and fostering the development of complementary soil micro-organisms. Microbial Inoculants including plant growth promoting rhizobacteria, arbuscular mycorrhizal fungi and *Trichoderma* spp. are applied to plants with the aim to enhance plant resilience and also to improve nutrient uptake and translocation. By embracing the power of microbial-inoculants, sustainable agriculture can be achieved, contributing to a more harmonious relationship between food production and the natural environment. To satisfy our agricultural requirements, beneficial microorganisms are better alternatives to conventional farming methods.

Keywords*: biostimulants, microbial-inoculants, nutrient uptake, translocation*

Microorganisms in the soil play a crucial role in soil biodiversity and coordinated nutrient management. They are essential to the growth and evolution of plants. Recent years have seen the use of chemical fertilizers in agriculture, making the nation more self-sufficient in food production, but at the expense of the ecosystem and the well-being of all living things. The excessive use of these fertilizers in agriculture is expensive and has several negative impacts on soil fertility (Suyal *et al.*, 2016). To satisfy our agricultural requirements, beneficial microorganisms are better alternatives to conventional farming methods. Biofertilizers are safer than chemical fertilizers because they cause less environmental harm, have more focused activity, and are more efficient when used in lesser amounts. Additionally, they have the capacity to multiply while being concurrently regulated by the plant and local microbes. Additionally, microbial inoculants have quicker decomposition processes and are less likely to cause pathogens and pests to develop resilience (Suyal *et al.*, 2016).

Microbial inoculants do not show any detrimental impact on the soil's plant and animal life as they are ecofriendly, highly efficient, and can be utilized as bio pesticides that do not affect any harmful influence on plant products. The plant requires mineral nutrients which can only be provided when chemical fertilizers are used directly or indirectly, along with organic manure and biofertilizers to increase the organic carbon in soil and uphold sustainability in a field and horticultural crop (Pathak *et al.*, 2016). Microbial inoculants are described as organisms that are introduced into an environment for a particular purpose, such as biocontrol or promoting plant growth, such as bacteria, fungi, and other microorganisms. The term bio-fertilizer refers to a wide range of products that contain living or dormant microorganisms, including bacteria, fungi, actinomycetes, and algae. Upon application, these microorganisms help to fix atmospheric nitrogen or solubilize/mobilize soil nutrients in addition to secreting substances that promote plant growth (Kaminsky *et al.*, 2019). Now a day, biofertilizers and bio pesticides are currently available as substitutes for conventional inorganic fertilizers and synthetic pesticides respectively along with a variety of other products.

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Organic farming has generated significant interest among consumers and scientists owing to their healthier and safer characteristics to human health. However, nutrient (N and P) availability has been identified to be a major yield-limiting factor in many organic farming systems (De Pascale *et al.*, 2017). Microbial Inoculants including plant growth promoting rhizobacteria, arbuscular mycorrhizal fungi and *Trichoderma* spp. are applied to plants with the aim to enhance plant resilience and also to improve nutrient uptake and translocation. Their application can overcome nutrient limitation typical of organic systems by improving nutrient availability, uptake and assimilation, and consequently reducing the gap between organic and conventional yields (De Pascale *et al.*, 2017).

Several studies conducted on greenhouse and open-field vegetables suggest that applications of biostimulant substances can promote nutrient uptake and assimilation (Catrock *et al.*, 2015). The increase of plant nutrient uptake has been often attributed to one or more of the following factors: an increase in soil enzymatic and microbial activities, modifications in root architecture as well as an enhancement in micronutrient mobility and solubility (Ertani *et al.*, 2009; Cartoux *et al.*, 2015, Lucini *et al.*, 2015). In addition to biostimulant substances, the use of microbial inoculants in horticulture has been also on the rise during the last two decades. Microbial inoculants mainly include plant growth promoting rhizobacteria (PGPR) *and* endophytic fungi such as AMF *and* *Trichoderma* spp. (Calvo *et al.*, 2014; Catroux *et al.*, 2015; Rouphael *et al.*, 2017b). These useful bacteria and fungi have the potential to contribute to sustainable plant growth promotion even under nutrient limitation typical of organic farming. The plant growth promotion resulting from better nutrient uptake induced by microbial based biostimulants has been associated to several mechanisms such as:

1) supplying soil nitrogen (i.e., by biological N2 fixation);
2) making soil nutrients more available to plant uptake (i.e., by solubilization of mineral phosphates and other nutrients through the production of small metal-binding molecules such as organic acids and siderophores, and the release of specific enzymes like phosphatases);
3) increasing plant access to soil nutrients (i.e., by increasing the volume of soil accessed by the root system) (Hayat *et al.*, 2010; Calvo *et al.*, 2014; Catroux *et al.*, 2015b; Rouphael *et al.*, 2015b)

Harnessing microbial-inoculants for sustainable agriculture offer a promising avenue towards ecological farming practices that promote environmental health, crop productivity, and long-term sustainability (Yadav *et al.*, 2018). This article explores the diverse ways in which microbial inoculants can be utilized to achieve these goals. Biofertilizers, such as nitrogen-fixing bacteria, contribute to nutrient management by reducing reliance on synthetic fertilizers and minimizing nitrogen runoff (Yadav *et al.*, 2018). Biopesticides derived from microorganisms provide effective alternatives to chemical pesticides, reducing environmental pollution and protecting beneficial organisms. Plant growth-promoting rhizobacteria (PGPR) and mycorrhizal fungi enhance nutrient uptake, promote plant growth, and increase crop resilience. Furthermore, they participate in bioremediation, aiding in the restoration of contaminated soils. By embracing the power of microbial-inoculants, sustainable agriculture can be achieved, contributing to a more harmonious relationship between food production and the natural environment (Yadav *et al.*, 2018).

Most of the microorganisms under development or already commercialized as microbial inoculants can be broadly classified as plant growth-promoting bacteria (PGPB) or root-colonizing rhizobacteria (PGPR) and their fungal equivalents. PGPB/PGPR promote plant growth via a range of both indirect and direct mechanisms, including providing plants with specific compounds, water or nutrients (biofertilizers) or by providing protection against pests and diseases (biopesticides) The level of understanding of the mechanisms underpinning these beneficial effects varies widely and single isolates can deploy multiple mechanisms for plant growth enhancement.

Insufficient nitrogen in soil severely limits crop productivity *and* the biofertilizer market remains dominated by nitrogen-fixing bacterial strains capable of forming symbiotic relationships with specific legumes. For many legume varieties, inoculation with the correct rhizobial partner is essential for crop establishment where the required strain is not already present in soil. Legume inoculation has been standard agricultural practice since the middle of the last century and can be considered 'the success story' of applied soil microbiology (Catroux *et al.*,2015).The legume inoculant industry is well established, and a wide range of rhizobia products are available around the world. Free-living N-fixing microorganisms

suchas *Azospirillum and Azotobacter* spp. have frequently been developed into inoculant products. *Azospirillum* is one of the most studied genera of PGPB in the world, and there are currently over 100 products based on this genus in South America alone; the majority are registered for use in wheat and maize, but there are recommendations for use in sixteen different crops (Cassan *et al.*, 2020).

Large reservoirs of phosphorus (P) are often present in agricultural soils but occur in forms that are unavailable for plant uptake, requiring ongoing application of P fertilizers. Given the current issues around security of supply, the cost of extraction, processing and shipping of P, and the environmental impacts of P fertilizers on water quality, there is increasing interest in better management of the pool of P existing in soil using P-solubilizing microorganisms. A wide range of microorganisms have been shown to play a role in biogeochemical cycling of inorganic and organic P in the plant rhizosphere and rapid commercial growth of inoculants based on P-solubilizing microorganisms is expected in the future (Rafi *et al.*, 2019).P-solubilizing bacteria (PSB) are heterotrophic bacteria selected for their capacity to solubilize sparingly soluble phosphate compounds in artificial media through secretion of low molecular weight organic ions, which acidify the medium (Barrow and Lambers, 2022). PSB are reported to aid in desorption of inorganic P *and* complex organic P compounds from clay particles in soil by acidifying the rhizosphere, thereby increasing the solubility of precipitated inorganic P salts. Organic ions also solubilize Ca, Fe, Al and Zn-phytate salts, which can increase access of these organic P compounds to enzyme hydrolysis. Like plant roots, rhizosphere microorganisms also produce various enzymes including phytases that enable microbes to access soil P but the relative contribution of microbial enzymes in comparison with plant-derived soil enzymes remains unknown. P-solubilizing bacterial strains have been isolated from a wide range of the genera including *Pseudomonas, Bacillus and Burkholderia* (Hsu *et al.*, 2015).

Microbial inoculants play a significant role in enhancing nutrient uptake *and* promoting plant health in organic agriculture. These inoculants contain beneficial microorganisms, such as bacteria, fungi, and archaea, that can improve soil fertility, suppress plant pathogens, and enhance nutrient availability to plants. In organic vegetable production, where synthetic inputs are limited, microbial inoculants offer a sustainable and environmentally friendly approach to improving crop yields. To harness the benefits of microbial inoculants in organic vegetable production, farmers should carefully select the appropriate inoculant based on the specific needs of their crops and soil conditions. Application methods vary but generally involve inoculating seeds, seedlings, or the soil directly. Some inoculants can also be applied as foliar sprays or through irrigation systems.

Thus, microbial inoculants offer a sustainable and environmentally friendly approach to enhancing nutrient uptake, promoting plant health, and improving crop yields in organic vegetable production. By harnessing the power of beneficial microorganisms, organic farmers can reduce their reliance on synthetic inputs *and* enhance the sustainability of their farming practices.

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RESEARCH ARTICLE

Optimization of Fermentation Parameters for Ethanol Production

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Abstract

One of the processes employed for manufacture of ethanol is fermentation of molasses by the action of enzymes. Generally the fermentation processes yield high value chemicals from low cost materials. Therefore, the present experiment is carried out to obtain optimum conditions for fermentation of synthetic molasses in the presence of *Saccharomyces cerevisiae* (yeast) enzyme. *Saccharomyces cerevisiae* is the cheapest strain available for the conversion of biomass substrate. In the present study, it is used for bio-ethanol production from sugar molasses. Synthetic molasses is subjected to fermentation in the presence of S. cerevisiae NCIM -2232 (yeast). The effects of pH, temperature, sugar concentration of solution and incubation period are studied. The pH is varied from 2 to 5.5. The temperature is changed from 20 to 52 °C. Sugar concentration of solution is taken from 1% to 30%. Addition of yeast is in the range of 1 to 8g per liter. From this study the optimum pH is found to be 4.25. The optimum initial solution temperature is 30°C. Optimum values of sugar concentration is 18% w/v and incubation period is 50 hours. Ethanol is a very important industrial chemical which has plenty of applications both as a base chemical and as an intermediate. As a solvent, it is found in paints, tinctures, markers, perfumes and deodorants. It is used in the manufacture of alcoholic beverages, as antifreeze in automobile radiators, as a preservative for biological specimens etc.

Keywords *Alcoholic fermentation, Saccharomyces cerevisiae NCIM-2232, molasses*

Introduction

Biofuel research gained huge importance due to the projected rapid decrease in fossil fuel reserves because of increased global demand (Campbell and Laherrere, 1998). Use of ethanol as fuel is expected to reduce climatic change and global warming (Sheehan and Himmel, 1999) by bringing about 86% reduction in greenhouse gas emissions (Wang, 2005) and increased interest to develop rural economies by establishing agro-dependent industries (Oscar and Carlos, 2008). Bioethanol is produced by the fermentation of sugars by microorganisms such as *Saccharomyces cerevisiae, Zymomonas mobilis* (Gi-Wook *et al.*, 2008), *Mucor indicus* (Anna *et al.*, 2005), thermophillic bacteria like *Clostridium thermocellum* and *Clostridium thermohydrosulfuricum* (Lovitt *et al.*, 1984), filamentous fungi-*Monilia* sp., *Neocallimastix* sp., *Trichoderma reesei* and *Fusarium oxysporum* (Xu *et al.*, 2009); and *Clostridium phytofermentans* has the ability use more number of carbohydrates (Cantarel *et al.*, 2009) and the feasibility of its industrial use is under study (Christian *et al.*, 2010). However, bacteria produce less ethanol in large-scale fermentation, by-products, susceptible to high ethanol concentrations, can only grow at narrow and neutral pH range of 6.0 to 8.0 (Bothast *et al.*, 1999) and are prone to more viral infections (Jones *et al.*, 2000). *Zymomonas mobilis* isolate can only ferment glucose, fructose and sucrose. On the other hand, genetically engineered *Saccharomyces cerevisiae* can consume more xylose than genetically engineered bacteria (Lau *et al.*, 2010). Commercial ethanol production with engineered microorganisms has not succeeded so far (Laluce *et al.*, 2012). Among these, *Saccharomyces cerevisiae* is the most preferred organism for industrial ethanol production. Therefore, though yeasts were isolated from number of sources, still search for new yeasts or *Saccharomyces cerevisiae* strains is on involving various carbon sources of ethanol production such as fruit juices (low glucose), starch (high initial glucose and high ethanol), lignocelluloses (multiple sugars). Moreover, high initial sugar level is vital to get more ethanol accumulation and to reduce production costs (Gírio *et al.*, 2010). Therefore, industrial strains should possess characters such as high tolerance towards carbohydrate, ethanol and salt, be able to produce ethanol from various sugars, good yield and so on.

Nowadays the petroleum products are running out of race due to unbalanced relation between supply and

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demand besides air pollution of sources. The hike in petrol cost is mainly due to shortage of resources which leads to search for alternate fuel to replace fossil fuels. An eco-friendly bio-ethanol is one such alternate fuel that can be used in unmodified petrol engines with current fueling infrastructure and it is easily applicable in present day combustion engine, as mixing with gasoline (Hansen *et al.*, 2005). Combustion of ethanol results in relatively low emission of volatile organic compounds, carbon monoxide and nitrogen oxides. The emission and toxicity of ethanol are lower than those of fossil fuels such as petroleum, diesel etc., (Wyman and Hinman, 1990). More than a few decades, though there have been several reviews of literature (Palmarola *et al.*, 2005, Dale, 1987, Ferrari *et al.*, 1992, Martin *et al.*, 2006, Nigam, 1992, Olsson and Hahn-Hagerdal, 1996) available for the production of bio-ethanol from various sources, only a very few authors (De Vasconcelos *et al.*, 1998; Doelle and Greenfield, 1985; Huertaz-Díaz *et al.*, 1991) have studied kinetics for the production of ethanol from sugar cane using yeast cells (*Saccharomyces cerevisiae*). Hence in this present research an attempt has been made to optimize the variables which affect the bio-ethanol production from sugar molasses and the experimental results are compared with the available reaction kinetics.

In the present communication the author has confined his study to optimize the parameters for maximum production of ethanol by fermentation using the yeast strain of *Saccharomyces cerevisiae* NCIM-2232.

Material and Method

Ethanol production by yeast *Saccharomyces cerevisiae* NCIM-2232. The composition of the medium employed for the study of the effect of different substrates are given as under:

Different substrates: 10% (w/v), Malt extract: 1.15%, Yeast extract: 1.15%, Peptone: 1.15%, $(NH_4)_2HPO_4$: 1.15%, pH: 4.25

The pH 4.25 of the above medium was adjusted and maintained by adding requisite amount of buffering agent lactic acid. The above composition medium was made 100 ml by adding requisite amount of distilled water. Now, the same composition medium was prepared for 48 conical flasks and these flasks were arranged in 16 each sets consisting of 3-flasks.

Now, the different substrates were added in an amount so as to give 10% of different carbohydrate sugars to each of the 16 sets of the flasks. All the experimental flasks and plugs were sterilized at 15 lbs steam pressure for 30 minutes and then inoculated with a 0.05 ml inoculum of *Saccharomyces cerevisiae* NCIM-2232 prepared in distilled water. The flasks were then incubated at 30 $\pm$ 1°C and contents of the flasks were analysed after 50 hours of optimum incubation period for ethanol produced and substrates left unfermented.

Study of the concentration of the sugar substrates for ethanol production by yeast *Saccharomyces cerevisiae* NCIM-2232

10-sets, each comprising 3-flasks were prepared as described in experimental portion of this chapter with the only differences that the concentrations of selected substrates in flasks of 1st to 10th set were : 1%, 2%, 4%, 6%, 8%, 10%, 15%, 18%, 25% and 30% respectively.

The fermentor flasks were then sterilized, cooled, aseptically inoculated, incubated and analysed for ethanol produced and molasses left unfermented.

Ethanol production by the yeast *Saccharomyces cerevisiae* NCIM-2232 at different pH

10-sets, each consisting of 3-flasks were prepared as described in experimental portion. Now, to the first five sets requisite amount of lactic acid solution were added to adjust the pH at 2.00, 2.50, 3.00, 3.50 and 3.70 . Similarly the pH values 4.00, 4.25, 4.50, 5.00 and 5.50 were kept in the set from 6th to 10th sets respectively. The pH values adjusted in each case was also ascertained by a pH meter. All the flasks were then sterilized, cooled, aseptically inoculated, incubated and analysed for ethanol produced and molasses substrate left unfermented as mentioned in experimental portion of this thesis.

Temperature

10-sets, each consisting of 3-flasks were prepared as described in experimental portion of this chapter. These flasks were sterilized cooled and inoculated with 0.05 ml inoculum of *Saccharomyces cerevisiae* NCIM-2232. Now, 1st to 10th sets of flasks were incubated at 20°C, 23°C, 25°C, 30°C, 35°C, 40°C, 42°C, 45°C, 50°C and 52°C respectively for 50 hours. The contents of the flasks were analysed for ethanol produced and substrate (selected) left unfermented as described in general experimental methods.

Incubation period

10-sets, each of 3-flasks were also prepared as described in experimental portion of this thesis. All the flasks were sterilized, cooled and Inoculated with 0.05 ml inoculum of *Saccharomyces cerevisiae* NCIM-2232 and were incubated at 30 ± 1°C in an incubator. The contents of the all, 10 sets (flasks) were analysed after 20, 25, 30, 35, 45, 50*, 55, 58, 60 and 62 hours of incubation period for the production of ethanol and substrate (molasses) left unfermented.

Experimental Conditions

Medium: The composition of production medium for ethanol production by the yeast *Saccharomyces cerevisiae* -2232 is prepared as under :

Molasses: 18%; Malt extract: 1.25%; Yeast extract: 1.25%; Peptone: 1.25%; $(NH_4)_2HPO_4$: 1.25%, pH: 4.25

Culture medium

The yeast *Saccharomyces cerevisiae* -2232 was periodically cultured on malt-agar media. The fresh culture media was prepared every fortnight as follows:

Sucrose : 2.5%, Malt extract : 0.20g, Yeast extract : 0.20g, Peptone : 0.25 g,

Agar-Agar : 0.25g, Distilled water : 100ml, pH : 4.25

Sterilization :

The growth and production media were sterilized in an autoclave maintained at 15 lbs steam pressure for 35 min.

Strain : The yeast *Saccharomyces cerevisiae* - 2232 was used in the present study. The strain *Saccharomyces cerevisiae* - 2232 was procured from NCL, Pune,India.

Assay methods:

Evalution of ethanol formed and molasses left unfermented was made colorimetrically *Mc.Closkey and L.L. Reploge (1974); Dubois et.al. (1956)*

Age of the inoculum: 48 hours old.

Quantum of the inoculum: 0.5 ml yeast suspension of *Saccharomyces cerevisiae* NCIM-2232

Molasses concentration : 1%, 2%, 4%, 6%, 8%, 10%, 15%, 18%*, 25% and 30%.

Temperature (in°C): 20, 23, 25, 30*, 35, 40, 42, 45, 50 and 52°C

Incubation period: 20, 25, 30, 35, 45, 50*, 55, 58, 60 and 62 hours

pH: 2.0, 2.50, 3.00, 3.50, 3.70, 4.00, 4.25*, 4.50, 5.00 and 5.50.

Results and Discussion

The results obtained from optimization of different parameters show that optimum values for ethanol production by the yeast *Saccharomyces cerevisiae* NCIM-2232 proceeds best when 18% (w/v) molasses solution is allowed to ferment for 50 hours at 30°C by maintaining the pH value of the fermentation medium at 4.25 in the presence of strain of yeast *Saccharomyces cerevisiae* NCIM-2232.

The results of ethanol production by the yeast *Saccharomyces cerevisiae* NCIM-2232 in the present investigation favours the view that in general ethanol production by the yeast *Saccharomyces cerevisiae* NCIM-2232 becomes more and more easier as the size, molecular weight and the structure complexity of the sugar molecules decreases.It is obvious that the simplest sugars glucose and fructose both are easily fermentable because both the monosaccharide sugars are easily phosphorelated. Galactose fermentability is also much similar to that of glucose and fructose while arabinose, rhamnose and sorbose were found to be least fermentable. Arabinose is an aldopentose – a monosaccharide containing five carbon atoms and including an aldehyde (CHO) functional group.

For biological and biosynthetic reasons, most of the saccharides are almost always more abundant in nature as the "D"-form, or structurally analogous to D-glyceraldehyde. However, L-arabinose is in fact more common than D-arabinose in nature and is found in nature as a component of biopolymers such as hemicellulose and pectin. The L-arabinose operon is a very important operon in molecular biology and bioengineering. Galactose, sometimes abbreviated Gal, is a monosaccharide sugar that is less sweet than glucose. It is a C-4 epimer of glucose. Galactan is a polymer of the sugar galactose found in hemicellulose. Galactan can be converted to galactose by hydrolysis.

Sucrose is made from glucose and fructose units. Sucrose is the organic compound commonly known as table sugar and sometimes called saccharose. A white, odorless, crystalline powder with a sweet taste, it is best known for its nutritional role. The molecule is a disaccharide composed of the monosaccharides glucose and fructose with the molecular formula $C_{12}H_{22}O_{11}$. The word was formed in mid-19th century from Latin sucrum = "sugar" and the chemical suffix -ose.

Lactose is a disaccharide sugar that is found most notably in milk and is formed from galactose and glucose. Lactose makes up around 2~8% of milk (by

weight), although the amount varies among species and individuals. It is extracted from sweet or sour whey. The name comes from lac or lactis, the Latin word for milk, plus the -ose ending used to name sugars. It has a formula of $C_{12}H_{22}O_{11}$.

Maltose is a disaccharide, two simple sugars in one molecule. In maltose, the two sugars are both glucose. White, odorless, sweet-tasting powder. Maltose also known as maltobiose or malt sugar, is a disaccharide formed from two units of glucose joined with an α(1-4)bond, formed from a condensation reaction. The isomer isomaltose has two glucose molecules linked through an α(1, 6) bond. Maltose is the second member of an important biochemical series of glucose chains. Maltose is the disaccharide produced when amylase breaks down starch. It is found in germinating seeds such as barley as they break down their starch stores to use for food. It is also produced when glucose is caramelized.

The addition of another glucose unit yields maltotriose; further additions will produce dextrins (also called maltodextrins) and eventually starch (glucose polymer). Maltose can be broken down into two glucose molecules by hydrolysis. In living organisms, the enzyme maltase can achieve this very rapidly. In the laboratory, heating with a strong acid for several minutes will produce the same result. Isomaltose is broken by isomaltase.

Raffinose is a trisaccharide composed of galactose, fructose and glucose. It can be found in beans, cabbage, brussels sprouts, broccoli, asparagus, other vegetables and whole grains. Raffinose can be hydrolyzed to D-galactose and sucrose by the enzyme α-galactosidase (α-GAL), an enzyme not found in the human digestive tract. α-GAL also hydrolyzes other α-galactosides such as stachyose, verbascose and galactinol, if present. The enzyme does not cleave β-linked galactose, as in lactose.

The raffinose family of oligosaccharides (RFOs) are alpha-galactosyl derivatives of sucrose and the most common are the trisaccharide raffinose, the tetrasaccharide stachyose and the pentasaccharide verbascose. RFOs are almost ubiquitous in the plant kingdom, being found in a large variety of seeds from many different families and they rank second only to sucrose in abundance as soluble carbohydrates.

Starch or amylum is a carbohydrate consisting of a large number of glucose units joined by glycosidic bonds. This polysaccharide is produced by all green plants as an energy store. It is the most common carbohydrate in the human diet and is contained in large amounts in such staple foods as potatoes, wheat, maize (corn), rice and cassava.

Pure starch is a white, tasteless and odourless powder that is insoluble in cold water or alcohol. It consists of two types of molecules: the linear and helical amylose and the branched amylopectin. Depending on the plant, starch generally contains 20 to 25% amylose and 75 to 80% amylopectin by weight. Glycogen, the glucose store of animals, is a more branched version of amylopectin.

Starch is processed to produce many of the sugars in processed foods. Dissolving starch in warm water gives wheatpaste, which can be used as a thickening, stiffening or gluing agent. The biggest industrial non-food use of starch is as adhesive in the papermaking process.

Inulins are a group of naturally occurring polysaccharides produced by many types of plants. They belong to a class of dietary fibers known as fructans. Inulin is used by some plants as a means of storing energy and is typically found in roots or rhizomes. Most plants that synthesize and store inulin do not store other forms of carbohydrate such as starch.

Dextrins are a group of low-molecular-weight carbohydrates produced by the hydrolysis of starch or glycogen. Dextrins are mixtures of polymers of D-glucose units linked by (α-1, 4) or (α-1, 6) glycosidic bonds.

Dextrins can be produced from starch using enzymes like amylases, as during digestion in the human body and during malting and mashing, or by applying dry heat under acidic conditions (pyrolysis or roasting). The latter process is used industrially and also occurs on the surface of bread during the baking process, contributing to flavor, color and crispness. Dextrins produced by heat are also known as pyrodextrins. During roasting under acid condition the starch hydrolyses and short chained starch parts partially rebranch with α-(1,6) bonds to the degraded starch molecule.

Dextrins are white, yellow, or brown powders that are partially or fully water-soluble, yielding optically active solutions of low viscosity. Most can be detected with iodine solution, giving a red coloration; one distinguishes erythrodextrin (dextrin that colours red) and achrodextrin (giving no colour).

Mannitol is a white, crystalline sugar alcohol with the chemical formula $(C_6H_8(OH)_6)$. It is used as an osmotic

diuretic agent and a weak renal vasodilator. It was originally isolated from the secretions of the flowering ash and called manna after its resemblance to the Biblical food. It is also referred to as mannite and manna sugar. In plants, it is used to induce osmotic stress.

The world produced about 168 million tonnes of table sugar in 2011. Sucrose a disaccharide sugar has been found suitable for production of ethanol while lactose was found to be unsuitable. However, maltose was fermented to some extent on the basis of total sugar taken. In the group of polysaccharides, starch was found to be least fermentable and unsuitable for ethanolic fermentation. However, raffinose, inulin, dextrin and starch produced no ethanol. Polyethanol mannitol also could not produce any ethanol by fermentation. On the basis of above observation it is concluded that glucose amongst monosaccharides and sucrose amongst disaccharides are most suitable and useful for ethanolic fermentation with the yeast *Saccharomyces cerevisiae* NCIM-2232.

It was interesting to note that in the case of molasses 6.50 ml of ethanol was produced from 18% solution of molasses. Since it is economical, cheapest and richest source of sugar substrate it has been employed as starting raw material for ethanolic fermentation during the course of present investigation. Different strains of *Saccharomyces cerevisiae* has been employed by Krumphazal and Singh *et al.* in ethanolic fermentations as enzymes sources and in the present investigation *Saccharomyces cerevisiae* NCIM-2232 was found most suitable and therefore, the author has selected *Saccharomyces cerevisiae* NCIM-2232 for ethanol production.

An interesting research field in alcoholic fermentation is the study of yeast strains able to utilize sugar solutions more concentrated than those generally fermented in usual practice and hence it is important to establish the limits of ethanol tolerance of the yeast strain. Therefore, the fermentation, were conducted with molasses concentrations at 18% with an intention to obtain high yield of ethanol in reasonable time.

In the present investigation different concentrations of molasses, i. e., from 1% to 30% was employed for ethanol production by the yeast *Saccharomyces cerevisiae* NCIM-2232 and it was observed that 18% molasses solution (w/v) was found to be most suitable for ethanolic fermentation. Different concentrations of molasses and yields of ethanol has been recorded in the Table-2. It has been observed that lower concentrations of molasses has been found insignificant and therefore, production of ethanol is negligible. On the other hand it has been observed that higher concentrations of molasses interferes with the enzymes activities of *Saccharomyces cerevisiae* NCIM-2232 and therefore, retards the ethanol production by the yeast *Saccharomyces cerevisiae* NCIM-2232.

The pH has a significant influence on fermentation due to its effect on yeast growth, fermentation rate and by product formation. Therefore, maintenance of pH is of paramount importance in any fermentation processes especially ethanolic fermentation with the yeast *Saccharomyces cerevisiae* NCIM-2232.

Hydrogen ion concentrations of the production medium also plays vital role in the ethanol production by the yeast *Saccharomyces cerevisiae* NCIM-2232. The results of the influence of hydrogen ion concentrations (pH) are recorded in table-3. It was observed that production of ethanol at the pH values 2.0, 2.50, 3.0, 3.50, 3.70 and 4.0 was found to be in increasing order. It was further observed that at pH value of 4.25 production of ethanol, i.e., 6.40ml/100 ml was recorded which is maximum. Therefore, it is clear from the table - 3 that the pH of 4.25 is optimum for the production of ethanol (6.40 ml/100 ml) using 18% molasses as a starting material. It was interesting to note that there was a gradual fall in the production of ethanol with the increase of hydrogen ion concentrations from 4.50 and onwards. It was thus, concluded that hydrogen ion concentrations of 4.25 (pH) was most effective and suitable for the optimum (maximum) ethanolic fermentation of molasses and therefore, the pH 4.25 was selected and maintained in the production medium for ethanol production by the yeast *Saccharomyces cerevisiae* NCIM-2232

The temperature has a marked influence on the production of ethanol with the yeast *Saccharomyces cerevisiae* NCIM-2232. Usually, the rate of ethanolic fermentation increases with temperature to an optimum between 20°C and 30°C using conventional yeast *Saccharomyces cerevisiae* NCIM-2232. Therefore, fermentation experiments were conducted under varying temperature in the range 20-52°C to see the effect of the newly developed strain towards ethanol production. Ethanolic fermentation is greatly influenced with the temperature. The results recorded in the table - 4 show that ethanol production with the yeast *Saccharomyces cerevisiae* NCIM-2232 increases with the increase in temperature from 20°C to 30°C.

The yields of ethanol at lower temperature was found to be minimum, i.e., 3.85 ml/100 ml at 20°C, while the maximum yield of ethanol, 6.57 ml/100 ml was recorded at 30°C. The yield of ethanol gradually decreases with increase in temperature from 35°C onwards. However, higher temperature, i.e., 35°C onwards were found to be insignificant for production of ethanol by the yeast *Saccharomyces cerevisiae* NCIM-2232. It was thus, concluded that the temperature 30°C was found most suitable and effective for maximum ethanol production by the yeast *Saccharomyces cerevisiae* NCIM-2232 and therefore, the temperature 30°C was selected and maintained throughout in the present investigation.

The fermentation reaction is a complex transformation of material via the metabolic activity of micro-organisms or via an enzymatic reaction using enzymes obtained from micro-organisms. In fermentation reactions, incubation period is very important in obtaining maximum ethanol production with minimum time. After the particular cell density is reached the growth phase slows and the life cycle of the yeast deviates from the growth path and produces ethanol. If the cell density is less, more time will be taken for complete fermentation. In fermentative processes incubation period plays vital role because it is directly related to the great economy of industry. The results recorded in the table-5 shows that the yields of ethanol increases with the increase in incubation period from 20 hours to 50 hours and then yield of ethanol gradually falls (from 55 hours to 62 hours of incubation period).

The study of the influence of different incubation periods on yield of ethanol from 18% molasses reveals that it proceeds in different phase .The very first phase completes in 20 hours where slow molasses consumption is accompanied by poor yields of ethanol, i.e., 3.85 ml/100ml. The next second phase occurs during 25 hours and 35 hours of incubation period where molasses consumption and yields of ethanol follows the first phase with slight improvement in the yield of ethanol. After 45 hours of incubation period that the 3rd important and effective last phase begins and the ethanol yields are maximum in this very phase, i.e., in 50 hours. In this way 50 hours of incubation period gives the maximum yield of ethanol, that is 6.62 ml/100 ml. Thus, ethanol production by the yeast *Saccharomyces cerevisiae* NCIM-2232 was optimized using 18% molasses, 4.25 pH, 30°C temperature and 50 hours of incubation period alongwith some other necessary growth ingredients.92°C

Table-1 : Parametric studies of ethanol production by yeast exposed to some sugary materials

S. No.	Substrates taken for ethanolic fermentation	Yield of ethanol* in ml/100 ml	Substrate left Unfermented in g/100ml
1	Arabinose	1.43	-
2	Rhamnose	1.10	-
3	Xylose	0.82	-
4	Glucose	5.80	2.36940
5	Fructose	5.10	2.61942
6	Galactose	4.05	3.18749
7	Sorbose	1.50	-
8	Lactose	3.40	-
9	Sucrose	5.70	2.38643
10	Maltose	1.35	2.10860
11	Starch	0.85	-
12	Inulin	-	-

13	Dextrine	-	-
14	Raffinose	-	-
15	Mannitol	-	-
16	Molasses	6.50	2.860

**Each value represents mean of three trials. S.No. 1–6 Monosaccharides, 8 - 10Disaccharides, 11-14 Polysaccharides, 15 Polyalcohol & 16 Molasses. Experimental deviation (+) 1.5 to 3.5%*

Table - 2 : Effect of different % of molasses substrates on the ethanol production by yeast Saccharomyces cerevisiae NCIM-2232 in 48 hrs of optimum incubation period.

S. No.	% Concentrations of molasses (in W/V)	Yield of ethanol* (in ml/100 ml)	Molasses * left Unfermented (in g/100 ml)
1	1%	0.25	-
2	2%	0.70	-
3	4%	1.35	-
4	6%	1.95	-
5	8%	2.80	-
6	10%	3.60	-
7	15%	5.05	3.80685
8	18%**	6.35**	2.34789
9	25%	6.05	2.86940
10	30%	5.75	-

**Each value represents mean of three trials.*
***Optimum concentration of molasses.*
**** Optimum yield of ethanol. Experimental deviation (+) 1.5 to 3.5%.*

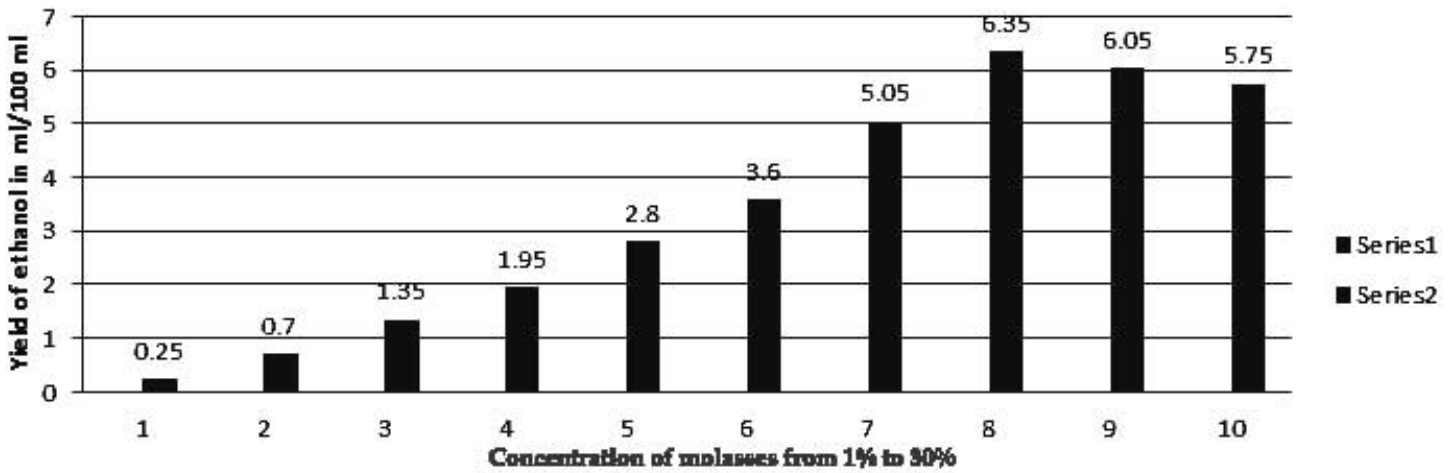

Fig.1 : Effect of different % of molasses substrates on the ethanol production by yeast Saccharomyces cerevisiae NCIM-2232 in 48 hrs of optimum incubation period

Table-3 : Effect of different pH on ethanol production by yeast Saccharomyces cerevisiae NCIM-2232 in 48 hours

S. No.	% Concentrations of molasses (in W/V)	Yield of ethanol* (in ml/ 100 ml)	Molasses * left Unfermented (in g/100 ml)
1	1%	0.25	–
2	2%	0.70	–
3	4%	1.35	–
4	6%	1.95	–
5	8%	2.80	–
6	10%	3.60	–
7	15%	5.05	3.80685
8	18%**	6.35**	2.34789
9	25%	6.05	2.86940
10	30%	5.75	–

**Each value represents mean of three trials. **Optimum concentration of molasses.*
**** Optimum yield of ethanol. Experimental deviation (+) 1.5 to 3.5%.*

Table-3 : Effect of different pH on ethanol production by yeast Saccharomyces cerevisiae NCIM-2232 in 48 hours

S. No.	Effect of pH	Yield of ethanol* (in ml/ 100 ml)	Molasses * left Unfermented (in g/100 ml)
1.	2.0	2.10	–
2.	2.50	2.20	–
3.	3.00	2.60	–
4.	3.50	3.25	–
5.	3.70	4.90	–
6.	4.00	6.21	2.45950
7.	4.25**	6.40***	2.23662
8.	4.50	5.75	3.12665
9	5.00	****	–
10	5.50	****	–

**Each value represents mean of three trials. Molasses substrate taken in (w/v) 18%.*
***Optimum pH.*
**** Optimum yield of ethanol.*
***** Insignificant values*
Experimental deviation (+) 1.5 to 3.5%.

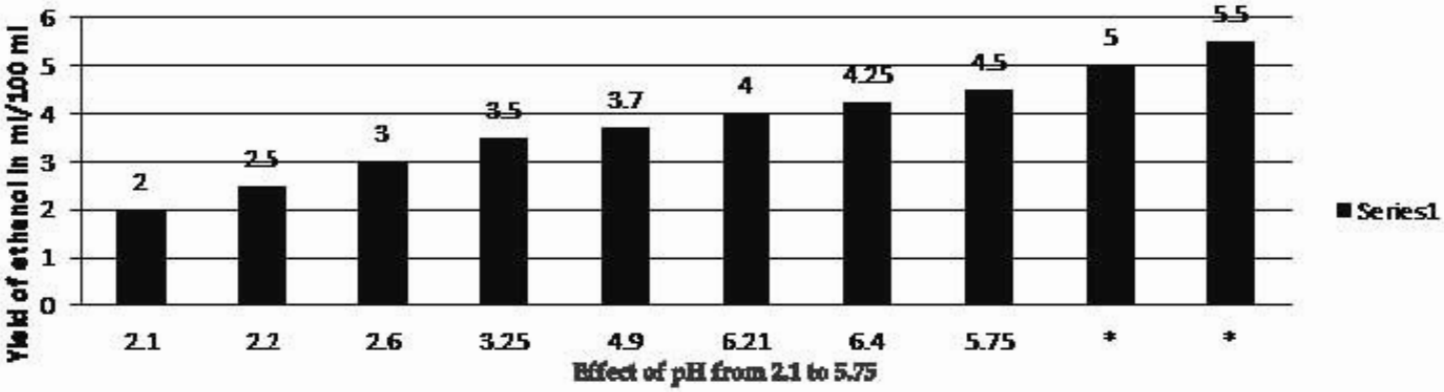

Fig. 2 : Effect of different pH on ethanol production by yeast Saccharomyces cerevisiae NCIM-2232 in 48 hours

Table - 4 : Effect of different temperatures on ethanol production by yeast Saccharomyces cerevisiae NCIM-2232 in 50 hours incubation period and 4.25pH

S. No.	Temperature in °C	Yield of ethanol* (in ml/ 100 ml)	Molasses * left Unfermented (in g/100 ml)
1	20	3.85	–
2	23	4.30	–
3	25	5.40	3.10739
4	30**	6.57***	2.09980
5	35	6.39	2.27865
6	40	5.68	3.20780
7	42	****	–
8	45	****	–
9.	50	****	–
10.	52	****	–

**Each value represents mean of three trials. Molasses substrate taken in (w/v) 18%.*
***Optimum temperature 30OC.*
**** Optimum yield of ethanol*
***** Insignificant values. Experimental deviation (+) 1.5 to 3.5%.*

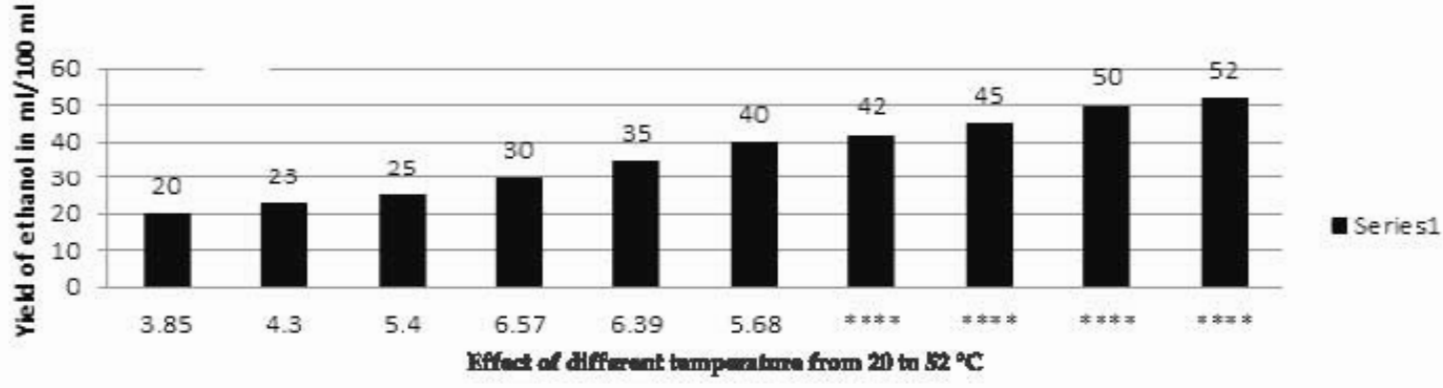

Fig. 3 : Effect of different on ethanol production by yeast Saccharomyces cerevisiae NCIM-2232 in 50 hours incubation period and 4.25pH

Table-5 : Effect of different incubation period on ethanol production by yeast Saccharomyces cerevisiae NCIM-2232 at pH 4.25 and temp. 30°C

S. No.	Incubation period in hours	Yield of ethanol* (in ml/ 100 ml)	Molasses * left Unfermented (in g/100 ml)
1	20	2.75	–
2.	25	2.90	–
3.	30	4.95	–
4.	35	5.15	–
5.	45	5.95	2.91863
6.	50**	6.62***	2.01630
7	55	6.32	1.88763
8	58	5.10	–
9	60	4.80	–
10	62	3.15	–

**Each value represents mean of three trials. Molasses substrate taken in (w/v) 18%.*
***Optimum incubation period in 50 hours.*
**** Optimum yield of ethanol*
Experimental deviation (+) 1.5 to 3.5%.

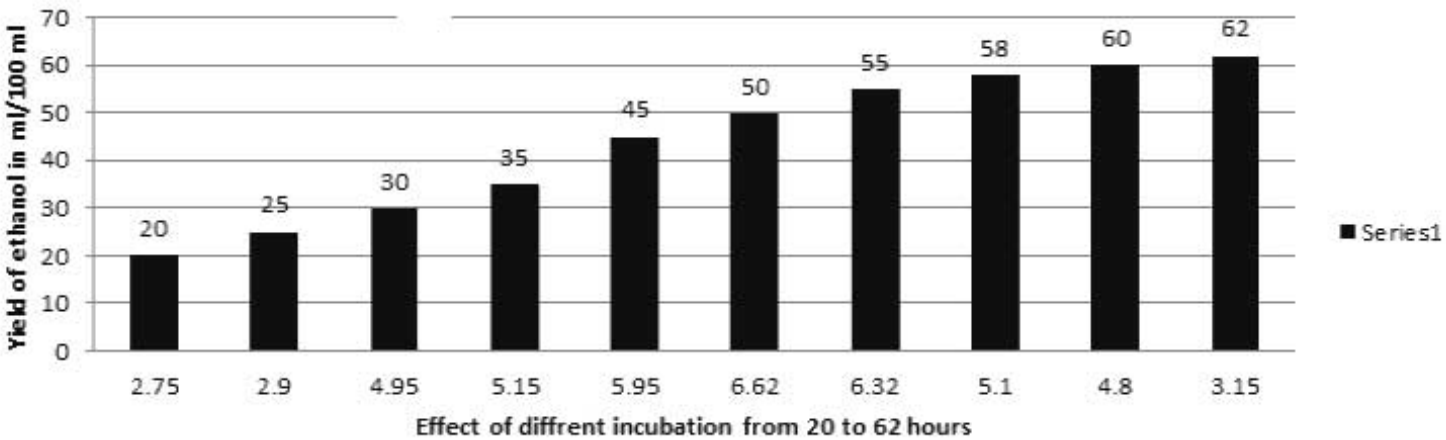

Fig. 4 : Effect of different incubation period on ethanol production by yeast Saccharomyces cerevisiae NCIM-2232 at pH 4.25 and temp. 300C

Table-6 : Table -6 Effect of concentration of molasses, pH, temperature and incubation period on ethanol production by yeast Saccharomyces cerevisiae NCIM-2232

% of molasses	pH	Temp. in (°C)	Incubation period in hours	Corresponding yield of ethanol* in ml/100 ml				Corresponding amount of molasses* left unfermented in g/100 ml			
1	2.00	20	20	.25	2.10	3.85	2.75	–	–	–	–
2	2.50	23	25	.70	2.20	4.30	2.90	–	–	–	–
4	3.00	25	30	1.35	2.60	5.40	5.95	–	–	3.107	–

6	3.50	30**	35	1.95	3.25	6.57***	5.15	–	–	2.099	–
8	3.70	35	45	2.80	4.90	6.39	5.95	–	–	2.278	2.918
10	4.00	40	50**	3.60	6.21	5.68	6.62***	–	2.459	3.207	2.016
15	4.25**	42	55	5.05	6.40**	****	6.32	3.805	2.236	–	1.887
18**	4.50	45	58	6.35***	5.75	****	5.10	2.347	3.126	–	–
25	5.00	50	60	6.05	****	****	4.80	2.469	–	–	–
30	5.50	52	62	5.75	****	****	3.15	–	–	–	–

** Each value represents mean of three trials ** Optimum values of molasses solution, pH, temp. and incubation period.*
**** Optimum yield of ethanol. **** Insignificant value.*

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RESEARCH ARTICLE

Overview of RNA Seq based Transcriptomics and Potential Intervention in Brinjal

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Abstract

RNA sequencing (RNA-seq) is an important technique of molecular and plant biotechnology. It has potential to explore signal transduction pathway of the metabolic pathway involved in biotic and abiotic resistance in the crop by studying the expression patterns and regulatory mechanisms of plantgenes. Next-generation sequencing technologies and expanding horizon of this technology in the field of plant transcriptomics has resulted significant development and has become an important component of plant biology research. The transcriptomic analyses through RNA-seq have been applied in many crops. Similarly, genetic information and new functional genes and their regulatory networks can be explored in brinjal too to analyze defence mechanism and stress response pathways utilizing this technique, leading to identifications of different regulatory genes. This manuscript deals with the techniques of RNA Seq based trnascriptomics and potential intervention in brinjal to address the problem as mentioned.

Keywords: *Brinjal, transcriptome, RNA Seq., pathogenesis, disease*

Introduction

Plants have to face both biotic and abiotic stresses and so the brinjal. Transcriptomic studies have been mainly applied to only a few plants species including the model plant, maize, barley, rice, wheat, tomato and Arabidopsis thaliana but now a day it has become an established technology. These studies have provided valuable insights into the cross talk of signal transduction pathway in response to stress in plant. Transcriptome sequencing (RNA-Seq) is a high-throughput, high-sensitivity, and high-resolution technique that can be used to study model and non-model organisms. Brinjal (*Solanum melongena*) is one of the most widely grown vegetable crops across the globe, including the Indian sub-continent. But this crop is prone to massive attacks by several species of fungi and bacteria that cause wilt, soft rot and root rot. These challenges activate a defence system that involves an array of induced mechanisms such as the hypersensitive response and the expression of pathogenesis related (PR) gene. PR genes are strongly induced in response to infection by pathogens, accumulate abundantly at the site of infection, and contribute to systemic acquired resistance. With the advent of next-generation sequencing and various tools of bio-informatics, RNA seqbased transcriptome analysis has become choice and promising field of molecular research to investigate the pathogenesis related genes involved in disease resistance. RNA-seq techniques involve RNA isolation, cDNA synthesis, Adaptor ligation, Library preparation, DNA fragmentation and sequencing while RNA-seq data analysis involves (Kukurba *et al.*, 2015)

1. Accurate mapping of millions of short sequencing reads to a reference genome, including the identification of splicing events or *de novo* assembly of short sequence reads in the absence of a reference sequence and read mapping.
2. Quantifying expression levels of genes, transcripts, and exons.
3. Differential analysis of gene expression among different biological parameters; and
4. Biological interpretation of differentially expressed genes.

Techniques and Methodology of RNA-seq: The standard RNA-seq methods involve several essential steps, including selection of sequencing platform, protocol of RNA extraction, library construction, sequencing and data analysis.

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Selection of Platform for RNA Seq: Sequencing platform selection is the first crucial step in the RNA-seq, in terms of budget and effectiveness of the results. Illumina short-readRNA sequencing technology has emerged as the dominant platform among others owing to its high throughput, cost-effectiveness and authenticity. This NGS platform is based on the principle of sequencing-by-synthesis and reversible dye-terminators technology where every single base is identified as it is synthesized into the DNA strand.

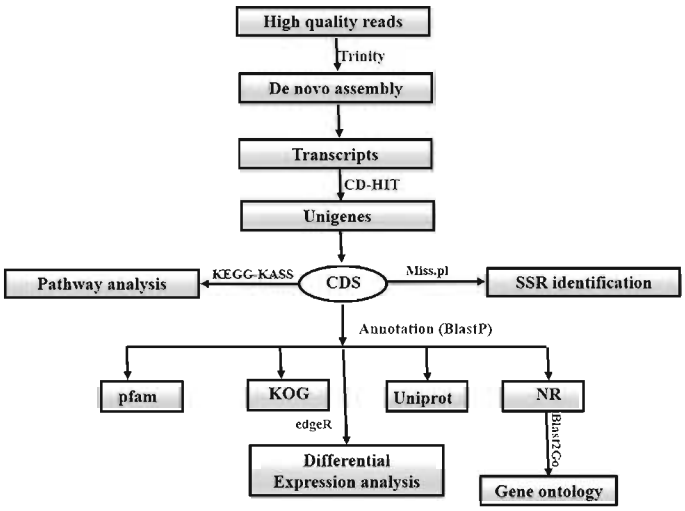

Fig 1. Flow Chart of RNA Seq based transcriptomics in plants and vegetables.

RNA extraction : RNA extraction is very sensitive process, therefore, this process must be carefully done to preserve RNA integrity and minimize degradation as well. High quality RNA is essential for reliable gene expression quantification. After isolation of RNA Samples are analyzed using 1% agarose gel and are processed using Alexgen Total RNA kit (Cat-AG-TR50). The samples are quantified by quibit 4. flurometer to screen the quality of the RNA before the library preparation and sequencing.

Library construction: Library construction is a crucial step and it is done to generate asequencing-ready library from the RNA samples. Currently, various libraries preparation methods are available (table 1). The choice of the specific library construction strategy depends on the biological question to be answered, including oligo-dT enrichment, specific selection of 30 or 5'ends, detection of PCR duplicates by using unique molecular identifiers (UMI), and the analysis of low-quality or degraded RNA (Stark *et al.*, 2019). RNA fragmentation and strand-specific library preparation occupy is an essential step regardless of the methods.(Mortazavi*et al.*, 2008). It is important to mention here that, RNA specific selective formation of libraries with different fragment sizes takes place while library construction.

Table-1 : Types of designed library (Kukurba et al., 2015)

S. No.	Library design	Description	Usage
1	Strand-specific method	It is based on preservation of strand information of the transcript	It is used in De novo transcriptome assembly
2	Long-read method	In this design, >1000 bp reads are produced ; it is advantageous for resolving splice junctions and repetitive regions	It is also used for De novo transcriptome assembly
3	Size selection method	It involves selection of RNA species using size fractionation by specific gel electrophoresis	miRNA Sequencing
4	Duplex-specific nuclease method	It is based on cleavage ofhighly abundant transcripts, including ribosomal RNA and other highly expressed genes	Reduction of highly abundant transcripts
5	Poly-A selection method	It involves selection for RNA species with poly-A tail and enrichment for mRNA	mRNA sequencing
6	Multiplexed method	It is Genetic barcoding method that enables sequencing multiple samples together	It can Sequence multiple samples together
7	Short-read methods	In this case there is production of 50–100 bp reads; generally higher read coverage and reduced error rate compared to long-read sequencing	It has higher coverage
8	Ribo-depletion method	It is based on Removal of ribosomal RNA and enrichment for mRNA , pre-mRNA, and non -coding RNA	Sequencing of mRNA, pre-mRNA and ncRNA

Generally paired-end sequencing library a prepared using NEBNext® Ultra™ RNA Library Prep Kit for Illumina. (NEB #E7770). The average size of libraries is 604bp, 409bp, 417bp and 495bp respectively for all samples. The library preparation process are initiated with 1000 ng input. Ribosomal RNA removed using depletion is carried out using Human specific ribodepletion kit (Cat.no. NEB #E6310) following the user manual. Ribo-depleted RNA was subjected to fragmentation, first and second-strand cDNA synthesis, end-repair, 3´ adenylation, adapter ligation, selective enrichment of adapter-ligated DNA fragments through PCR amplification, followed by validation of Library on Agilent 4150 tape station. The final library are pooled with other samples, denatured and loaded on to flow cell.

***De novo* assembly of transcripts and Unigene prediction:** Master/Combine assembly are performed taking high quality adapter trimmed reads together using Trinity (at default parameters, kmer 25) to generate common assembly for annotation as well as sample comparison. Unigenes Prediction from master assembly Transcripts are further processed for unigenes prediction with the help of CD-HIT package. CD HIT-EST executable issued to remove the shorter redundant transcripts when they were 100% covered by other transcripts with more than 90% identity (default parameter). The non-redundant clustered transcripts are called unigenes which are deduced for further information (Mishra *et al.*, 2025).

RNA Seq Data analysis : Transcriptomics is the study of full range mRNA molecules expressed by an organism under particular condition. It involves isolation and quantitative analysis of RNA from samples under normal and controlled conditions followed by the preparation of library and its quality level. RNA-seq data analysis involves typical key steps, including data quality control (QC), reads comparison, transcript assembly, expression quantification and differential gene expression analysis (DGE). Libraries are sequenced under NGS platform, particularly *Illumina*. Generated data are derived in clean reads and assembled into transcripts, generally *de novo* by *Trinity*. CD-HIT is applied for clustering transcripts into UniGene. These are used for the prediction of CDS with the help of *Transdecoder*. After that functional and metabolic pathway analyses of the identified differentially expressed genes can be done using GO enrichment and KEGG pathway enrichment analysis (Das *et al.*, 2020). The protein sequence corresponding to the predicted coding regions within the UniGenes are subjected to similarity search by *BlastP* against Non Redundant (NR) Database of NCBI. Unigenes from which CDS were predicted using transdecoder and having length less than 200nt were removed. These predicted unigenes were then searched for similarity against different protein databases like Pfam, Uniprot and NR using blastx (Stupnikov *et al.*, 2021).

Simultaneously, all protein sequences are searched by BlastP for similarity against *UniProt*, *KOG* and *Pfam*. EuKaryotic Orthologous Groups (KOG) is a eukaryote-specific version of the Clusters of Orthologous Groups (COG) tool to identify Ortholog and Paralog proteins while Pfam is a database of curated protein families, each of which is defined by alignments and a Hidden Markov Model. Gene Ontology (GO) mapping is carried out by BLAST2GO to retrieve GO terms for all the *BlastP* functional annotated proteins against NR database (Ghelfi *et al.*, 2025). CDS is also used for differential gene expression by edgeR, metabolic pathway analysis by KEGG-KASS and SSR marker identification by *Misa.pl*. *EdgeR* is the tool for differential expression (DE) analysis of CDS derived from RNA-Seq. The algorithm of this tool cash in on information from all the genes, computes the dispersion using a weighted likelihood and F-test techniques. KEGG Automatic Annotation Server (KAAS) provides functional annotation of genes by Blast comparisons against the manually curated Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Zhangh *et al.*, 2023). DGE analysis is one of the most common components of transcriptomics to determine genotypical differences between two or more conditions of cells as mentioned in objectives. List of Software used in the analysis is mentioned in table 2.

Differential gene expression analysis: Here the plants are grown in different conditions with control and RNA samples are collected under each condition from the plant for sequencing. Reads of each sample are mapped separately to CDS sequences obtained from master assembly using bwa. PCR duplicate reads are removed from alignment file using Picard's Mark Duplicates.jar. Reads mapping to each CDS are calculated for each sample using idxstats program of Samtools v1.4. Finally these mapped reads in above mentioned combination were considered for differential gene expression (DGE) analysis. The read mapped count from each sample was given as an input for differential expression analysis using edge Rbioconductor package in R (v3.6.2). Differential gene

Table-2 : List of Software used in different application of RNA Seq based transcriptomics.

S. No.	Software Used	Version	Application
1	Trinity	2.14.0	Denovo Assembly of RNA Sequence
2	Cd-hit	4.8.1	Generation of Unigenes from transcript clustering
3	TransDecoder	5.6.0	Prediction of CDS from unigenes
4	Blast	2.13.0+	Functional annotation of proteins against NR, Uniprot, KOG, Pfam and transcription factor database
5	Blast2Go cli	1.4.1	Gene Ontology (GO) mapping and annotation
	KAAS	Web server	Pathway analysis against KEGG database
6	MISA	A pearl script	Identification of SSR
7	BWA	0.7.17	Mapping of reads to CDS for expression Profiling
8	Picard-tools	2.4.1	Removal of multi mapped reads from BAM file
9	Samtools	1.14	For getting reads mapped count
10	EdgeR	3.6.2	Analysis of differentiation analysis and its visualization.

expression is inferred between samples by applying the R package edgeR. edgeR is a bioconductor package based on negative binomial distribution method (Shahjaman *et al.*, 2020). The statistical criterion used to identify both upregulated and downregulated transcripts along with the significance level are up-regulated log2FC > 0, down-regulated log2FC < 0, significantly up-regulated log2FC > 0 and q-value <0.05 and significantly down-regulated log2FC < 0 and q-value (Rosati *et al.*, 2024).

Application of RNA-seq in plant sciences and vegetable crop (Brinjal): RNA-Seqtranscriptomics in plants has many applications such as understanding of gene expression, identification of differentially expressed genes, revealing of regulatory networks, and exploration of plant evolution. It also helps in identification of co-expressed or co-regulated gene groups and detection of structural changes in plant transcriptomes. The brief of major application are mentioned here.

Analysis of differentially expressed genes and their regulatory networks: Tissues, organs or temporal transcriptomic data allows researchers to identify the differences in gene expression patterns, to detect the differentially expressed genes in addition to know the function and regulatory mechanisms of the genes. 125 ZmWRKY genes detected from the analysis of transcriptomic data in maize exhibited different expression patterns across different developmental stages (Hu *et al.*, 2021).

Study of plant evolution and its origin

The techniques of Illumina sequencing, PacBio sequencing, and high-quality optical mapping have been employed and a superior genome of *Mesostigma viride*, the most ancient single-cell green algae were obtained. The evolutionary pathway of plants from single cells to plants as well as the evolution of photosynthetic pathways and mechanisms underlying stress response and environmental regulation of gene expression were identified (Liang *et al.*, 2020). The transcriptomes of more than thousand plant species have been analysed and concerned databases have also been created One Thousand Plant Transcriptomes Initiative (1 KP). It has led to construction of a comprehensive framework for exploring genetic relationships and phylogeny among different species under study (Wong *et al.*, 2020).

Strengthening of gene annotation and transcriptomic database creation: *De novo* assembling of RNA-seq data is more empirical than that of reference genome based assemblage. Many analytical tools have been developed for plant RNA-seq data for precise identification of full transcript information either in tissues or cells irrespective of availability of reference genome (Tu *et al.*, 2022). RNA-seq data collection is being utilized to construct comprehensive transcriptome databases for a number of plant species. Many of the databases of plant species such as Arabidopsisthaliana (Berardini *et al.*, 2015), maize (Portwood *et al.*, 2019), rice (Sato *et al.*, 2013) wheat

(Borrill *et al.*, 2016),barley (Lee *et al.*, 2020), tomato (Zouine, 2017) and *Brassica napus*(Liu *et al.*, 2021) have been develpoed. These databases are the source of potential information to do studies onexpression and regulation of genes later. It can be helpful in the elucidating gene interactions under differential physiological conditions.

Unwinding of complexity of the plant transcriptome: Full-length sequencing of transcriptome mediated by long-read RNA-seq is helpful in the exhaustive exploration and investigation of transcriptomes. It allows identification of long non-coding RNA (lncRNA) and various co/post-transcriptional events such as alternative splicing and polyadenylation beneficial for the study of mechanism of plantgrowth, development and stress resistance (Budak *et al.*, 2020). Scientist hasalso developed a multitude of computational instruments to detect and measure co/post-transcriptional events(Zhao *et al.*, 2019). This invention can facilitate long-read RNAsequencing can resolve numerous facets of transcriptome.

Single-Cell Transcriptomics: Single-cell RNA-seq has otential to analyze the whole mRNA profile of a single cell. This special type of transcriptomics can lead to the discovery of new cell types, cellular variation and differentiation of special cells. With the help of Single-cell RNA sequencing, it has been identifies that eggplant has similar regulator as of germ cell development in Drosophila (Sun *et al.*, 2021).

Studying Plant-Microbe Interactions : RNA-Seq can also be used to study the interaction between plants and microorganisms including pathogens. Thus molecular mechanisms underlying plant immunity, disease resistance, and interactions with beneficial microbes like rhizobia can be unravelled (Zhang *et al.*, 2020).

Breeding and Crop Improvement: Transcriptome analysis elucidates the molecular mechanisms of abiotic and biotic stress resistance in plants. It is also helpful to identify linked genes with desirable quantitative traits, such as yield, quality, or stress tolerance. This identification helps to develop molecular markers for marker-assisted selection and molecular breeding leading to genetic improvement of crops to meet the consequence due to climate changes (Yang *et al.*, 2023, Wang *et al.*, 2023).

Role of Transcriptomics and is intervention to cope up with Biotic stresses in Brinjal

Eggplant (*Solanum melongena* L.) is a major vegetable crop widely grown in tropics and subtropics region , but the yield of eggplant are being affected by both biotic and abiotic stresses. These biotic challenges not only reduce yields in egg plant but also fruit quality, shelf-life, and nutritional content. To date, some disease resistance genes have been utilized in commercial cultivars, but much less progress has been achieved for arthropods resistance (Arafa, 2022). Bacterial wilt in eggplant caused by *Ralstonia solanacearum* is a major disease causing heavy losses in eggplant production in the tropical, sub-tropical and temperate regions. Phomopsis fruit rot is another detrimental disease in brinjal which is caused by *Phomopsis vexans*. It is also known as phomopsis blight in brinjal. Fusarium wilt in brinjal is caused by *Fusarium solani* (Singh *et al.*, 2015) is one of the most devastating diseases of eggplant (*Solanum melongena* L.) causing heavy yield and quality loss. The development of high-yielding and resistant varieties of brinjal using regulatory and candidate genes based on the informations from trascriptomics is the most sustainable approach. Although, resistance sources have been identified by many researchers but only limited success has been attained in developing high-yielding resistant variety(s). Wide range of genetic diversity and recent genome sequencing of eggplant has made it possible to a few extents to accelerate the development of resistant variety(s). Use of wild species showed their possible use in grafting technology as potential root stocks (Saha *et al.*, 2021). RNA Seq based transcriptomics holds much scope and appear highly economic to investigate the relation between gene and resistance against disease in the wild resistant variety. Identification of target and regulatory gene(s) will help in the introgression of gene(s) into the sensitive cultivars as a part of crop improvement programme. In one of the study, the transcriptomes and metabolomes of entire root of brinjal infected by bacterial wilt-resistant eggplant, a total of 2,896 differentially expressed genes and 63 differences in metabolites were identified. There was also alteration of biosynthesis pathways of secondary metabolomes and phytohomone after inoculation with *R. Solanacearum,* the causal agent of swilt. This investigation inferred that phytohormones played a key role in eggplant response to bacterial wilt (Xiao *et al.*, 2023). Moreover, identification of genes involved in plant and beneficial microbe interactions can improve plant health and nutrition (Zhang *et al.*, 2020).

Conclusion and Future Perspective

RNA-Seq based transcriptomics is a high-resolution, sensitive and high-throughput next-generation

sequencing (NGS) approach used to study non-model plants and other organisms. It is an assemblage of RNA transcripts either from individual or whole samples of different physiological and developmental stages. RNA-*Seq* is a significant technique for mining functional analysis in addition to identification that improves gene ontology understanding mechanisms of biological processes, molecular functions, and cellular components. But still, there is limited information available on this topic (Tyagi *et al.*, 2022).

Research on Transcriptomics in different crops can give cue to scientist to understand functional genes in and regulatory processes in more empirical to develop good yielding variety of crops with desirable traits. Several advancements in RNA-*Seq* technology have been made for the characterization of the transcriptomes of distinct cell types in biological tissues in an efficient manner (Xi-Tong *et al.*, 2024). Single-cell RNA sequencing technology and spatial transcriptome technology are two among others. Single Cell RNA sequencing technology is more efficient and effective in analysis of gene expression in cell with greater impact.

On the other hand, Spatial Transcriptomics is one step ahead to single-cell sequencing in terms of loss of spatial information. It enables scientists to grasp the spatial distribution of gene expression within tissues. Consistent advancement in transcriptomics technologies has revolutionized the field of Molecular Biology but yet to be utilized in plant science for major outcome (Tyagi *et al.*, 2022).

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BIOTECHNOLOGY DISCOVERIES

Gene Editing and CRISPR Advances

- HIV Eradication in Cell Cultures: In March 2024, researchers at the University of Amsterdam successfully eliminated HIV from cell cultures using CRISPR-Cas gene editing, marking a significant step toward potential cures.
- CRISPR in Cancer Therapy: Advancements in base and prime editing have enhanced CAR-T cell therapies, enabling more potent and less toxic treatments for cancer patients.

Synthetic Biology and Protein Engineering

- AI-Designed Proteins: In January 2025, scientists developed esmGFP, an artificial green fluorescent protein, using the AI model ESM3. This protein, not found in nature, was created by simulating 500 million years of evolution, showcasing AI's potential in protein design.
- Customized Microorganisms: Researchers engineered microorganisms capable of converting organic waste into biofuels and biodegradable plastics, offering sustainable solutions for energy and materials.

Cancer Therapies and Immunotherapy

- Cell Therapy Breakthroughs: Significant advancements in cell and immunotherapy have brought us closer to potential cures for cancer. Notably, a clinical trial at the Melanoma Institute Australia demonstrated the potential to "cure" advanced melanoma using a combination of nivolumab and ipilimumab immunotherapy drugs.
- Vir Biotechnology's Progress: Vir Biotechnology reported promising results for their experimental cancer treatments targeting HER2 in solid tumors and PSMA in prostate cancer. In the HER2 trial, 10 of 20 patients experienced tumor shrinkage. In the prostate cancer trial, all 12 patients saw reduced PSA levels, with seven experiencing a decrease of at least 50%.

AI in Drug Discovery and Protein Design

- AI-Powered Drug Discovery: AI tools like DeepVariant are accelerating genomic analysis, aiding in the rapid identification of genetic variations linked to diseases, and streamlining the development of targeted therapies.
- Advancements in Protein Structure Prediction: Deep learning models such as AlphaFold3 and RoseTTAFold have significantly improved protein structure prediction, facilitating the design of novel proteins for therapeutic applications.

Sustainable Biotech and Environmental Applications

- Plastic-Degrading Microbes: In April 2024, the startup Breaking, developed within Colossal Biosciences, discovered X-32, a microbe capable of breaking down various plastics in as little as 22 months, leaving behind only carbon dioxide, water, and biomass.
- Biomanufacturing for Food Systems: Innovations in biomanufacturing, including cultured meat and fermentation technologies, are advancing sustainable food production, reducing greenhouse gas emissions, and meeting the growing demand for alternative protein sources.

Longevity and Aging Research

- Senolytic Compounds: Researchers at RWTH Aachen University identified four senolytic compounds—JQ1, RG7112, nutlin-3a, and AMG232—that can decrease epigenetic age in vitro, offering potential avenues for anti-aging therapies.
- Cellular Senescence Regulation: A study from the University of Osaka discovered that the protein AP2A1 plays a role in cellular senescence. Suppressing this protein in older cells reversed senescence and promoted rejuvenation, while overexpression in young cells advanced senescence.

De-Extinction and Conservation Efforts

- Thylacine Genome Sequencing: In October 2024, Colossal Biosciences announced the reconstruction of a 99.9% accurate genome of the thylacine (Tasmanian tiger) using a 110-year-old fossilized skull, marking the most complete ancient genome sequenced to date.
- Dodo Bird Revival: Colossal's Avian Genomics Group is working on reconstructing the DNA of the extinct dodo bird, aiming to create a hybrid with traits associated with the dodo and reintroduce it into its natural habitat.

PLANT BIOTECHNOLOGY

Gene-Edited Crops and Regulatory Progress

- UK Advances Gene-Edited Superfoods: The UK is moving forward with legislation to permit the sale of gene-edited crops, such as non-browning bananas, long-lasting strawberries, and vitamin D-enriched tomatoes. This follows the Genetic Technology (Precision Breeding) Act of 2023, which allowed field trials of gene-edited crops. The upcoming secondary legislation aims to authorize consumer sales of such foods grown in England or imported from abroad.
- EU Debates Patent Ban on Gene-Edited Crops: The European Union is considering a proposal to deregulate gene-edited crops, which includes banning patents on these crops to help European farmers adapt to climate change and improve agricultural yields. This move has sparked a dispute over intellectual property, with concerns about potential impacts on innovation and investment.
- Global Adoption of Genome Editing: Countries like Ghana, Thailand, and New Zealand have introduced or are planning regulations to facilitate the development and commercialization of genome-edited crops, reflecting a global trend towards embracing new breeding techniques for agricultural innovation.

CRISPR and Synthetic Biology Innovations

- CRISPR-Enhanced Disease Resistance: European researchers have utilized CRISPR technology to enhance resistance to mildew in crops, offering an alternative to chemical fungicides and promoting sustainable agriculture.
- Biofortified Crops for Nutrition: Advancements in biofortification have led to the development of crops like iron-rich beans, zinc-rich rice, and vitamin A-rich sweet potatoes, aiming to address micronutrient deficiencies and improve global nutrition.
- Synthetic Biology in Solanaceae Crops: Researchers are applying virus-based biotechnologies to enhance the performance and diversity of Solanaceae crops (e.g., potatoes, tomatoes, eggplants, peppers), focusing on disease resistance, nutritional enhancement, and environmental adaptability.

Sustainable Agriculture and Climate Resilience

- Drought-Resistant Varieties: Biotechnology has led to the creation of drought-resistant crop varieties that offer enhanced resilience and productivity, crucial for maintaining global food security amid climate change and water scarcity.
- • Biological Agri-Inputs: The agricultural sector has seen significant innovations in biological inputs, including biopesticides, biostimulants, and biofertilizers, contributing to sustainable farming practices and reduced reliance on chemical inputs.

AI-Driven Plant Phenotyping Tools

- ChronoRoot 2.0: An open-source platform combining affordable hardware with advanced AI to enable sophisticated temporal plant phenotyping, facilitating studies on plant development and adaptability.
- PhenoAssistant: An AI-driven system that streamlines plant phenotyping through natural language interaction, supporting automated phenotype extraction, data visualization, and model training.

International Research Highlights

- Silicon in Plant Synthetic Biology: Explorations into incorporating silicon into plant biology suggest potential for creating silicon-based life forms, with applications in medicine, sustainable agriculture, and environmental sustainability.
- Plant-Based Pharmaceutical Production: A team of University of Ottawa students developed "Phytogene," a project using the *Nicotiana benthamiana* plant to produce GLP-1 receptor agonists, potentially allowing for sustainable and accessible pharmaceutical manufacturing.

Pocket-Sized Cancer Detector: Tackling Osteosarcoma in the Field

- Researchers at IIT-BHU, Varanasi, have engineered a pioneering portable bioelectronic device capable of detecting osteopontin (OPN), a biomarker for osteosarcoma, without requiring complex lab setups. This reagent-free immunosensor, akin to a mini glucometer, employs gold- and redox-nanomaterial.enhanced electrodes, delivering rapid, accurate results with just a buffer solution. This innovation marks a breakthrough in point-of-care oncology. The device's low-cost, reagent-less design makes it especially suited for rural clinics and remote areas often overlooked in diagnostic outreach. A patent has already been filed, and the team is integrating smartphone-based reporting for remote monitoring in later stages. Beyond early detection, this tool could revolutionize pediatric healthcare with rapid referrals and early interventions. Guarded by the ethos of "Make in India" and aligned with Start-up India goals, IIT-BHU's initiative showcases.

UNIVERSITY-ORGANIZED NATIONAL SEMINARS

1. **Utkal University, Odisha**
   - **Theme :** *Indian Textual Tradition: Native and Hybrid Engagements*
   - **Dates :** February 28–March 1, 2025
   - **Organized by :** Department of English
   - **Focus :** Exploration of Indian literary traditions and their hybrid forms.
2. **Jawaharlal Nehru University (JNU), New Delhi**
   - **Theme :** *Impact of Mobile Phone Radiation and Nanotechnology on Environment and Public Health*
   - **Dates :** March 21–22, 2025
   - **Organized by :** School of Environmental Sciences
   - **Focus :** Assessing the environmental and health implications of emerging technologies.
   - **Details:** Jawaharlal Nehru University
3. **Banaras Hindu University (BHU), Varanasi**
   - **Theme:** *Social Sciences and Sustainable Development*
   - **Dates:** April 11–12, 2025
   - **Organized by:** Centre for the Study of Social Inclusion
   - **Focus:** Role of social sciences in achieving sustainable development goals.
   - **Details:** Banaras Hindu University Indian Institute of Advanced Study
4. **Central University of Tamil Nadu**
   - **Theme:** *Institutions for Viksit Bharat: Challenges and Opportunities*
   - **Dates:** February 4–5, 2025
   - **Organized by:** Department of Economics
   - **Focus:** Institutional reforms and policy frameworks for India's development.
5. **Govt. Home Science College, Chandigarh**
   - **Theme:** *Contribution of Community Science in Indigenous Technology for Developing Sustainable Strategies for Viksit Bharat*
   - **Date:** February 28, 2025
   - **Focus:** Leveraging community science and indigenous technologies for sustainable development.

GOVERNMENT-ORGANIZED NATIONAL SEMINARS

1. **18th National Seminar on National Sample Surveys**
   - **Date:** March 2025
   - **Organized by:** Ministry of Statistics and Programme Implementation
   - **Focus:** Discussing advancements and methodologies in national sample surveys.
   - **Participants:** Researchers, academicians, policymakers, and stakeholders.
   - **Details:** Press Information Bureau Press Information Bureau
2. **IIAS-DARPG India Conference 2025**
   - **Theme:** *Next Generation Administrative Reforms – Empowering Citizens and Reaching the Last Mile*
   - **Dates:** February 10–14, 2025
   - **Organized by:** International Institute of Administrative Sciences (IIAS) and Department of Administrative Reforms and Public Grievances (DARPG)
   - **Venue:** Bharat Mandapam, New Delhi
   - **Focus:** Exploring innovative administrative reforms to enhance citizen engagement.
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Research articles (not exceeding 5000 words) report research result of major significance. They should include an abstract, an introductory paragraph and brief subheads.

News (not exceeding 1000 words) can cover important scientific events or any other news of interest to scientist in general.

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Rynold, M.P. (1994). The Archaean grey gnesisses and the genesis of continental crust. In: *Archean Crustal* **Evolution** (ed. Candie, K.C.) Elsevier, Amsterdam, pp. 205–259.

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