Microbial Solutions

for Advancing Nature-Positive and Regenerative Agriculture in Drylands

ICRISAT

Transforming Dryland Agriculture

CGIAR

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Microbial Solutions for Advancing Nature-Positive and Regenerative Agriculture in Drylands

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ICRISAT
INTERNATIONAL CROPS RESEARCH INSTITUTE FOR THE SEMI-ARID TROPICS
Transforming Dryland Agriculture

2025


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CONTENT

FOREWORD

For a long time, microorganisms have been the unseen architects of nature, subtly influencing plant health, soil richness, and ecosystem balance. These microscopic companions, which range from plant growth promoters to biocontrol agents, provide a potent way to improve crop output, restore soil vitality, and lessen the negative effects of conventional farming practices. We may shift to an agricultural paradigm that places a higher priority on soil health, biodiversity, and sustainability by utilizing the potential of microbial-based solutions. The importance of microbial solutions in revolutionizing agriculture has never been greater in light of the growing environmental problems and the pressing need for sustainable food systems. For academics, practitioners, and politicians committed to creating a more resilient and ecologically sound agricultural landscape, this compilation, "*Microbial Solutions for Advancing Nature-Positive, and Regenerative Agriculture in Drylands*", is a pertinent resource.

This collection includes cutting-edge studies, case studies, and real-world applications of microbial solutions in regenerative agriculture, agroecology, and Nature-Plus approaches. In order to reduce reliance on chemical inputs, it emphasizes novel approaches that use microbial consortia for carbon sequestration, pest control, and nutrient cycling. The information presented here shows the path to a future in agriculture that will not only provide for the needs of the current generation but also protect the environment for future generations.

We envision that this compilation of ICRISAT's work act as a catalyst for innovation, knowledge sharing, and the broad use of microbial-based farming techniques. In order to promote a cooperative endeavor toward a more sustainable and regenerative future, we encourage scholars, farmers, legislators, and environmentalists to interact with the insights offered in this compendium.

Dr Himanshu Pathak
Director General
ICRISAT

MESSAGE

Adopting holistic strategies that complement nature's complex web of life is essential as we negotiate the challenges of climate change, soil degradation, and food security. The journey toward a sustainable and resilient agricultural future necessitates a paradigm shift—one that embraces the power of microbial solutions as a cornerstone of regenerative practices. This compendium is an important addition to the expanding volume of research on nature-inspired farming practices that improve biodiversity, restore soil health, and lessen environmental impact. Recent advances in our knowledge of microbial interactions in agroecosystems have highlighted the significant influence that microscopic organisms have on soil fertility, plant resilience, and the overall balance of ecosystems. Microbes have the potential to revolutionize current agriculture in a variety of ways, from microbially driven carbon sequestration and nutrient cycling to biofertilizers, biocontrol agents, and biopesticides. Leveraging the power of microbes presents a feasible and scalable answer to the growing global issues of climate change, soil degradation, and food security concerns. We believe that this compilation "*Microbial Solutions for Advancing Nature-Positive, and Regenerative Agriculture in Drylands*" will work as a springboard for innovation, knowledge sharing, and the broad use of microbial-based techniques in agriculture. Pioneering research, useful insights, and practical applications of microbial solutions in various agricultural environments are all brought together in this compilation. It emphasizes the function of microbes in promoting climate resilience, restoring degraded soils, and lowering dependency on synthetic inputs. This volume presents a comprehensive guidance of microbial solutions for building regenerative and nature plus nutri-dense food systems.

Dr Stanford Blade
Deputy Director General - Research & Innovations
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1. Background

Global community specially in drylands is currently confronted with rapidly evolving climate crisis, environmental degradation, loss of agro-biodiversity, malnutrition, health, and global economic shifts. In present-day agriculture, irrational (too much and too little) use of critical production inputs especially agro-chemicals have not only created sustainability challenges to agricultural production systems but also to biodiversity, environment, and health. Nearly two billion people live in drylands, which make up more than 40% of the planet's total area. Meeting the food production demand for an exponentially growing population elevates issues related to soil, human, and environmental health, ecological balance, and soil biodiversity. Long-term indiscriminate agrochemicals use has impacted soil health resulting in variation of nutrient availability to native microorganisms. The persistence of these recalcitrant agrochemicals in the environment significantly impacts soil microbial communities, which regulate various nutrient cycles, and their transfer through the food chain results in health hazards to humans. The deficiency of minerals and nutrients in food leads to malnutrition which is a major challenge, and the deteriorating soil health is worsening the situation. Hence, there is a need to look at soil, plant, animal, environment, and human health under a single lens – "One health" (CGIAR System Organization, 2022).

The conventional agricultural practices further exacerbate emission of greenhouse gases (GHG) and the depletion of natural resources. One third of all anthropogenic greenhouse gas emissions come from agri-food systems (Crippa et al. 2021) and the emissions due to agri-food system reached to 16.2 billion tons of carbon dioxide equivalent (Gt CO_2eq) by 2022 (FAO, 2022) due to the world's agricultural activities and food production. Agriculture contributes to climate change but also gets impacted by it, causing more frequent extreme weather events, such as droughts, floods, and uneven precipitation, which lower crop yields. Global warming also upsets the ecological balance and impacts crop pests and disease prevention and control. Additionally, soil degradation and land resource scarcity brought on by climate change affect the sustainable growth of agricultural production.

Increasing fertilizer consumption by advocating and doling out subsidies on fertilizer production and distribution is not the appropriate approach to tackle the problem of soil fertility and sustainability in agriculture. Using beneficial microorganisms has emerged as an innovative and eco-friendly technology for sustainably improving soil fertility, plant growth, and therefore food production (Kumar et al. 2022). Microbes improve soil health (nutrient cycling, soil structure, organic matter buildup), enhance plant growth and development, and lessen the need for synthetic inputs (nitrogen fixation, phosphate solubilization, indole acetic acid, and siderophore production), making them indispensable to agroecology, regenerative agriculture, and nature-positive farming. In a nutshell, using biological inputs in agriculture represents a revolutionary and long-term strategy for tackling the interrelated problems of soil health, environmental deterioration, climate change, and human welfare. The "One Health" approach, which highlights the interconnectedness of soil, plant, animal, environmental, and human health, is in line with biological solutions since they improve overall soil health and decrease the dependency on synthetic agrochemicals.

ICRISAT's capability on research and innovations on microbial solutions

ICRISAT promotes agroecology, regenerative agriculture, and nature-positive farming practices for transforming dryland agriculture across Asia and Africa. It continues to pave the way towards sustainable agriculture by harnessing underutilized biological solutions including, plant growth promoting microorganisms (PGPMs), biocontrol agents, aerobic composting, and embarking on study of soil microbiome-based on metagenomics. ICRISAT has shown how biological innovations can restore soil health, and mitigate environmental degradation by utilizing PGPMs to improve soil fertility and crop resilience, encouraging environmental-friendly biocontrol agents to reduce pesticide use, and empowering

farmers to turn waste into nutrient-rich compost. These initiatives highlight ICRISAT's dedication to developing sustainable, resilient farming practices that aligns with the environment and concept of one health.

Highlights of research on microbial solutions @ ICRISAT

- Plant growth promoting bacteria, biocontrol agents for pest and disease control, and microbial consortium for recarbonization of soil through aerobic composting are exemplary, resonating the importance of biologicals in agriculture.
- Rhizobia-like bacteria isolated at ICRISAT 's state-of-art microbiology laboratory were found to improve nitrogen fixation (>4 nmol ethylene g^{-1} fresh nodules per hectare), improve yield by 25 to 27%, nitrogen by 9 to 11% and phosphorus by 14 to 29% in the rhizosphere.
- Microbial-based biofortification using bacteria (Gopalakrishnan et al. 2016) Streptomyces spp. were found to improve the micronutrients like iron, zinc, manganese, magnesium, and calcium content (2 to 39%) in Chickpea, Pigeonpea, and pearl millet (Srinivas et al. 2022).
- Microorganisms like *Actinomycetes, Penicillium citrinum*, *Streptomyces griseoplanus, Actinomycetes* sp. were found to effectively control *Fusarium wilt*, Botrytis gray mold, cotton bollworm, Lepidopteran insects respectively (Gopalakrishnan et al. 2011, Sreevidya et al. 2005, Sathya et al. 2006, Vijayabharathi et al. 2014).
- Nucleopolyhedrosis virus (HaNPV) isolated at ICRISAT's was found to be more effective in controlling *Helicoverpa* armigera compared to other HaNPV isolates from different locations of India (Sireesha et al. 2015).

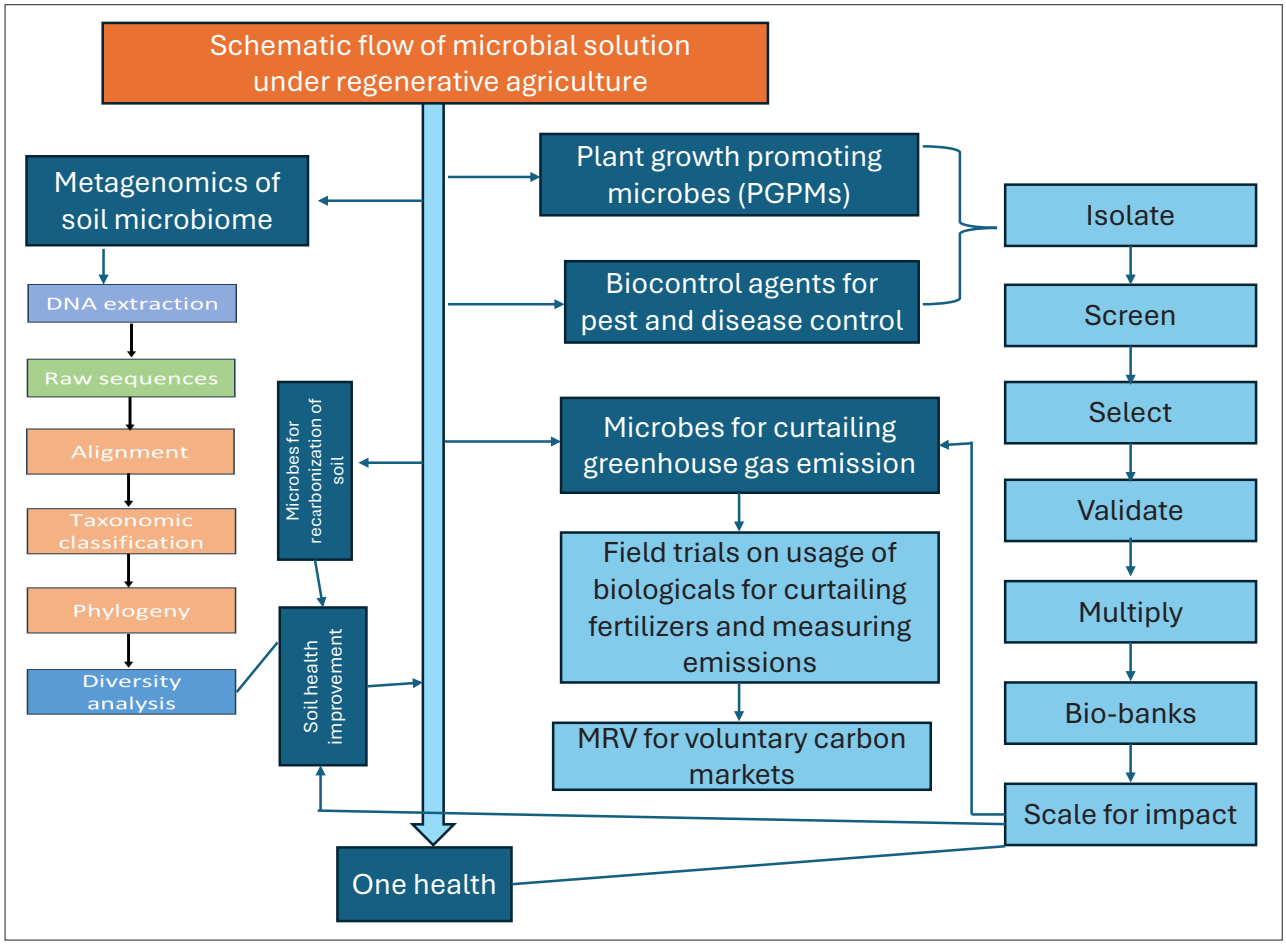
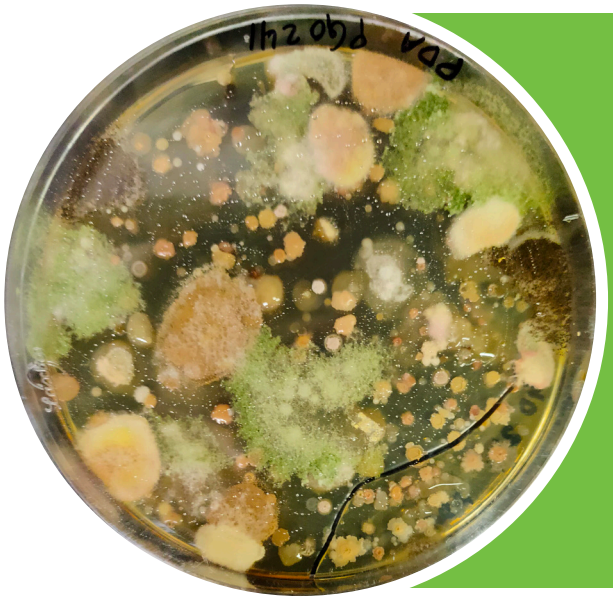


Figure 1. Microbial solution under regenerative agriculture.

2. Biology for sustainable soil and plant health



The soil microbial community includes protozoa, viruses, fungi, and bacteria. These microbes perform the essential functions that enable the nutrient cycles in soil, the breakdown of organic matter, the definition of soil texture, the soil's ability to retain water, the degradation of toxic materials, and the prevention of plant pathogens. Most nutrients, including nitrogen, phosphorus, and sulphur (N, P, and S), are bound in organic molecules in soil and are therefore barely accessible to plants.

Plants depend on soil microorganisms like bacteria and fungi, which have the metabolic capacity to mineralize organic forms of N, P, and S to make these nutrients available to plants.

Synthetic fertilizers supply macronutrients in most modern agricultural systems, substantially increasing productivity and playing an essential role in addressing food security. However, through processes including soil deterioration, eutrophication, and greenhouse gas emissions, unsustainable fertilizing methods are causing significant changes to the Earth's biogeochemical cycles, destabilizing microbial populations and functions. To restore soil health, regenerative agriculture combines environment-friendly farming techniques such as crop rotation, cover crops, no-till, agroforestry, and the use of biologicals.

Malnutrition, often called hidden hunger, represents a significant global challenge for humanity. It affects over one billion individuals worldwide, spanning developed and developing nations. This condition encompasses chronic diet-related diseases and apparent nutrient deficiencies, resulting in adverse health outcomes and hindered physical and mental development. Despite efforts to improve mineral supplementation and food fortification, these strategies have not consistently yielded positive results. Research indicates that plant growth promoting microorganisms can enhance the micro- and macro-nutrient profiles of staple crops through various mechanisms, including the production of siderophores (improves iron absorption), solubilization of zinc and phosphate, and nitrogen fixation. The processes through which plant growth promoting (PGP) bacteria enhance plant growth and productivity involve nitrogen fixation, the capacity to produce compounds such as indole acetic acid (improves root growth), siderophores (improves iron absorption), organic acids (improves bio-availability of insoluble form of nutrients), and exopolysaccharides (improves soil structures, biofilm formation, and plant systemic response), as well as the ability to solubilize phosphorus and other essential nutrients, thereby improving the uptake of micronutrients (Ahmad et al. 2008, Gopalakrishnan et al. 2014, 2016).

Applying these beneficial microorganisms in conjunction with mineral fertilizers can improve the uptake of essential minerals, thereby improving crop growth and yield. Consequently, the biofortification of staple food crops through plant growth promoting microorganisms is proposed as an innovative and sustainable approach to elevating the micronutrient content in crops and boosting yields in less fertile soils.

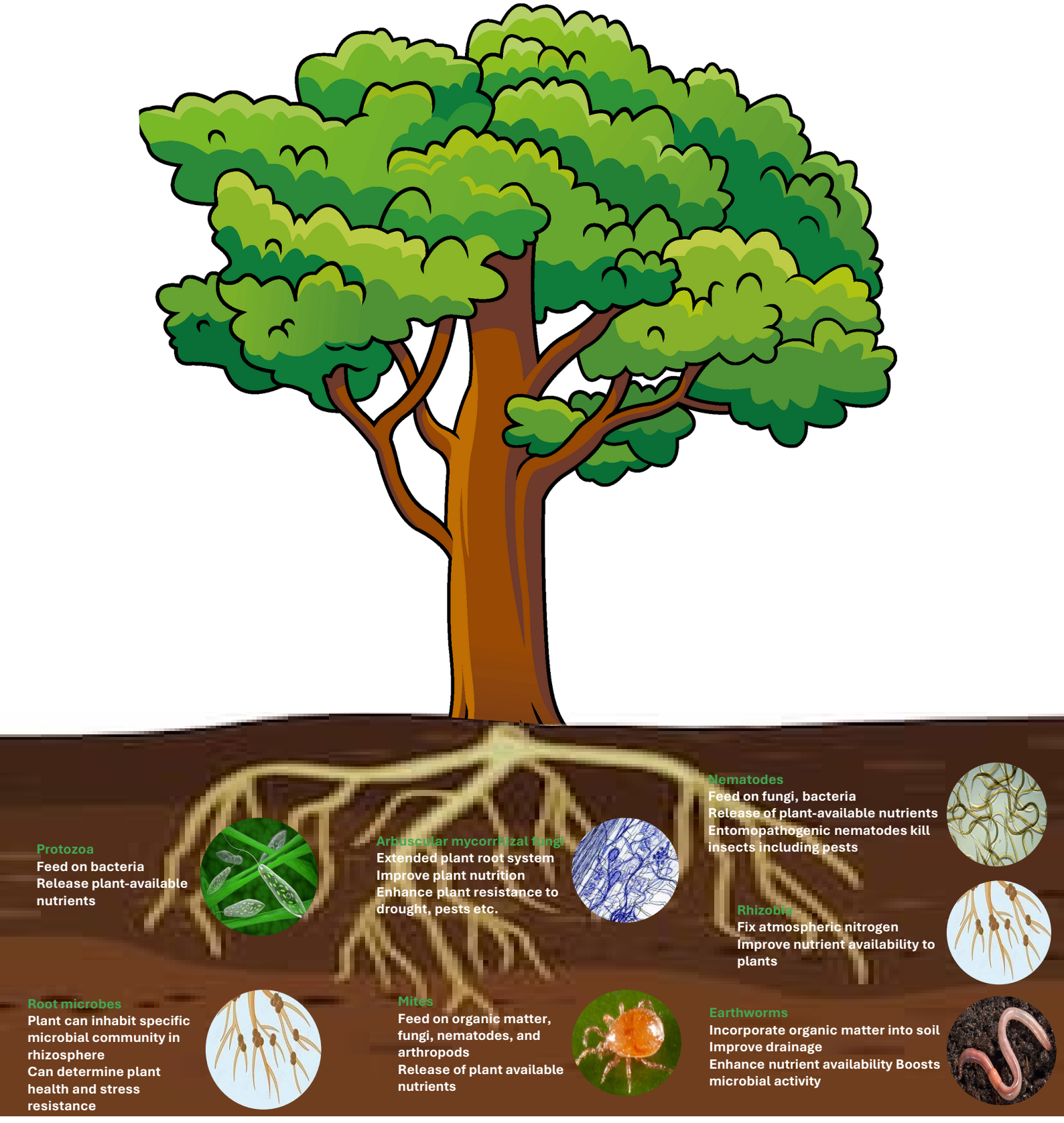


Figure 2. Life underneath the soil.

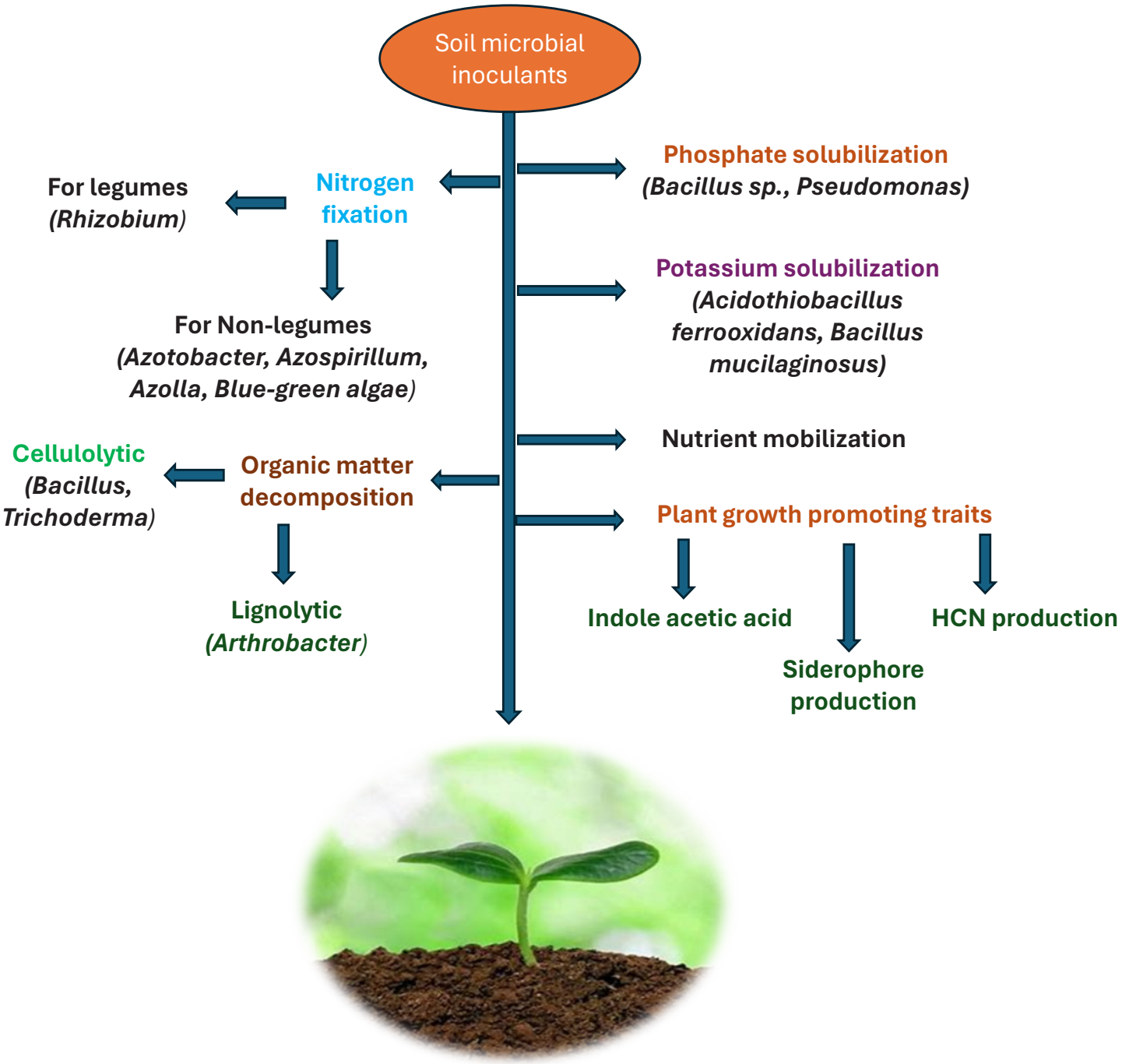


Figure 3. Role of bioinoculant in improving soil health.

4. Plant growth promoting trait-based classification of bioinoculants

The rhizosphere is micro-environment of soil surrounding plant roots, which plays a crucial role in root activity and plant metabolism. The rhizosphere is a niche supporting diverse microorganisms, including bacteria, fungi, protozoa, and algae. Bacteria are the dominant group of microorganisms that inhabit rhizosphere soils. Depending on their interactions with roots and their effects on plants, microorganisms that invade the rhizosphere can be categorized as pathogens and beneficial organisms. The bacteria that inhabit soil and exert beneficial effects on plants are called plant growth promoting bacteria.

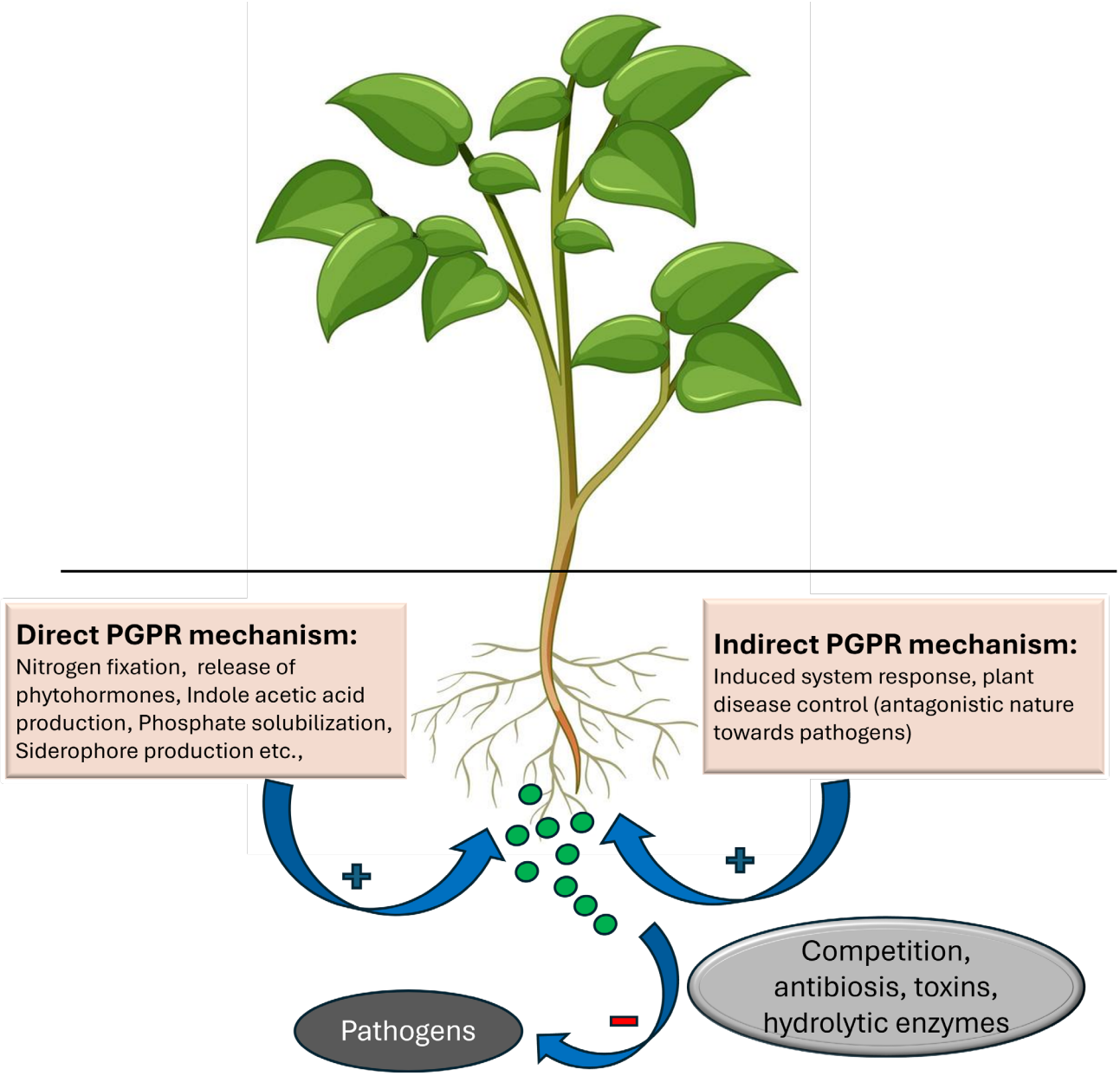


Figure 4. Plant growth promoting trait-based classification of bioinoculants.
(Source: Adopted and modified from Chandran et al. 2021)

- **Bioprotectants** are the microorganisms that supress plant diseases. The advancement of inoculants has proven to be highly effective in providing biological control agents for plant diseases, specifically organisms that can eliminate other pathogenic entities harmful to crops. *Agrobacterium, Bacillus, Pseudomonas, Streptomyces,* and *Burkholderia* are known to mitigate plant diseases through various mechanisms. These include systemic resistance development and the generation of siderophores or antibiotics.
- **Biofertilizers** are proven to improve plant growth through improved nutrient availability, such as nitrogen fixation, phosphate solubilization, and potassium mobilization etc,.
- **Biostimulant** bacteria produce phytohormones (gibberellins, cytokinins, indole-acetic acid, and ethylene production inhibitors). Phytohormones control a number of physiological and developmental functions. These hormones are vital for plant productivity and sustainable agriculture because they affect growth, stress responses, nutrient allocation, and defence systems.

4.1. Applications of plant growth promoting microorganisms

4.1.1. Biological Nitrogen Fixation (BNF)

Applying bio-fertilizers and bioenhancers, including nitrogen-fixing bacteria and beneficial microorganisms, can diminish the reliance on chemical fertilizers, thereby reducing production costs. *Azospirillum*, *Azotobacter*, *Cyanobacteria*, *Rhizobia*, and *Frankia* are genera of bacteria reported to perform biological nitrogen fixation.

Symbiotic nitrogen fixation is carried out through symbiotic inhabitation of bacteria in plant roots. These encompass symbiotic nitrogen-fixing (N_2-fixing) organisms, such as *Rhizobium* and *Frankia*, essential symbionts found in leguminous plants and associated with non-leguminous trees, respectively. Free-living N_2-fixing microbes such as *Cyanobacteria, Azospirillum*, and *Azotobacter* are known for non-symbiotic nitrogen fixation. The nitrogen fixers belonging to different domains of bacteria are given in Fig. 5.

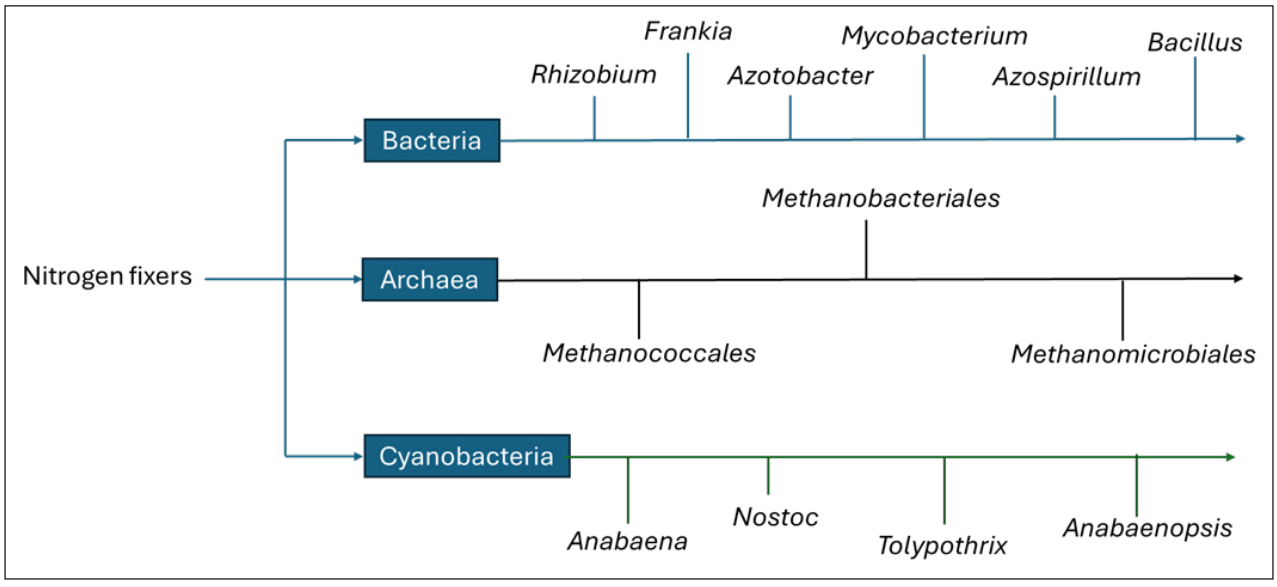


Figure 5. Different domains of microorganisms involved in biological nitrogen fixation.
(Source: Soumare et al. 2020)

4.1.2. Phosphate solubilization

Phosphorus (P) is one of the essential nutrients for a plant's growth. Microorganisms facilitate a biological process that solubilizes the inorganic phosphorus, an insoluble form of phosphorus in soil, and releases it for plant uptake. One crucial characteristic of plant growth promoting microbial inoculants for boosting plant yields is the capacity of certain microbes to transform insoluble phosphorus (P) into orthophosphate, an accessible form for plants. Fungi like *Aspergillus* and *Penicillium* and bacteria like *Pseudomonas, Bacillus*, and *Microbacterium* are the most effective phosphate-solubilizing microorganisms.

4.1.3. Potassium solubilization and mobilization

According to Gallegos-Cedillo et al. (2016), potassium (K) is a fundamental nutrient crucial for metabolic activities, viz., enzyme activity, starch synthesis, breakdown of sugars, photosynthesis, disease resistance, etc. Plants only take up exchangeable and soil solution form of potassium from the soil, which comprises approximately 2% of total K in soil and approximately 98% is in non-exchangeable or mineral form (Kirkby et al. 2001). The availability of potassium is determined by the rate at which non-exchangeable potassium is released into the soil which is mediated by potassium solubilizing bacteria. Examples: *Bacillus mucilaginosus*, *Acidothiobacillus ferrooxidans.*

4.1.4. Sulphur oxidation

Sulphur is a crucial nutrient for plants, regarded as equally vital as nitrogen, phosphorus, and potassium for their growth. It is essential to use sustainable agricultural management techniques to guarantee a sufficient supply of sulphur for healthy crops and yield. Understanding and measuring the sulphur dynamics in soil, including sulphur immobilization, mineralization, oxidation, and reduction, is a prime step for sustainable sulphur supply in the field. Environmental factors, soil conditions, and microbiological diversity affect how easily sulphur oxidizes. The activities of various microflora, particularly sulphur-oxidizing bacterial groups (SOB), play a vital role in the biological oxidation of sulphur compounds, which results in the loss of sulfides in agroecosystems. Chemolithotrophic SOB get their energy needs from process of oxidizing reduced sulphur compounds, whereas phototrophic SOB use light to power their metabolism—examples: *Chlorobium limicola, Oscillatoria sp., Thiobacillus, Sulfolobus,* etc.

4.1.5. Hydrogen cyanide (HCN) production

Certain soil-dwelling microorganisms (bacteria, algae, fungi), plants, and insects have the distinctive capability to synthesize hydrogen cyanide (HCN), which is significant in the ecological interactions among these organisms. Notably, cyanogenic bacteria have demonstrated the ability to suppress the growth of numerous pathogenic fungi, weeds, insects, termites, and nematodes. Consequently, employing HCN-producing bacteria as biopesticides presents an eco-friendly strategy for promoting regenerative agricultural practices. *P. aeruginosa*, *P. fluorescens*, *Rhizobium leguminosarum* are bacteria reported to produce HCN (Jayaprakashvel et al. 2010).

4.1.6. Siderophore production

Siderophores are small, highly effective iron-chelating substances made by various microbes, including fungi and bacteria. For every living thing to grow, iron is a critical component which manifests intense competition owing to shortage of bioavailable iron in soil environments. Microorganisms that promote plant development under iron-limiting conditions produce siderophores and also outcompete the pathogenic microorganisms. Siderophores are produced by gram-positive bacteria belonging to the genera *Pseudomonas* and *Enterobacter*, as well as *Bacillus* and *Rhodococcus*.

5. Direct plant-growth promotion mechanisms (Learnings @ ICRISAT)

5.1. Nitrogen fixation: Diazotrophic bacteria for nitrogen fixation

Diazotrophs are the primary group of microorganisms that fix nitrogen as biological nitrogen fixation in biosphere. Diazotrophs share minimal characteristics aside from their classification as prokaryotes, existing within the microbial domains of Eubacteria and Archaea. They are typically categorized according to their host association, which may be free-living, symbiotic, or characterized by a loose association, often with plant roots.

- Yield, plant growth promoting (PGP), nodulation, and nitrogen fixation characteristics of three bacteria—IC 59, IC 76A, and IC 2002—that were isolated from chickpea nodules were examined in five different chickpea cultivars, including BG 256, RSG 888, Subhra, K 850, and ICCV2.
- Bacteria produced β-1,3-glucanase, 1-aminocyclopropane-1-carboxylate deaminase, cellulase, protease, siderophore, hydrocyanic acid, and indole acetic acid.

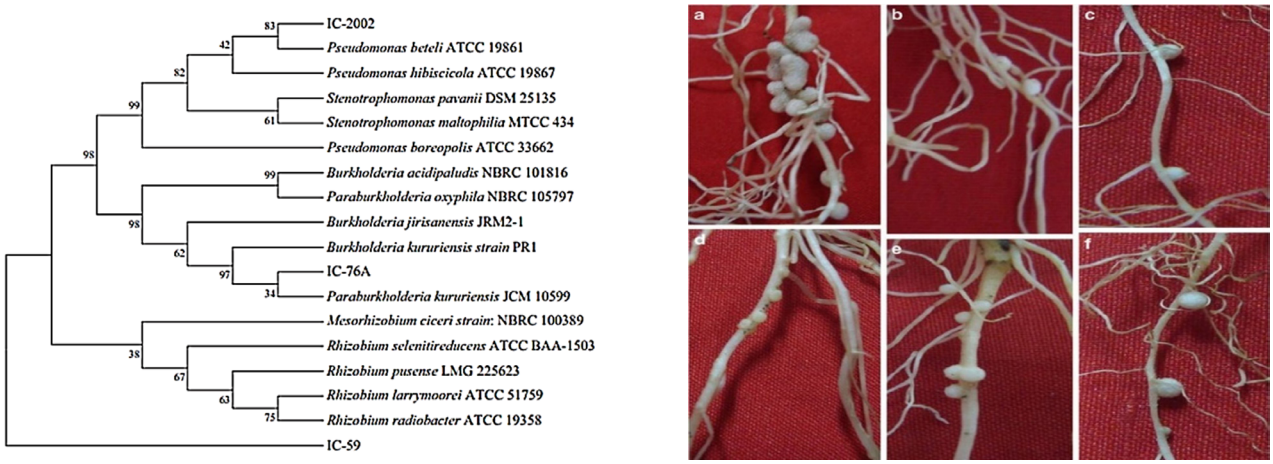


Figure 6. Molecular identification of the diazotrophic isolates (Gopalakrishnan et al. 2018), and enhanced root nodulation by diazotrophs.

- All five chickpea cultivars were nodulated by the three bacteria (*Pseudomonas beteli*, *Paraburkholderia kururiensis*, and *Rhizobium*), which fixed nitrogen and increased the nifH gene count (nitrogenase reductase (nifH) marker gene is used to identify nitrogen fixing bacteria).
- Compared to the uninoculated control, five chickpea cultivars treated with bacterial cultures (IC 59, IC 76A, and IC 2002) under greenhouse conditions increased their nodule number (up to 45%, 38%, and 43%), nodule weight (up to 31%, 15%, and 39%), shoot weight (11%, 16%, and 14%), and root weight (37%, 48%, and 62%), respectively at 30 and 45 days after sowing (DAS) (Gopalakrishnan et al. 2018).

5.1.1 Rhizobia for improved plant growth and nodulation (in chickpea)

Traditionally, it was believed that only *Rhizobium* sp. present in root nodules can fix nitrogen in legumes. But recently, a variety of α-, β-, and γ-Proteobacteria have been discovered from legume nodules, mostly belonging to genera including *Bacillus, Burkholderia, Pantoea, Serratia, Pseudomonas*, and *Enterobacter* (Gopalakrishnan et al. 2018, Martinez-Hidalgo and Hirsch, 2017; Saidi et al. 2013) that are capable of nitrogen fixation in legumes.

- Six rhizobia-like bacterial strains were isolated from leguminous plants (chickpea, pigeonpea, rostrate sesbania, and *shola pith*/Indian cork) in Indian agricultural field sites

- The isolates were identified to be *Rhizobium tropici* (IHRG), *Rhizobium* sp. (IHSR), *Rhizobium multihospitium* (IHAA), *Mesorhizobium* sp. (HGN-3), *Burkholderia cepacia* (IHCP-10), and *Rhizobium pusense* (IHCP-2)
- Polymerase chain reaction (PCR) amplification of nifH genes confirmed nitrogen-fixing ability

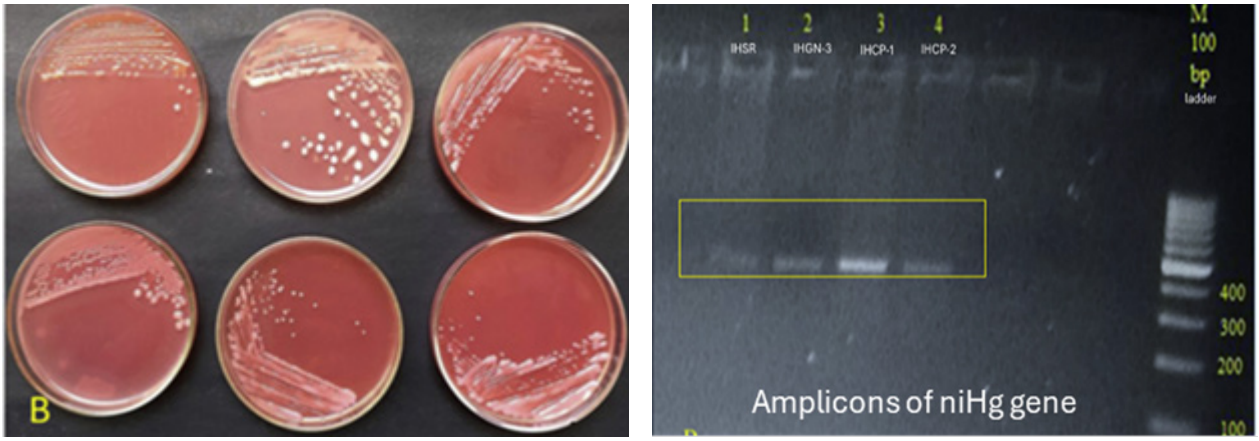


Figure 7. Isolated pure culture and amplification of nifH gene.

Colonization studies of chickpea roots by SEM analysis

- The capacity of the six bacterial isolates and the consortium (IHGN-3 combined with IHRG) to colonize the root tissues was examined using scanning electron microscopy (SEM) in 14 day old chickpea seedlings.
- In contrast to the uninoculated control seedlings, all bacterial isolates firmly adhered to the root surfaces of the inoculated seedlings.

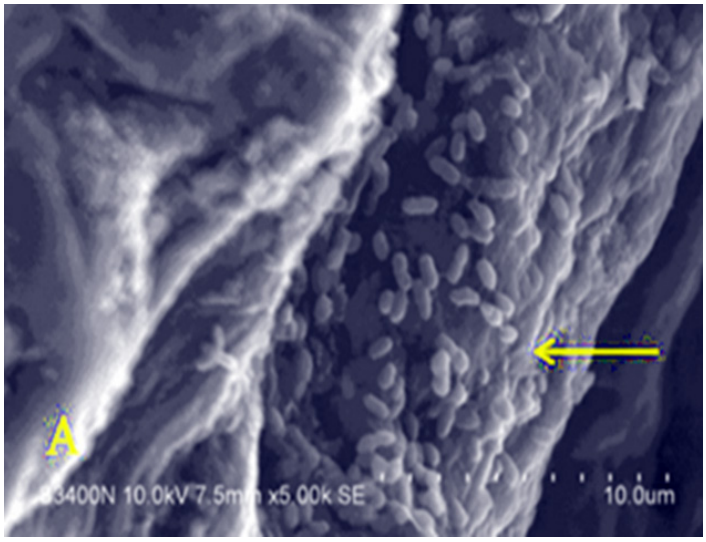


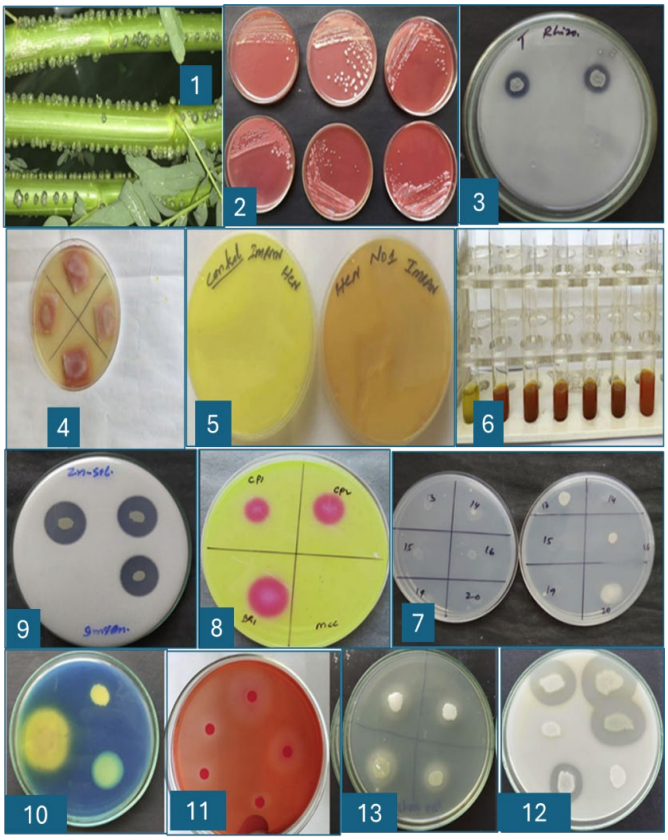
Figure 8. Roots colonized by bacterial strains.

Comparison of symbiotic parameters (nodule number and nodule dry weight) by various species

Chickpea plants treated with separated bacteria individually have been shown to increase pod number, seed number, and total nitrogen, phosphorus, and potassium at the crop maturity stage compared to the control plants.

Table 1. Comparison of symbiotic parameters (nodule number and nodule dry weight) by various species of *Rhizobium, Mesorhizobium*, and the six isolates from ICRISAT (adopted from Mir et al. 2021 and modified)

Microbial inoculant	No. of nodules/ plant	Nodule dry weight (mg plant^{-1})	Reference	Microbial inoculant	No. of nodules/ plant	Nodules dry weight (mg plant^{-1})
Rhizobium pusense	25	27	Gopalakrishnan et al. 2018	*Rhizobium* sp. strain IHSR	27	24
Paraburkholderia kururiensis	34	37				
Stenotrophomonas maltophilia	38	31				
Mesorhizobium sp.	35	86	Verma et al. 2023	*Rhizobium tropici* strain IHRG	27	20
Mesorhizobium sp. + A. chroococcum	41	103				
Mesorhizobium sp.+ B. megaterium	46	94				
Mesorhizobium sp.+ P. aeruginosa	62	132				
Rhizobium sp.	135	157	Rudresh et al. 2005	*Rhizobium multihospitium* strain IHAA	29	28
Bacillus megaterium sub sp. phospaticum + T. harzianum þ Rhizobium sp.	144	178				
R. leguminosarum	35	86	Yadav and Verma 2005	*Mesorhizobium* sp.IHGN-3	30	53
R. leguminosarum + A. chroococcum	41	103				
R. leguminosarum + B. megaterium	46	94				
R. leguminosarum + P. aeruginosa	62	132				
Rhizobium	19	67	Tagore et al. 2014	*Burkholderia cepacia* strain IHCP-1	23	20
Rhizobium + PSB	21	95				
Mesorhizobium strain Ca181	49	1312	Sindhu et al. 2002	*Rhizobium pusense* strain IHCP-2	27	26
Ca181 + *Pseudomonas* strains MRS13	71	1612				
Ca181 + *Pseudomonas* strains CRS55b	56	1482				
Ca181 + *Pseudomonas* strains CRS68	58	1420				
				Rhizobium tropici strain IHRG + *Mesorhizobium* sp. strain IHGN-3	36	99



Plant growth promoting traits of the isolates:

1) Stem nodules of Sesbania rostrata
2) Pure cultures on Yeast mannitol agar media
3) Halo zones on National Botanical Research Institute's phosphate growth medium (NBRIP) media indicated tricalcium solubilization
4) Red colour reaction on plate indicates Indole acetic acid (IAA) production
5) Brown colour development in test plate indicates HCN production
6) Colour development indicates ammonia production
7) Halozone indicates ACC deaminase activity
8) Colour development on Rock phosphate solubilization
9) Growth of colony indicates zinc solubilization
10) Halozone indicates siderophore production
11) Halozone indicates cellulase production
12) Halozone indicates Protease production
13) Halozone Lipase production

Figure 9. In vitro plant growth promoting traits of root and stem nodule bacteria.

Plant growth-promotion in greenhouse conditions

Chickpea plants (ICCV 2) treated with six specific bacterial strains, both individually (IHSR, IHRG, IHAA, IHGN 3, IHCP 1, IHCP 2) and in a consortium (IHGN 3 + IHRG), demonstrated a significant improvement in plant growth parameters and yield traits when grown under greenhouse conditions.

- The isolates' effect on the chickpea crop's nutrient traits is given in Fig. 11. The nutrient (N, P, K) content was higher when treated with the isolates.

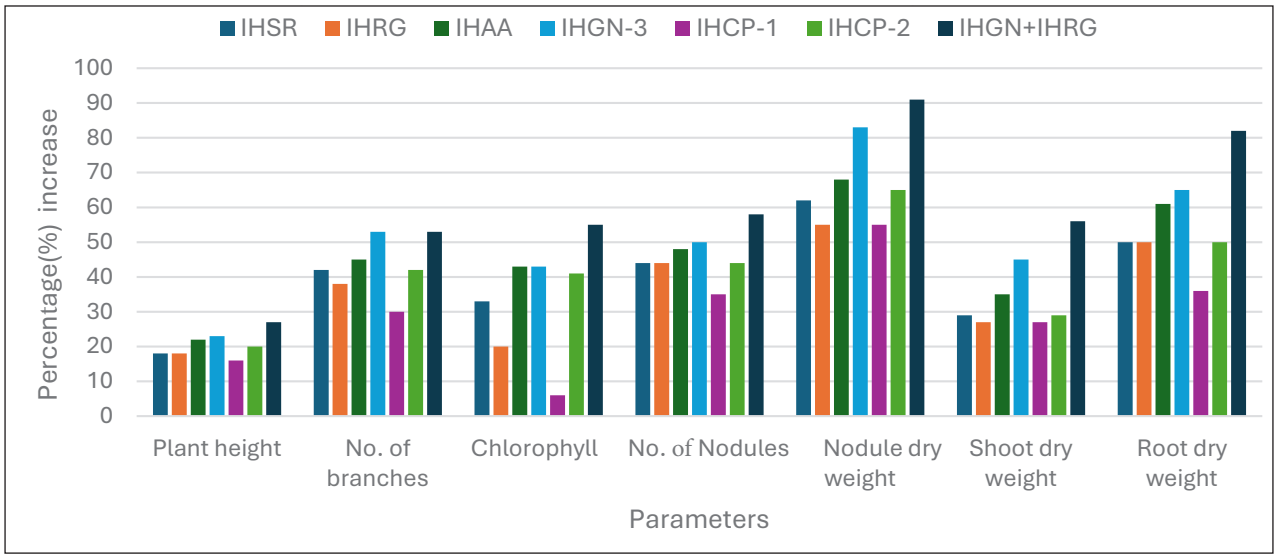


Figure 10. Plant growth-promotion by six rhizobia like isolates in greenhouse conditions.

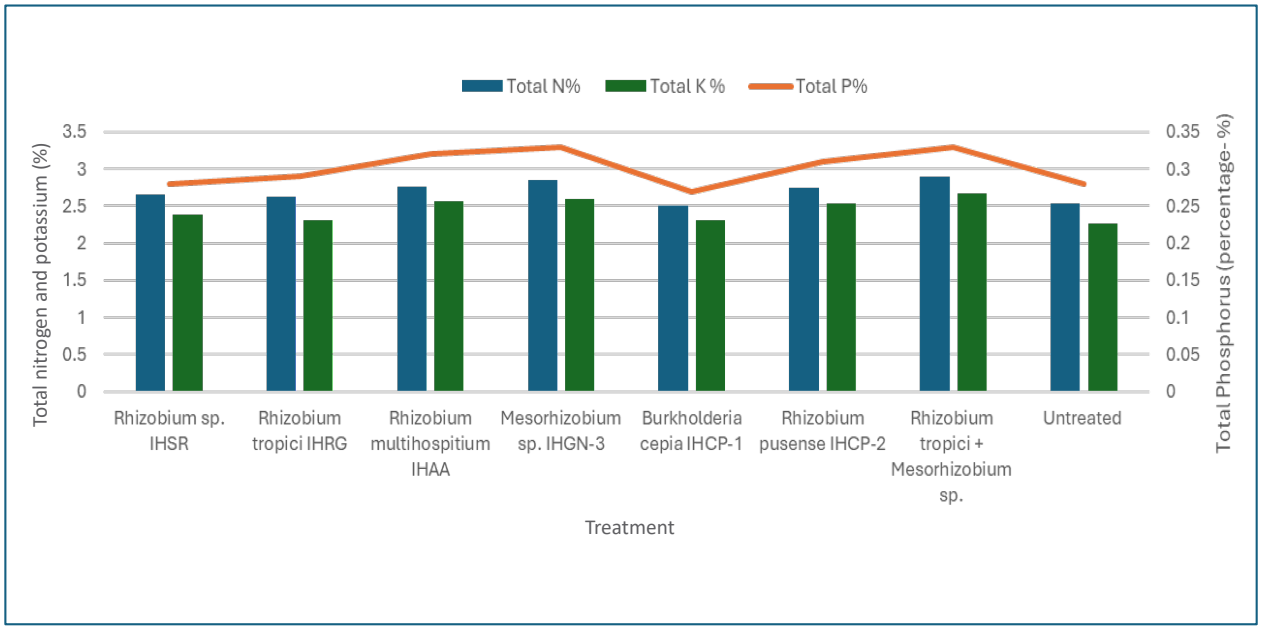


Figure 11. Effect of isolated bacteria on nutrient traits of chickpea (ICVV2) under glasshouse conditions.

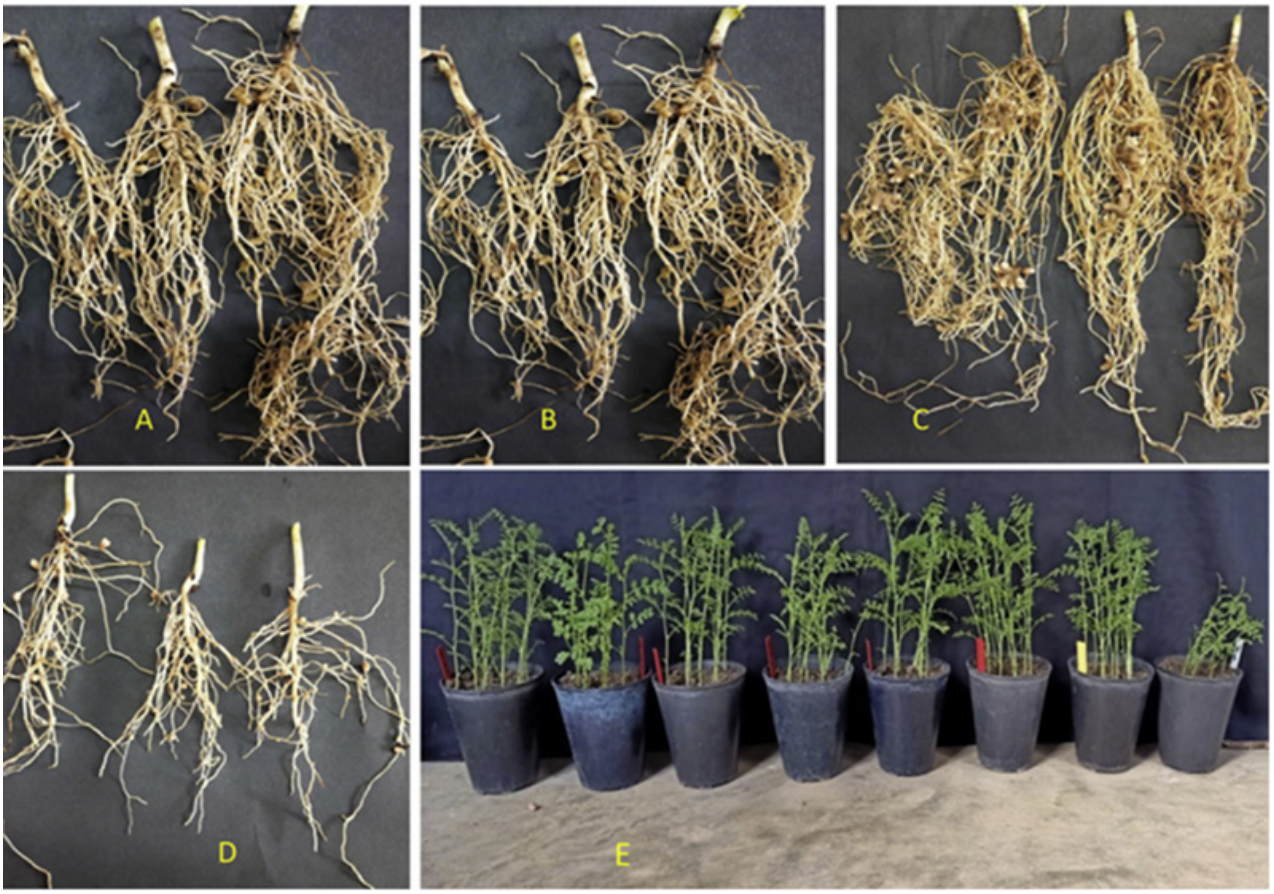


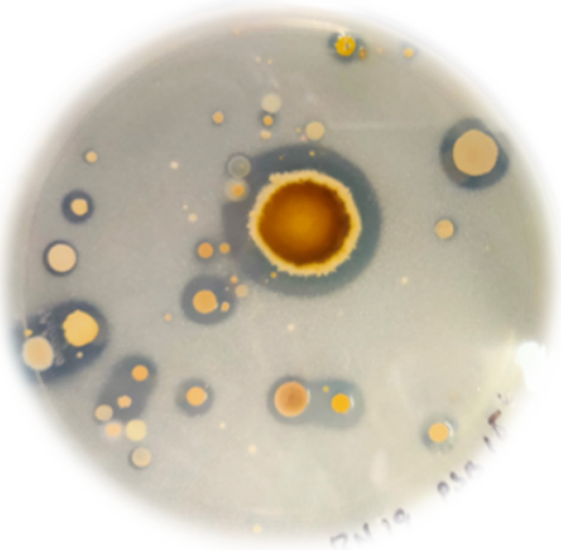
Figure 12. Results of diazotrophic bacteria application to chickpea plant (A) Treatment with Mesorhizobium sp. strain IHGN-3 (A) Rhizobium multihospitium strain IHAA (B) and Consortium of Rhizobium tropici strain IHRG + Mesorhizobium sp. strain IHGN-3 (C) enhanced root nodulation in chickpea (D) chickpea plants without microbial inoculation-Control (E) 15 days old chickpea plants treated with root and stem nodule bacteria.

5.2. Phosphate solubilization

Growth promotion by phosphate solubilizing bacteria isolated from composts and macrofauna

Phosphorus (P), the world's second most common agricultural chemical, is a necessary mineral fertilizer for plant growth and development after nitrogen. In natural environments, soluble P frequently serves as the limiting mineral nutrient for biomass formation. Plants use fewer phosphatic fertilizers, and what is left over is quickly transformed into insoluble complexes in the soil. This necessitates the frequent use of phosphate fertilizers.

These insoluble phosphate forms are changed into soluble forms by phosphate solubilizing microorganisms (PSMs) through chelation followed by exchange reactions, acidification, and gluconic acid synthesis. **Organisms: *Serratia marcescens* EB 67 and *Pseudomonas sp*. CDB 35**



Preparation of peat-based formulation

Pre-sterilized neutralized peat HDPE bags (30g) were inoculated with mid-log phase culture through the injection point under aseptic conditions and incubated for 10 days. These packets can be preserved at either room temperature or at 4°C based on further use.

Evaluation of PSB EB 67 and CDB 35 in field conditions

- The experiment was conducted in field BP2C at ICRISAT on maize.
- Nitrogen was applied as urea at 80 kg N ha^{-1} in two split doses (initially during the sowing and flowering stage). Based on the treatments, P was applied as 20 kg P ha^{-1} either as single superphosphate (SSP) or rock phosphate (RP).
- The P uptake by maize crop improved; the details are in Fig. 13.

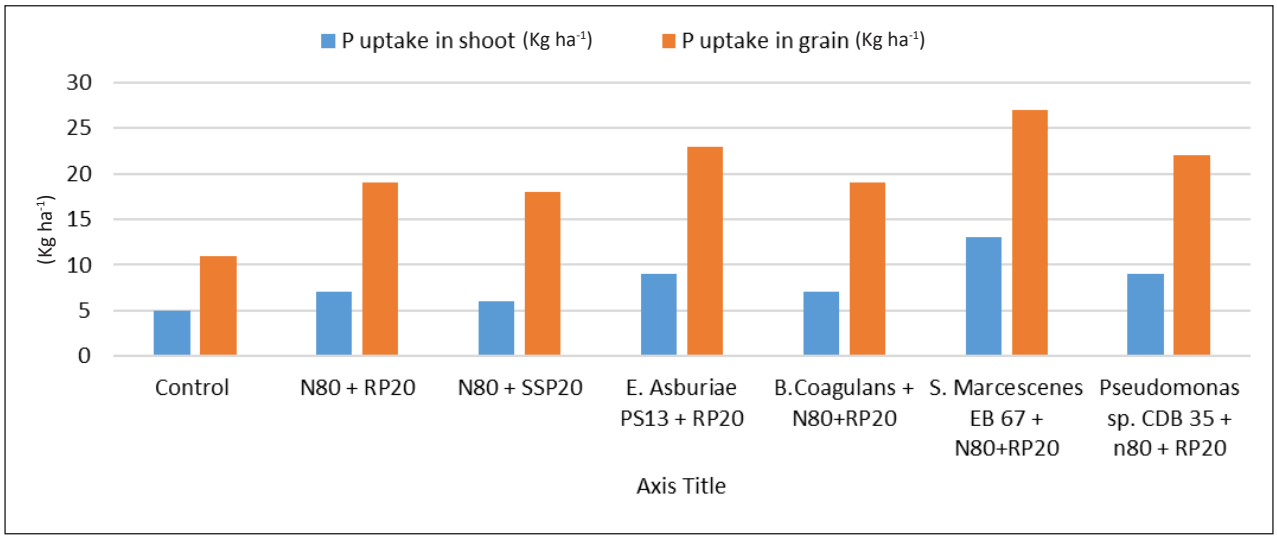


Figure 13. Uptake of phosphorus by maize crop (N80: 80 kg N ha^{-1}, SSP20: 20 ha^{-1} kg single super phosphate, RP: 20 rock phosphate ha^{-1})

- Maize seeds were treated with a peat-based formulation of EB 67 and CDB 35, resulted in increase of grain yield, dry biomass, shoot length, and seedling emergence.
- Phosphate solubilizing bacteria (PSB) was not present in the control plots till day 24 according to plate count (plate count is technique used for enumerating culturable microorganisms). However, the soil population from the control plot was 3.8 $\log_{10} g^{-1}$ at 48 DAS (Days After Sowing), 3.2 $\log_{10} g^{-1}$ at 72 DAS, and remained the same until 96 DAS.
- Population in the EB 67 inoculated plots was 6.0 $\log_{10} g^{-1}$ soil at 12 DAS, which was increased to 4.7 $\log_{10} g^{-1}$ soil at 96 days, and population in CDB 35 inoculated plot was 6.9 $\log_{10} g^{-1}$ soil at 12 DAS and 5.1 $\log_{10} g^{-1}$ soil at 96 days (Hameeda et al. 2008), (Fig. 14).

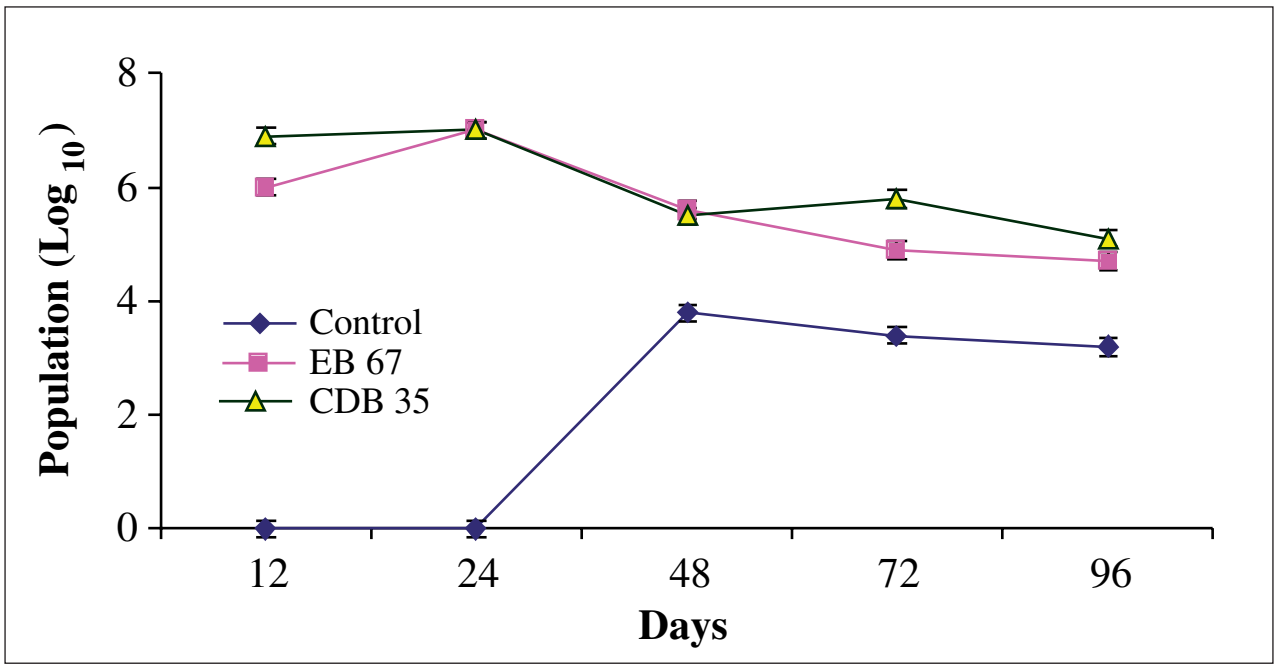


Figure 14. Colonization of PSB in field conditions, Serratia marcescens: EB 67 and Pseudomonas sp.: CDB 35 (source: Hameeda et al. 2008)

6. Plant growth promoting bacteria for chickpea

One of the significant legume food grains, or pulses, in South Asian cuisines is chickpea. With more than half a billion people living in extreme poverty, this is the largest concentrated area of poverty in the world.

About 11 Rhizobia-like bacteria were isolated and tested for plant growth promoting traits and tested with chickpea crops in field conditions, and the results are detailed in Table 2.

Table 2: Plant growth promoting traits of 11 Rhizobia-like isolates (Srinivas et al. 2022)

S. no	Culture ID	Organisms	PGP traits
1	ICKM 1	*Pantoea dispersa*	Siderophore
2	ICKM 4	*Chryseobacterium indologenes*	Cellulase, lipase, protease,Indole acetic acid (IAA) beta 1-3 glucanase, chitinase, siderophore
3	ICKM 7	*Pseudomonas geniculata*	Siderophore, phosphate solubilization
4	ICKM 9	*Stenotrophomonas pavanii*	Cellulase, lipase, protease, Indole acetic acid (IAA), beta 1-3 glucanase, chitinase, siderophore, phosphate solubilization
5	ICKM 12	*P. geniculata*	Cellulase, lipase, protease, Indole acetic acid (IAA), beta 1-3 glucanase, chitinase, siderophore, phosphate solubilization
6	ICKM 14	*P. geniculata*	Cellulase, lipase, protease, Indole acetic acid (IAA), beta 1-3 glucanase, chitinase, siderophore
7	ICKM 15	*Stenotrophomonas maltophilia*	Phosphate solubilization
8	ICKM 17	*Chryseobacterium sp*.	Cellulase, lipase, protease, Indole acetic acid (IAA), beta 1-3 glucanase, chitinase, siderophore
9	ICS 30	*P. geniculata*	Siderophore, phosphate solubilization
10	ICS 31	*Chryseobacterium indologenes*	Phosphate solubilization
11	ICS 32	*Stenotrophomonas acidaminiphil*	Cellulase, lipase, protease, Indole acetic acid (IAA), Beta 1-3 glucanase, chitinase, siderophore

Findings based on the work carried out on the eleven microbial isolates carrying PGP traits for chickpea

- Eleven Rhizobia-like bacteria were isolated from chickpea nodules, and their capacity to fix nitrogen and promote growth was evaluated.
- Four isolates (ICKM 9, ICKM 15, ICS 31, and ICS 32) were discovered to fix nitrogen greater than 4.0 nmoles of ethylene g^{-1} fresh weight of nodules ha^{-1}. All isolates nodulated chickpea, tested PCR amplification positive for nifH gene (marker gene for nitrogen fixation capability of microbes), and observed to fix nitrogen.
- In contrast to the uninoculated control, ICCV 2 and JG 11 chickpea seeds treated with the bacteria in the field enhanced the number of nodules (up to 46% and 46%), nodule mass (up to 76% and 50%), shoot mass (up to 21% and 42%), and grain yield (up to 27% and 25%). In comparison to the uninoculated control, the treated (inoculated with bacteria) rhizospheres of ICCV 2 and JG 11 exhibited higher levels of organic carbon (up to 7% and 24%), total nitrogen (up to 11% and 19%), and accessible phosphorous (up to 14% and 29%) at harvesting time.
- Every isolate exhibited characteristics that promoted plant growth, such as hydrocyanic acid (except from ICKM 17 and ICS 31), Siderophore, indole acetic acid, and β-1,3-glucanase (except ICS-31).

7 . Plant growth promoting bacteria for pearl millet

Actinomycetes have been known to promote plant development, exhibit antagonistic properties against plant diseases and insect pests, and have biofortification qualities in various crops vital to agriculture. The current study aimed to describe the probiotic qualities of the actinomycete(s) that were isolated from samples of pearl millet flour and batter. Actinomycetes isolation agar (AIA) was prepared using selective and particular media and the most notable actinomycetes that were detected in large quantities on the AIA plate were isolated. The most significant actinomycete was characterized using various techniques, including gram staining, morphological analysis, antibiotic tolerance, probiotic potential, and antimicrobial activity against human pathogens.

The *Streptomyces* sp. isolated carried PGP traits, antibiotic resistance, and probiotic and antagonistic properties (Kunchala et al. 2017). **Findings are given in Table 3.**

Table 3. Probiotic, PGP, and biocontrol properties of *Streptomyces* species isolated from batter and pearl millet flour (adopted and modified, Kunchala et al. 2017)

Probiotic Properties	Units or rating scale (RS)	Result
Acid tolerance	pH	2
Bile tolerance	(%)	0.5
Phenol tolerance	(%)	0
NaCl tolerance	(%)	6
PGP and biocontrol traits		
Indole acetic acid	(µg/ml)	0.06
Siderophore	(% units)	0.5
Lipase (halo zone) *	No halo zone-0	4
Cellulase (halo zone) *	1-10 mm halo zone – 1	2
Chitinase (halo zone) *	11-20 mm halo zone – 2 21-30 mm halo zone -3 31-40 mm halozone-4	3
Hydrocyanic acid (rating) *	No color change-0 Light reddish brown-1 Medium reddish brown-2 Dark reddish brown-3	2

*Halo zone is the zone of clearance in the petri dish appeared due to enzyme activity (lipase, cellulase, chitinase). Hydrocyanic acid test is based on the intesntiy of color developed in the test. Source: Kunchala et al. 2017 .

8. Bioinoculants for millet production

Twelve bacterial isolates were characterised using staining, morphology, culture, growth, and biochemical characteristics and identified referring to Bergey's manual of determinative bacteriology (Krieg and Holt 1984). Three possible isolates were found at the Microbial Type of Culture Collection (MTCC) in Chandigarh, India, for verification. Table 4 provides specifics about their PGP characteristics.

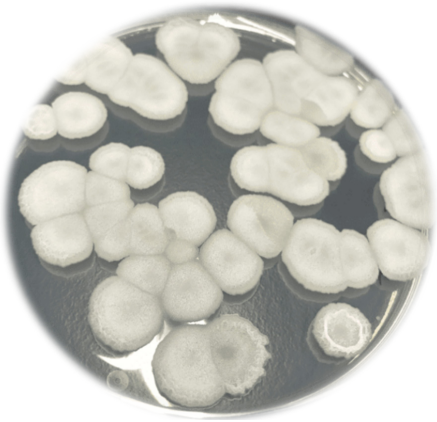


Table 4. Plant growth promoting traits of twelve isolates (Bioinoculants for millet production)

S.no	Isolate	P solubilization	Phytase	Siderophore	Chitinase	ACC deaminase	IAA	HCN	Biocontrol
1	*B. licheniformis* - EB 13								
2	*E. cloacae* - EB 27								
3	*B. circulans* - EB 35								
4	*S. marcescens* - EB 67								
5	*Serratia* sp. - EB 75								
6	*K. oxytoca* - EB 77								
7	*Pseudomonas* sp. - CDB 35								
8	*Pseudomonas* sp. - CDB 36								
9	*B. licheniformis* - CDB 47								
10	*Pseudomonas* sp. - BWB 21								
11	*Pseudomonas* sp. - BWB 36								
12	*Pseudomonas* sp. - BWB 40								

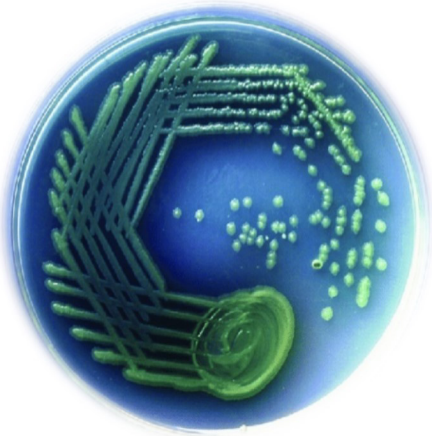
Red is negative (-), light green is positive (+), thick green is strongly positive (++), blue is very strongly positive (++++)

9. Plant growth promoting bacteria for pigeonpea

PGP microorganisms are widely recognized for their benefits in protecting crop production and preserving the soil's health. These microorganisms are frequently found in compost, decomposing organic materials, and soil. They also generate secondary metabolites that are important for agriculture. PGP bacteria, among which *Bacillus* species, *Pseudomonas* species, *Enterobacter* species, *Brevibacterium* species, and *Streptomyces* species are significant, have been shown to control insect pests and plant diseases and enhance plant growth (Gopalakrishnan et al. 2016).

PGP bacteria used in the study are enlisted below (Gopalakrishnan et al. 2016)

1. *Pseudomonas plecoglossicida* (SRI 156)
2. *Brevibacterium antiquum* (SRI 158)
3. *Bacillus altitudinis* (SRI 178)
4. *Enterobacter ludwigii* (SRI 211)
5. *E. ludwigii* (SRI 229)
6. *Acinetobacter tandoii* (SRI 305)
7. *P. monteilii* (SRI 360)



Salient findings

- The details of the influence of seven bacterial strains on the germination of pigeonpea are given in Table 5 and Fig. 15 (Gopalakrishnan et al. 2016).

Table 5. Influence of the seven bacterial strains on germination of pigeonpea

Bacteria	Shoot height (cm)	Root length (cm plant^{-1})
SRI 156	13.3	10.3
SRI 158	14.8	12.5
SRI 178	13.2	10.4
SRI 211	13.4	12.7
SRI 229	12.8	11.1
SRI 305	12.3	9.9
SRI 306	12.2	9.9
Control	10.5	9.8

- Since 100% germination was seen in both bacteria-treated and untreated control plants, the seven bacterial strains did not affect germination. Nonetheless, the seedlings' shoot height and length increased considerably, reaching up to 29% and 22% for pigeonpea, respectively.
- In comparison to the uninoculated control, it was discovered that the bacterial treatments SRI 158 and SRI 211 in pigeonpea considerably increased both height of shoot and length of root ($p < 0.05$).

Figure 15. Effect of PGP bacteria on root and shoot length of pigeonpea.

10. *Streptomyces spp.*: Plant growth promoting agent for sorghum

Sorghum has served as a vital staple food in the semi-arid regions of Asia and Africa for many centuries and is known to be the fifth most significant cereal crop globally. Its uses are diverse, encompassing food production, starch production, adhesives, alcoholic beverages, and biofuels. The relatively low yields of sorghum can be linked to both abiotic and biotic stresses, as well as the poor agronomic management practices, and soil fertility.

Microorganisms can positively impact plants by enhancing the availability of essential macro (N,P) and micro (Fe, Zn) elements, within the rhizosphere (Cakmakci et al. 2006) or by synthesizing substances that promote plant growth.

PGP microbes

Table 6. PGP microbes used in the study		
1	BCA-546 (KF770898)	Source: Alekhya and Gopalakrishnan, 2016
2	BCA659 (KF770889)	
3	BCA-667 (KF770888)	
4	BCA-667 (KF770888)	
5	BCA-698 (KF770900)	
6	CAI-133 (KF770895)	
7	CAI-8 (KF770890)	

Field studies

- Plots consisted of 4 × 3 m ridges in a randomized complete block design (RCBD). The seven *Streptomyces* strains that were selected (BCA 546, BCA 659, BCA 667, BCA 689, BCA 698, CAI 133, and CAI 8) were cultivated in starch casein broth (SCB) for five days, soaked for one hour with sorghum seeds (SPV 1411) before sowing, and then manually sown at a depth of 5 cm.
- The soil was treated with a booster dosage of *Streptomyces spp*. (10^8 cfu ml^{-1}) every 15 days till the flowering stage. There were no *Streptomyces* species in the control plots.
- Plant growth indicators, such as height, leaf area, root weight, shoot weight, and leaf weight, were measured at 60 days after sowing.
- Plant height, panicle length, 1000 seed weight, grain yield, and stover yield were among the growth and yield parameters measured at the final harvest.
- Using the established procedures, soil samples (from the 0 to 15 cm soil profile) were taken during the flowering phase (60 DAS) and harvesting phase, and analyzed for organic carbon, available P, and total N.

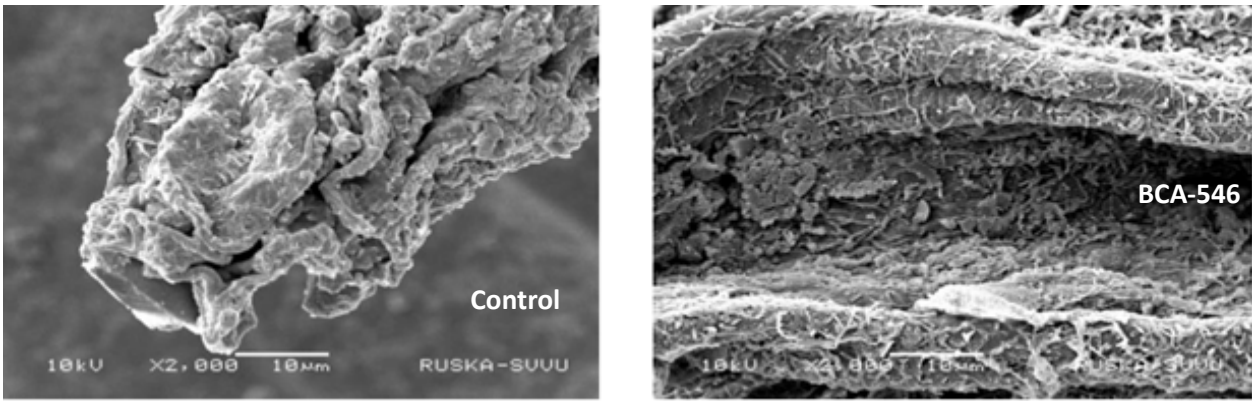


Figure 16. Scanning electron microscopy images revealing excellent colonization of Streptomyces BCA 546 on sorghum roots.

Salient findings

- According to scanning electron microscopy observations, *Streptomyces spp.* was found to colonize sorghum plant roots (Fig. 16).
- *Streptomyces spp*. was found to improve nutrient uptake, organic carbon, grain, and stover yield (Fig. 17, 18).

The seven chosen *Streptomyces* species improved sorghum growth in both greenhouse and field settings. Additionally, these isolates expressed PGP genes and had good colonization potential for the sorghum plant's root surface. Therefore, these isolates are the most effective for PGP in sorghum.

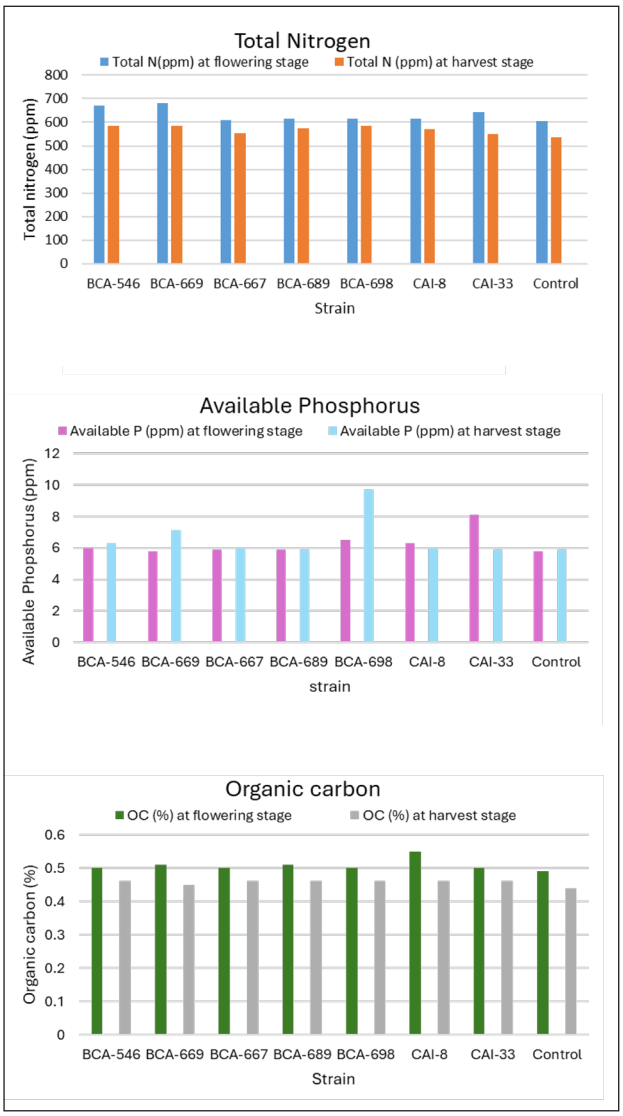


Figure 17. Influence of Streptomyces spp. (seven nos.) on the soil mineral properties.

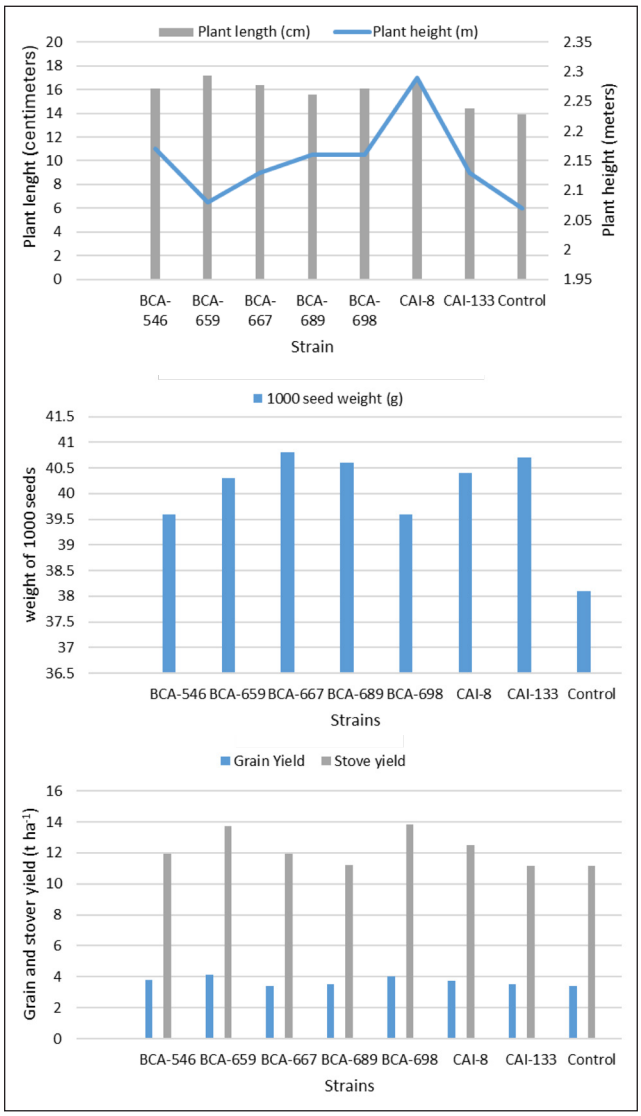


Figure 18. Influence of Streptomyces spp. (seven nos.) on the morphological observations and yields of sorghum under field condition.

11. Microorganisms-based bio-fortification of chickpea, pigeonpea, and pearl millet

Micronutrient deficiency is a pressing challenge and majorly contributing to malnutrition. Two billion people experience hidden hunger, according to the global hunger index 2014 (von Grember et al. 2014). Among the micronutrient deficiencies, iron and zinc deficiencies are considered the most prevalent mineral deficiencies ranked as 9th and 11th in the 20 major health risks. Iron deficiency is cause for anemia, still births, problems in mental development and child deaths. Zinc deficiency causes immune suppression, and growth disorders. Fortification of crops through conventional genetic breeding, transgenic and chemical amendment approaches were partly successful. Microorganisms-based biofortification through their inoculation into soil not only decreases the demand for synthetic fertilizers but also plays an important role in plant uptake of trace elements and their bioavailability in a cost-effective manner (Mishra et al. 2023).

ICRISAT has tested the biofortification ability of seven bacteria (*P. plecoglossicida, B. antiquum, B. altitudinis, E. ludwigii- SRI 211, E. ludwigii- SRI 229, A. tandoii, P. monteilii*) in Chickpea and Pigeon pea, and three *Streptomyces* spp. (CAI 24, KAI 27 and MMA 32) in Pearl millet in field conditions. The results are tabulated below.

Table 7. Microorganism-based biofortification of different crops

Crop	**Microorganisms used: *P. plecoglossicida, B. antiquum, B. altitudinis, E. ludwigii- SRI 211, E. ludwigii- SRI 229, A. tandoii, P. monteilii***				
	Percentage of increase of micronutrient content (in grains)				
	Iron (%)	**Zinc (%)**	**Copper (%)**	**Manganese (%)**	**Calcium (%)**
Chickpea	18	23	19	2	22
Pigeonpea	12	5	8	39	11
	Microorganisms used: *Streptomyces spp.* (CAI 24, KAI 27 and MMA 32)				
	Iron (%)	**Zinc (%)**	**Magnesium (%)**	**Calcium (%)**	
Pearl millet	24	10	25	17	

- Microbial-based biofortification using seven bacteria (Gopalakrishnan et al. 2016) and three Streptomyces spp. were found to improve the content of micronutrients like iron, zinc, manganese, magnesium, calcium content (2 to 39%) in chickpea, pigeonpea, and pearl millet (Srinivas et al. 2022).
- Recent studies proved that biofortified food might be able to influence the gut microbial taxa of hosts, as the microbes colonising the gastrointestinal tract need minerals for their metabolic activities. Therefore, cost-effective microorganism-based biofortification of staple food crops becomes imperative and highlights ICRISAT's contribution in strengthening the one-health concept.

12. Harnessing the potential of biological consortia for curtailing GHG emissions

A revolutionary strategy for reducing greenhouse gas (GHG) emissions and improving the sustainability and resilience of farming methods is the incorporation of biological consortia into agricultural systems. By lowering emissions, storing carbon, and enhancing ecosystem health, these microbial solutions provide a multipronged approach to combating climate change in the context of regenerative agriculture.

Microbial Consortia Granules (MCG)

Advanced bioformulations called Microbial Consortia Granules (MCG) combine benificial microorganisms into a single, simple-to-apply solution to improve soil health and plant development. Plant growth and soil health are enhanced by Microbial Consortia Granules, which contains a pre-mix of free-living nitrogen fixers, phosphate-solubilizing bacteria (PSB), and potassium-mobilizing bacteria (KMB). MCGs encourage beneficial interactions between different organisms, which helps ensure that farming is sustainable and that agro-ecosystems are resilient to climate change. The concepts of regenerative agriculture are perfectly aligned with their capacity to increase biodiversity and reduce reliance on artificial inputs.

While certain bacteria, such as Rhizobia, fix atmospheric nitrogen and reduce the need for synthetic fertilizers, others control denitrification and inhibit nitrification to reduce the amount of nitrous oxide (N_2O) released. Microbial consortia improve soil organic matter by encouraging carbon sequestration, decomposing, and using root exudate. In rice ecosystems, methanotrophs oxidize methane (CH_4), while microbial inoculants optimize anaerobic digestion to lower emissions. These remedies support regenerative agriculture, helping to mitigate climate change while enhancing soil health, resilience, and sustainability.

Salient findings

- Validation trials on Zero-till chickpea with biological consortia + lime showed promising results in *rabi* rice fallow systems across four districts of Odisha, India
- Chickpea equivalent yields under regenerative agriculture practice in millet-based cropping systems of *Vertisols* (Dryland Living Lab, ICRISAT) were greatly enhanced by the combination of mulching, biological consortia, and soil test-based fertilizer (STBF)
- Rooting density and root/shoot ratio of rice plants are significantly improved by application of microbial consortia granules in rice ecologies of Odisha

Source: https://hdl.handle.net/10568/168565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13. Impact of Natural Farming on soil microbial diversity

Community-managed natural farming (CMNF), formerly known as zero-budget natural farming (ZBNF), is a type of regenerative agriculture that was introduced by the Andhra Pradesh government in 2016. It is based on agroecology. The goal of CMNF is to completely give up synthetic fertilizers and insecticides. In order to combat a range of environmental and economic challenges, such as deteriorating soil quality, depleting water supplies, biodiversity loss, and loss of farmer livelihoods, the initiative aimed to encourage the adoption of comprehensive natural farming practices.

As a part of CGIAR Initiative on Agroecology, ICRISAT focused on studying the microbial diversity of soils from natural farming sites of Andhra Pradesh, Inda. The study encompasses collection of soil samples, followed by metagenomics. The Fig. 19. shows the schematic flow of the DNA extraction, sequencing, and microbial diversity analysis processes. The ITS2 region of the fungal 18S rDNA and the V4 portion of the bacterial 16S rDNA were amplified and sequenced. R (4.2.0) was used for all of the analyses. Read quality control, read filtering, dereplication, sample inference, paired-end read merging, and chimera identification removal were all accomplished using the software dada2 (v1.24.0). To conduct taxonomic assignment, the SILVA (138.1) database was used. Using the R package phyloseq (v1.40.0), the identified taxa were grouped according to the various taxonomic levels and utilized to create bar graphs.

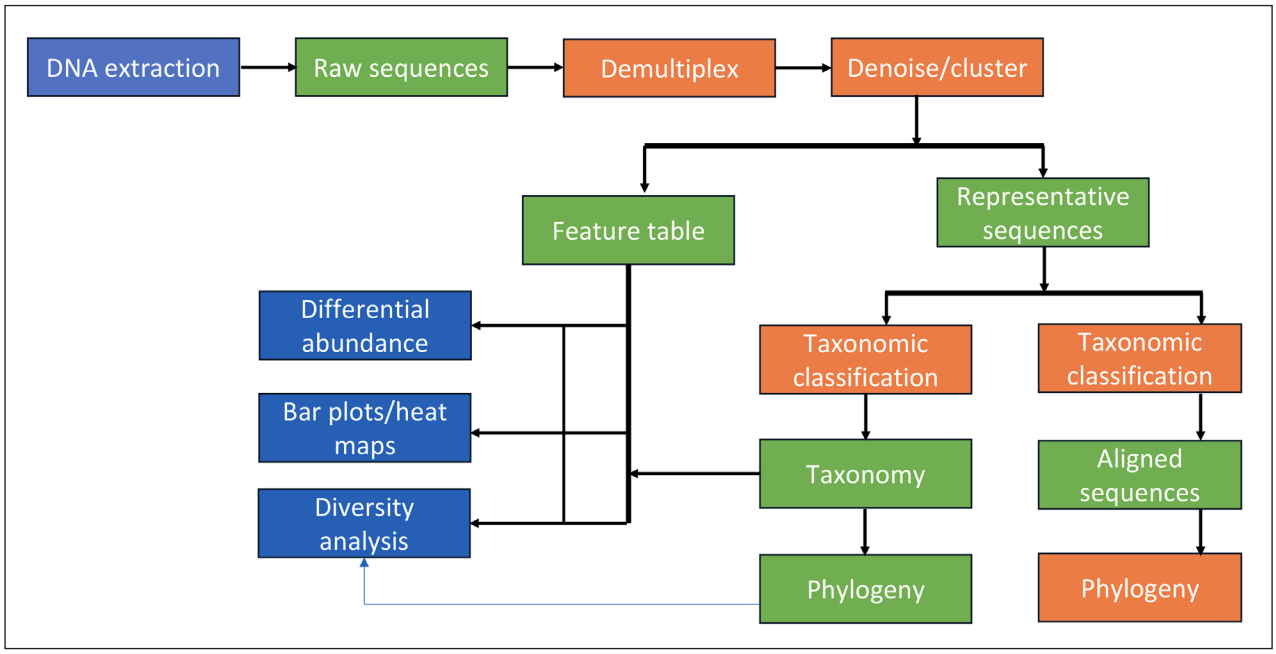


Figure 19. Schematic work flow of metagenomics-based microbial diversity study.

Salient findings

- It was discovered that the bacterial genera *Candidatus nitrososphaera*, *Nitrospira, Stenotrophobacter*, *Dunganella*, and *Pseudomonas* were prevalent in Andhra Pradesh's natural agricultural systems
- The nitrogen cycle depends on the ammonia-oxidizing archaeon (AOA) *Candidatus Nitrososphaera* and the bacterium *Nitrospira*, particularly for nitrification, which converts ammonia (NH_3) to nitrite (NO_2). This procedure is essential for agricultural systems to preserve soil fertility and nitrogen availability
- *Stenotrophobacter* breaks down organic materials in soils to release nutrients including carbon, phosphorus, and nitrogen that are vital for plant development.

- The rhizosphere, or soil region around plant roots, is commonly home to *Duganella* and *Pseudomonas* present in the rhizosphere are responsible for synthesis of phytohormones, nutrient solubilization, antagonistic action against soil-borne diseases, and organic matter breakdown
- *Alternaria* and *Aspergillus* are common fungi found in the soils of natural farming systems which can break down organic materials in soil. Beneficial fungi include *Aspergillus* species; for example, the non-virulent *Aspergillus flavus* serves as a biocontrol agent.

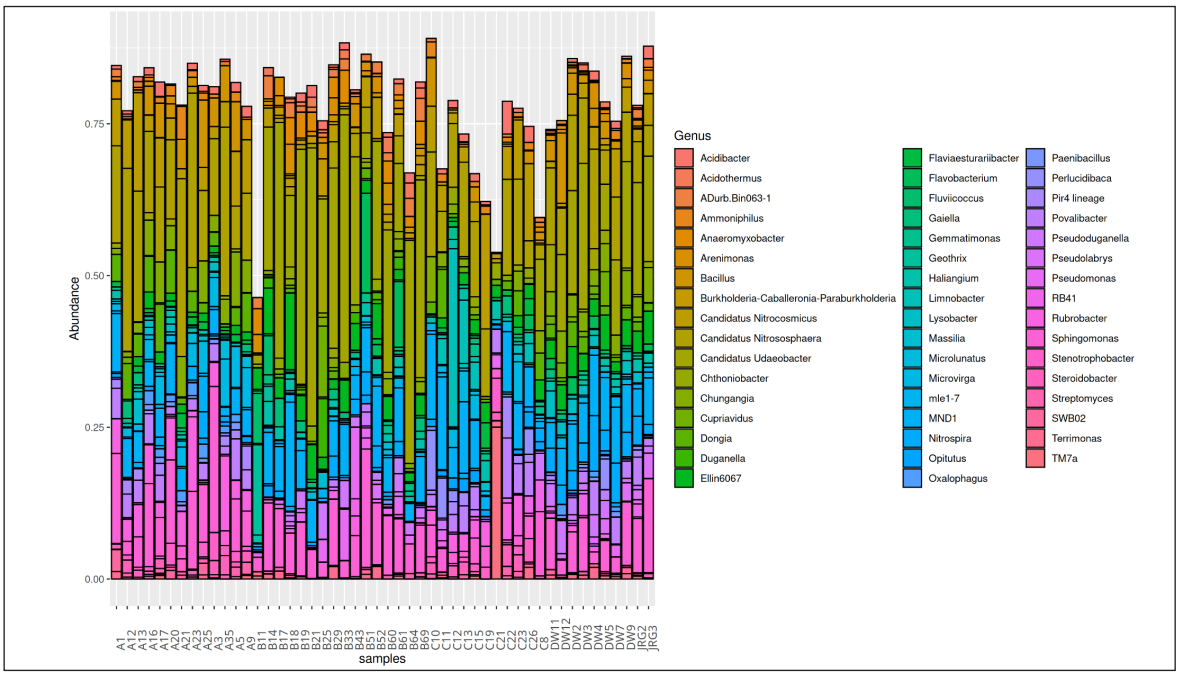


Figure 20. Bar plot showing the top 50 most abundant bacterial genera

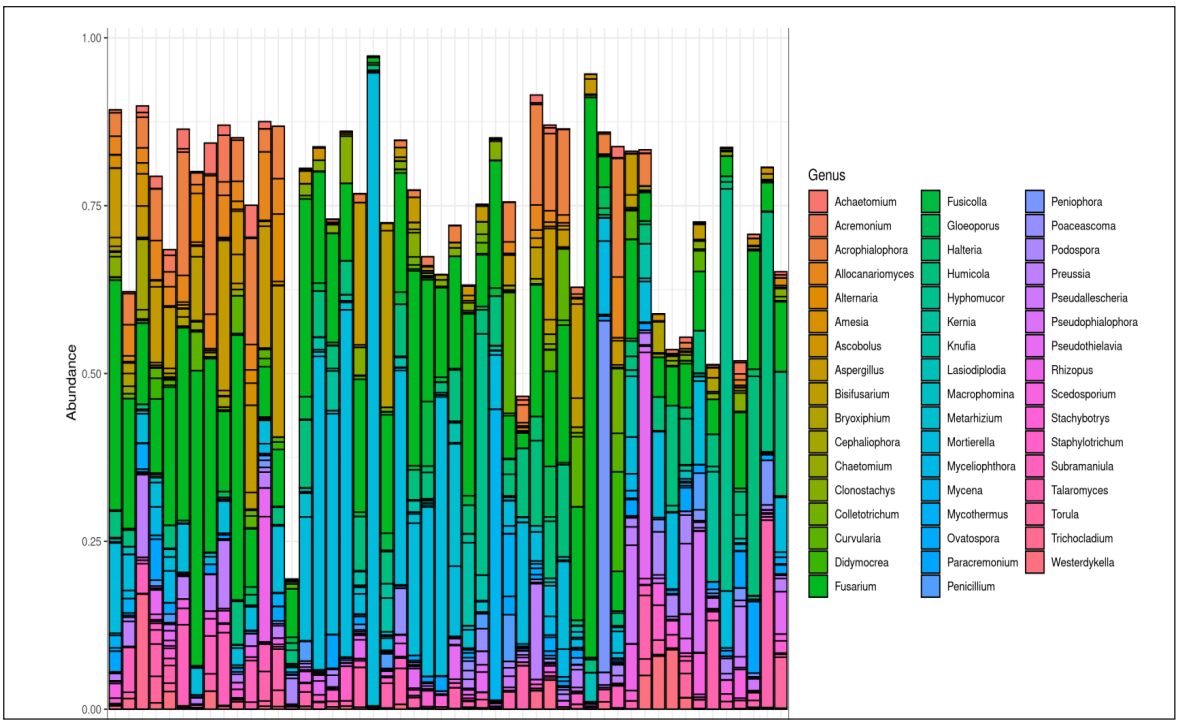


Figure 21. Bar plot showing the top 50 most abundant fungal genera.

Source: https://hdl.handle.net/10568/168632

14. Agriculturally important strains of *Streptomyces spp.* at ICRISAT

Actinomycetes or Actinobacteria, are the diverse and extensively dispersed class of gram-positive bacteria. These rod-shaped, filamentous, mold-like bacteria tend to branch and constitute a significant portion of the endophytic and rhizosphere microbial communities (Gayathri and Muralikrishnan, 2013).

Actinomycetes aid in carbon (C)-cycling by breaking down recalcitrant polymers found naturally in soil and plant litter, such as lignocelluloses, chitin, and pectin. *Streptomyces* and *Micromonospora* are the two *Actinomycetes* genera that have historically been described the most. Many studies have been conducted to find bioactive natural compounds in the genus *Streptomyces*. Members of the genus *Streptomyces* produce around 75% of the natural antibiotics isolated from *Actinomycetes*, accounting for about two-thirds of all antibiotics.

This bacterial group has drawn a lot of interest lately because of its dominance in the soil, intense antibacterial activity, and ability to promote plant growth. Table 8 gives details of *Streptomyces* isolates carrying plant growth promoting traits.

Table 8. Agriculturally important strains of *Streptomyces* isolated and tested at ICRISAT (Gopalakrishnan et al. 2014).

Accession number	Plant growth promotion (PGP) and biocontrol traits								Entomopathogenic potential			Field trials conducted on			
	Siderophore	Chitinase	Cellulase	Lipase	Protease	IAA	Hydrocyanic acid	β 1-3 glucanase	*H. armigera*	*S. litura*	*C. partellus*	Sorghum	Chickpea	Pigeonpea	Rice
KF770898	+	+	+	+	+	+	+	+	+	+	+	✓	✓		
KF770889	+	-	+	+	+	+	+	-	+	+	+	✓	✓		
KF770888	+	+	+	+	+	+	+	+	+	+	+	✓	✓		
KF770899	+	+	+	+	-	+	+	+	+	+	+	✓	✓		
KM191337	+	+	+	+	+	+	+	+	+	+	+	✓	✓		
KF770900	+	+	+	+	+	+	+	+	+	+	+	✓	✓		
KF770890	+	+	+	+	+	+	+	+	+	+	+	✓	✓		
KF770891	+	+	+	+	-	+	+	-	+	+	+	✓	✓	✓	✓
JQ682619	+	+	+	+	-	+	+	-	-	-	-	✓	✓	✓	✓
JQ682620	+	-	+	+	-	+	+	-	-	-	-	✓	✓	✓	✓
JN400112	+	+	+	+	-	+	+	-	-	-	-	✓	✓	✓	✓
JQ682621	+	+	-	-	-	+	+	-	-	-	-	✓	✓	✓	✓
JQ682622	+	-	+	+	-	+	+	-	-	-	-	✓	✓	✓	✓
KF770892	+	-	+	-	-	+	+	-	+	+	+				
JQ682623	-	-	+	+	-	+	+					✓	✓	✓	✓
KF770897	+		+			+	+		+	+	+	✓	✓	✓	✓
KF770893	+	+	+			+	+					?	?		✓
KF742498	+	+	+			+	+					✓	✓	✓	✓
JN400113	+	+	+			+	+					✓	✓	✓	✓
JN400114	+	+	+	+		+	+					✓	✓	✓	✓
KF770894		+	+			+	+		+	+	+	✓	✓		✓
KF770895	+		+	+		+	+	+	+	+	+	✓	?		
KF742497	+		+	+		+	+					✓	✓	✓	✓
KF770896	+		+	+		+	+					✓	✓	✓	✓
KF770896	+	+	+	+		+	+		+	+	+	✓	✓	✓	✓
JQ682624	+	+	+	+		+	+					✓	✓	✓	✓
JQ682625	+	+	+	+		+	+					✓	✓	✓	✓
JN400115	+	+	+	+		+	+					?	?	?	?

15. Bioinoculants as biocontrol agents

Beneficial microorganisms called bioinoculants, sometimes referred to as microbial inoculants, are employed in agriculture to promote plant growth and shield crops from pests and diseases. Through mechanisms like competition, antibiosis, induced resistance, and parasitism, bioinoculants are essential biocontrol agents that inhibit plant diseases. In addition to lowering environmental pollution and improving soil health and crop yield, these natural remedies provide a sustainable substitute for chemical pesticides. Because of their potential to enhance plant resilience, promote organic farming, and aid in integrated pest management (IPM) techniques, bioinoculants have drawn attention in response to the growing demand for nature-positive regenerative agriculture practices.

14.1. Actinomycetes isolates control *Fusarium* wilt in chickpea

Fusarium wilt is considered a significant disease of chickpea, caused by *Fusarium oxysporum* (Padwick; FOC). Chickpea is affected by other *Fusarium* species leading to wilt and mycotoxin production.

Controlling *Fusarium* wilt in chickpea is challenging because no single method is effective. Some commonly used measures to manage Fusarium wilt in chickpea include solarizing the soil, advanced sowing, using *Fusarium* wilt pathogen-free seed, and treating seeds with fungicide. However, these methods have had limited success.

Pseudomonas spp., *Trichoderma spp*., *Bacillus spp*., and non-pathogenic isolates of *F. oxysporum* were isolated from composts and rhizospheres of crop plants. These microorganisms were found to control plant pathogens and improve plant nutrient access and utilization. These novel microorganisms, possessing traits for promoting plant growth and an antagonistic nature towards pests and pathogens, are present in significantly higher levels in organic manures like compost, forest soils, and pasture soils.

Evaluation of *Actinomycetes* isolates in greenhouse conditions

The incidence of *Fusarium* wilt was found to be 45-76% lower at 29 DAS (days after sowing) in greenhouse experiments where five actinomycetes strains were used, compared to the *Fusarium oxysporum* FOC-inoculated control.
The *Fusarium oxysporum* FOC-inoculated control had disease incidence of 100% within 20 DAS (days after sowing). The *Actinomycetes* strain CAI-24 showed a significant reduction of 76% in wilt incidence, followed by 72% by CAI 127, 67% by CAI 121, 56% by KAI 32, and 45% by KAI 90.

Enumeration of *Actinomycetes* in *Fusarium* wilt experiment field

- Upon further evaluation of the five *Fusarium oxysporum* (putative FOC) antagonistic *Actinomycetes* under wilt sick field conditions, a decrease in the incidence of *Fusarium* wilt (4–19%) was noted at 28 DAS in comparison to the control group, which did not receive any *Actinomycetes*.
- In the control field, about 100% of disease incidence was recorded.
- The enumeration of *Actinomycetes* from both inoculated and uninoculated control plots' rhizosphere soils at 30 DAS revealed the presence of *Actinomycetes* (up to 106 values) in inoculated plots and no *Actinomycetes* were observed in the control plots. (Gopalakrishanan et al. 2011).

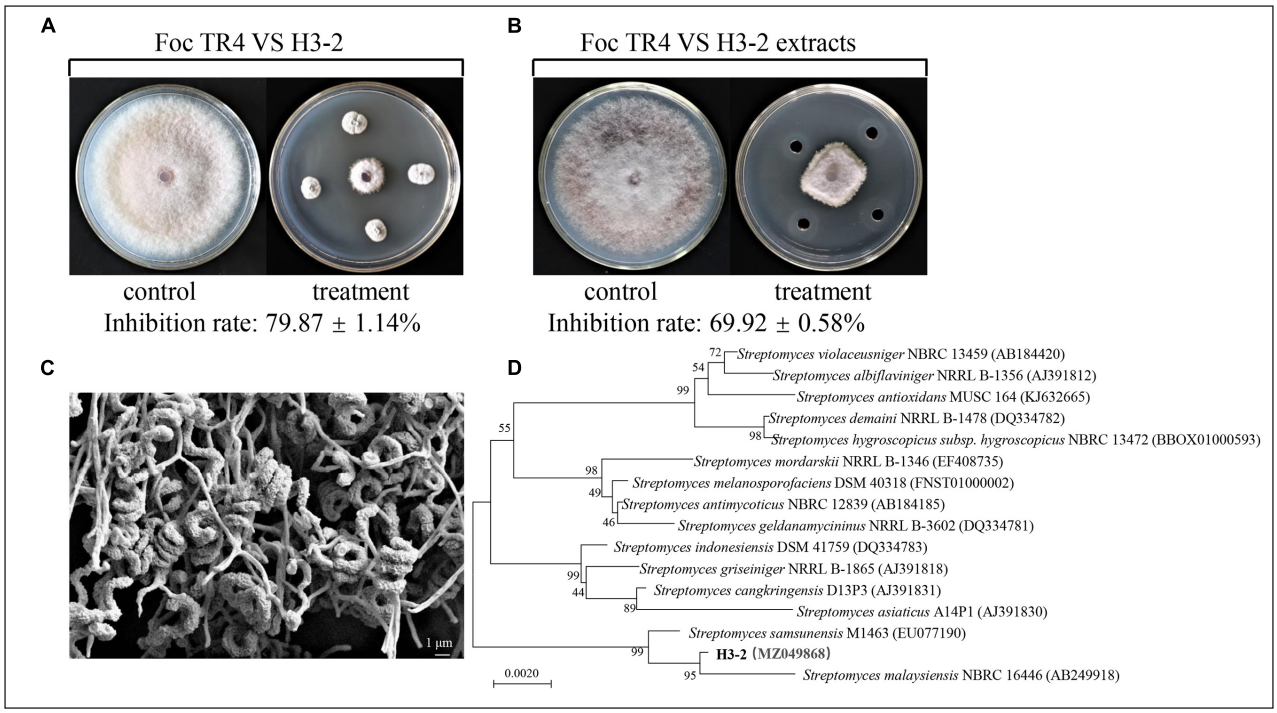


Figure 22. A similar result was noted by Zuo et al. 2021, using a novel antifungal Streptomyces species.

14.2. Penicillium citrinum for biological control of Botrytis gray mold in chickpea

Botrytis cinerea is a fungal pathogen responsible for Botrytis Gray Mold (BGM). This fungus impacts over 200 significant agricultural plant species, including chickpea, potentially leading to complete (100%) crop loss (Pande et al. 2005). The management of BGM presents significant challenges, as no singular control method proves to be entirely effective. Practices such as delayed sowing, increased spacing, intercropping with cereal or oilseed crops, and using pathogen-free seeds have been implemented to mitigate BGM (Pande et al. 2005). Yet, these strategies have yielded only moderate results. While seed treatments with fungicides like thiabendazole, thiram, and mancozeb have demonstrated efficacy in controlling BGM, the widespread application of these fungicides remains limited among economically disadvantaged farmers.

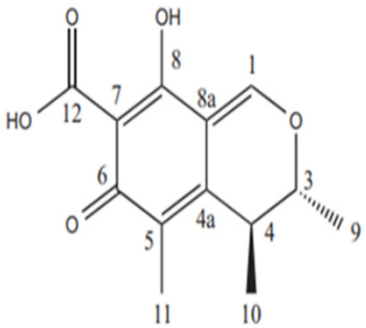


Figure 23. Structure of citrinin.

Salient findings

- Antagonistic capability of forty eight fungi against *Botrytis cinerea* was tested using co-culture and metabolite production tests, and *Penicillium citrinum* was screened for further evaluation
- Culture filtrate from *Penicillium citrinum* (VFI-51) was purified and identified as 'citrinin' using chromatographic and nuclear magnetic resonance (NMR) techniques, respectively (Fig. 23).

- Citrinin application has resulted in controlled BGM in chickpea under greenhouse conditions. The results are given in table 9.

Table 9. Control of *B. cinerea* by Citrinin in greenhouse experiement

Treatment	BGM severity (1 to 6 scale)
Control	1.0
Botrytis Gray Mold (BGM)	6
Citrinin	1.0
BGM + Citrinin at 0 hrs	1.7
Citrinin + BGM at 0 hrs	2.0
Botrytis Gray Mold (BGM) at 0 hrs + Citrinin at 24 hrs	3.3
Botrytis Gray Mold (BGM) at 0 hrs + Citrinin at 48 hrs	4.7
Botrytis Gray Mold (BGM) at 0 hrs + Citrinin at 72 hrs	6.0
After 20 days of inoculation, the BGM severity of the disease was measured using a 1-6 scale, where 1 indicates no infection on any plant components and 6 indicates extensive soft rotting and fungal growth on over 70% of the leaves, branches, and stems.	

SEM of chickpea roots revealed a significant level of colonization by *Penicillium citrinum (*VFI-51). Roots from plants inoculated with *Penicillium citrinum* (VFI-51) demonstrated considerable surface colonization by the fungus, in contrast to those from non-inoculated plants, which showed no such colonization (Fig. 24). Sporulation on the external cell layer of chickpea roots was distinctly observable, and the fungal hyphae had penetrated the peripheral cell layer of the chickpea roots (Sreevidya et al. 2015).

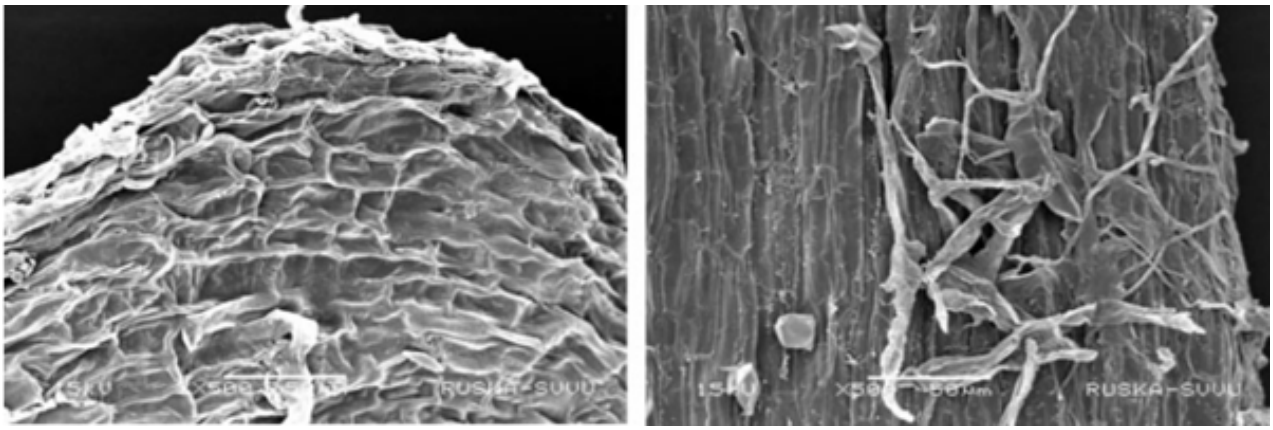
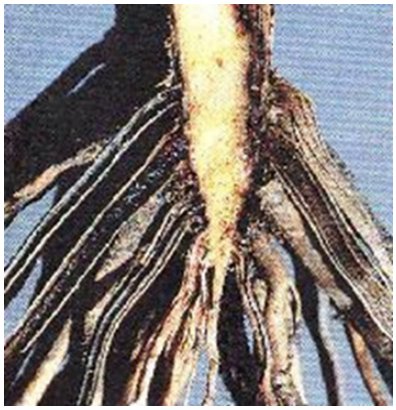


Figure 24. Scanning electron microscopy proving colonization of VFI-51 strain (source: Sreevidya et al. 2015).

14.3. *Amycolatopsis* sp. for bio-control of Macrophomina phaseolina: charcoal rot in sorghum



Charcoal rot, which is attributed to the pathogen *Macrophomina phaseolina* (Tassi) Goid., is a significant soil-borne disease affecting post-rainy sorghum, particularly in tropical and temperate regions globally. In India, this disease has been documented to result in substantial yield reductions exceeding 60% when conditions are conducive to its development. Furthermore, *M. phaseolina* can infect over 500 plant species, encompassing both leguminous and cereal crops, potentially leading to complete yield losses under optimal conditions (Patil and Kamble 2011).

The genus *Amycolatopsis* belongs *to the family Pseudonocardiaceae.* It is aerobic, gram-positive, and non-motile. It is recognized for its ability to synthesize antibiotics like rifamycin and vancomycin, which are produced by *Amycolatopsis rifamycin* and *Amycolatopsis orientalis*, respectively.

Organism used: *Amycolatopsis sp*. BCA 696 (KM191337- Genebank accession number).

Salient findings

- *In vitro* and *in vivo* antifungal activity tests were conducted. It was observed that BCA 696 inhibited *M. phaseolina*.
- In contrast to the pathogen-inoculated control, which showed more disease symptoms and significantly higher root infection (97%) in the *in vivo* experiment, BCA 696-treated roots showed very low disease symptoms and reduced root infection (17%).

Table 10. *Amycolatopsis* sp. BCA 696 activity against against *M. phaseolina in vivo* and *in vitro* conditions

Treatment	Dual culture Assay	Metabolite Production assay	Blotter paper assay	
			Visual rating	% inhibition
BCA-698 + *M. phaseolina*	12.7	60	1	83
M. phaseolina (positive control)	-	-	4	3
Water (negative control)	0	0	0	100

Scanning electron microscopy images show infection in control, very low infection when treated with *Amycolatopsis* sp. BCA 696, and high-intensity infection in positive control inoculated with *M. phaseolina* (Fig.25).

Greenhouse and field studies revealed control of charcoal rot of sorghum by *Amycolatopsis* sp. BCA 696 (Fig.25).

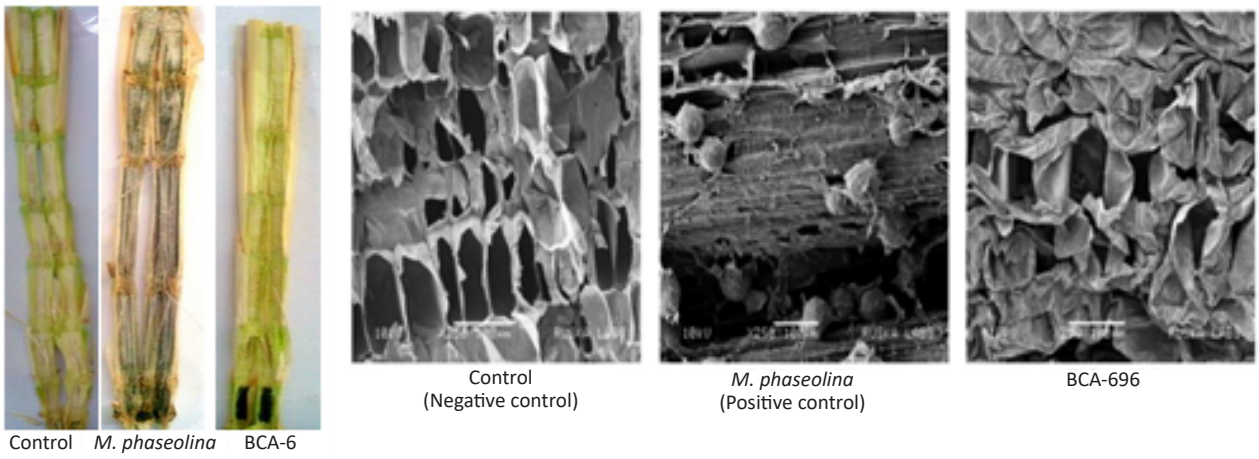


Figure 25. Control of charcoal rot in sorghum by Amycolatopsis sp. and electron microscope images representing the control of infection.

16. Bioinoculants for pest control

The extensive use of conventional pesticides has resulted in pollution because of the numerous chemicals used, many of which are recalcitrant and take decades or more to break down. In addition to humans, microbes, beneficial insects, animals, and plants are harmed by the synthetic properties of pesticides. Thankfully, there are a variety of natural pesticides, often known as "biopesticides," that are efficient against pests and, more significantly, do not negatively impact the functioning of ecosystems. These biopesticides are natural substances produced in nature, particularly by organisms like bacteria, fungi, plants, etc.

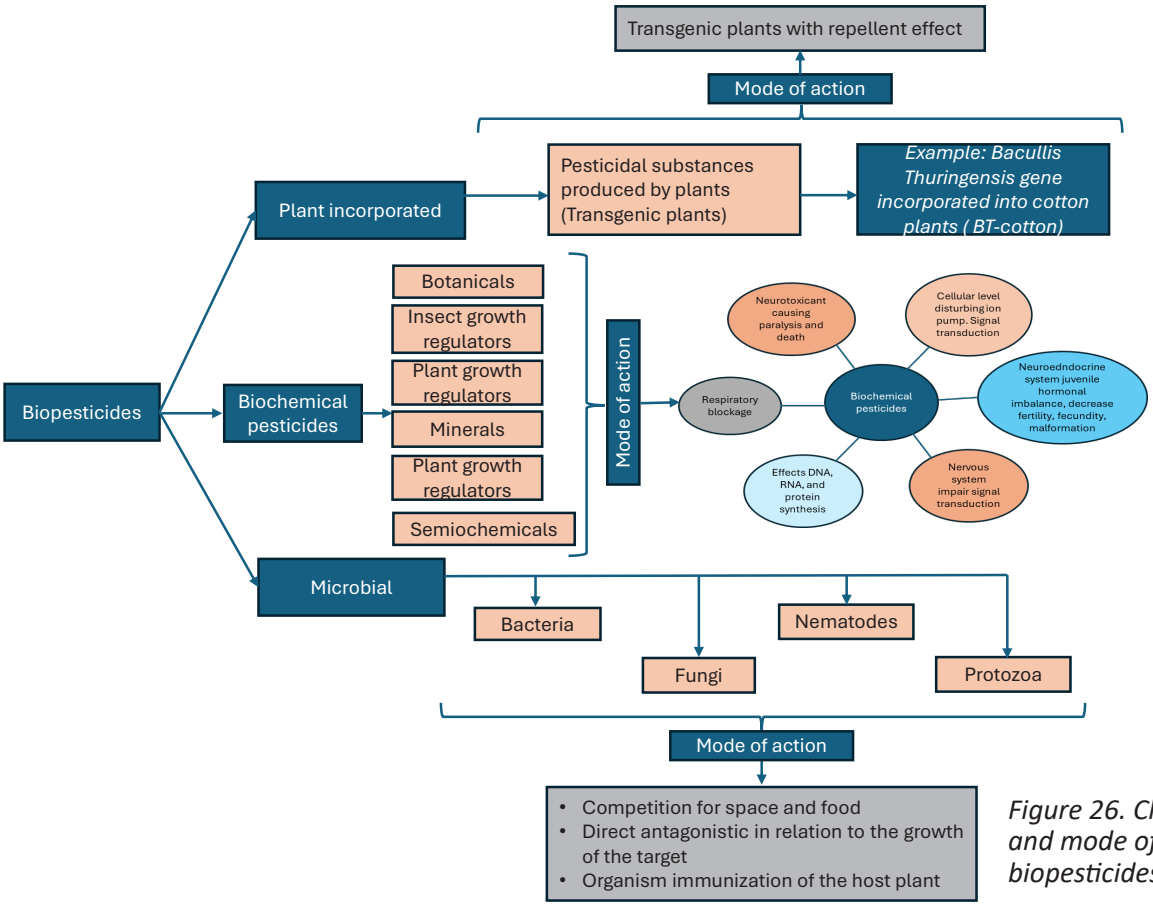
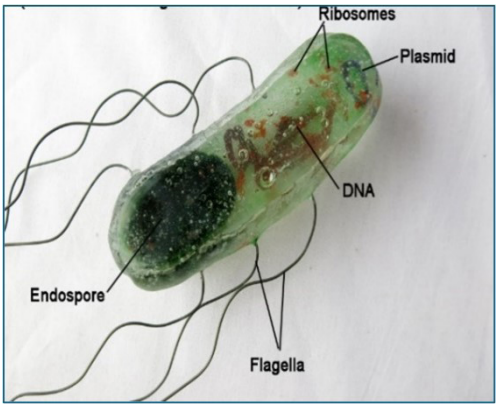


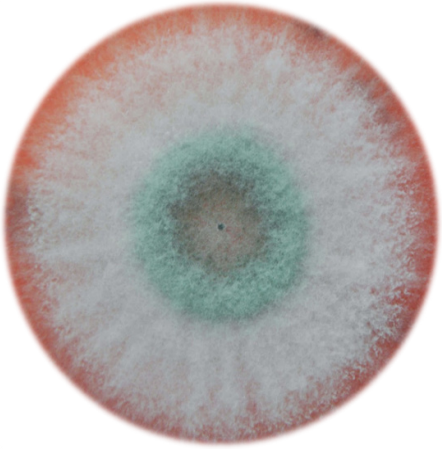
Figure 26. Classification and mode of action of biopesticides.

Bacillus thuringiensis (Bt)



The widely spread soil-dwelling bacteria *Bacillus thuringiensis* produces a protein crystal inclusion during sporulation that is insecticidal when consumed by the larvae of several insect groups. Lepidoptera, Diptera, and Coleoptera are among the vulnerable orders. In 1901, the action of *B. thuringiensis* was initially identified as the cause of a silkworm disease. Insects such as cabbage loopers, tobacco budworms, American bollworms in cotton, caster semi-loopers, diamondback moths, and paddy stem borers are susceptible to several strains of bacteria. *Bacillus thuringiensis* is perfect, with 300 times more toxicity than commercially available chemical pyrethroids. However, spraying must be scheduled during egg-hatching stages because it works best against neonates and early larval instars and should be used carefully.

Trichoderma viride



The filamentous fungus *Trichoderma viride* is a naturally occurring member of the *Trichoderma* genus. It is frequently found in root ecosystems, soil, and decomposing plant matter. Because of its capacity to function as a biological control agent against plant pathogens (*Fusarium, Pythium, Rhizoctonia, Phytophthora, Verticillium, Rhizopus, Thielaviopsis*, and nematodes including *Meloidogyne*), this species has drawn interest in the agricultural field.

Metarrhizium anisopilae

Three significant species viz., *M. anisopliae, M. album*, and *M. flavoviride* are found in the genus *Metarhizium*. The fungus *Metarhizium anisopliae* has the potential to be entomopathogenic. Conidiophore branching, dense interwinding, and conidiogenous cell alignment are characteristics of this genus. Conidia are cylindrical or ovoid, aseptate, and typically aggregate into a dense mass of parallel chains that range from light to brilliant green to golden green. More than 200 insect species from seven orders have been recorded to be attacked *by Metarrhizium anisopliae* var. *anisopliae*.

Beauveria bassiana

Agostino Bassi identified *Beauveria bassiana* from silkworm cadavers in the 19th century. It may infect over 200 insect species across 15 families and six orders (Nakahara et al. 2009). A variety of sucking insects, including aphids, thrips, and whiteflies, are susceptible to the white muscardine illness caused by the entomopathogenic fungus *B. bassiana*. Entomopathogenic fungi penetrate the insect's cuticle and destroy it by releasing enzymes like proteases and hydrolases.

Lecanicillium lecanii

The white halo fungus was previously known by the name *Verticillium lecanii* in the family Clavicipitaceae. This fungus is known to parasitize certain insect pests such as aphids, whiteflies, thrips, grasshoppers, flies, grubs, and caterpillars. In addition to attacking insects, it is also known to counteract disease-causing pathogenic fungi. Therefore its role as a biocontrol agent becomes even more important.

Nematodes

Entomopathogenic nematodes form an important component of the soil biology. These round worms are grouped in two genera *Steinernema* and *Heterorhabditis*. Their ability to attack a wide range of insects coupled with their high potency makes them an invaluable biocontrol agent in IPM.

Baculoviruses

The baculoviruses (family: *Baculoviridae*) are a group of viruses containing large DNA (large DNA virus) that infect insects. These viruses are widely recognized for their effectiveness and adaptability as biological insecticides, vectors for gene expression, and vectors for mammalian cell transduction. These are target-specific viruses that can infect and eradicate a variety of significant plant pests. They are efficient against the lepidopteran pests of cotton, grains, and vegetables. Utilizing a naturally occurring disease brought on by the nuclear polyhedrosis virus (NPV) as a new integrated pest management (IPM) alternative has grown significantly in recent years. Due to its popularity, farmers have begun applying it as a biopesticide to a variety of crops. This insect pathogen is very specific and does not harm species that are not its intended target. Both domestic and foreign markets have a strong need for commercially accessible *Helicoverpa* and *Spodoptera* NPVs.

15.1. *Streptomyces griseoplanus* SAI-25 controls cotton bollworm

Microorganisms have the peculiar property of producing metabolites with varying pest control properties. About 15 *Streptomyces spp*. strains that can act against *Chilo partellus*, *Spodoptera litura*, and *Helicoverpa armigera* were isolated and identified at ICRISAT.

An insecticidal compound was purified from the extracellular extract of *S. griseoplanus* SAI-25.

The purified product was identified as Cyclo (Trp-Phe) by spectral analyses using techniques like infrared (IR), nuclear magnetic resonance (NMR), and electron spray ionization mass spectroscopy (ESI-MS) (Fig. 27).

Cyclo (Trp-Phe) displayed dose-dependent antifeedant larvicidal and pupicidal activity of 70%, 67%, and 59%, respectively against *H.armigera*. The lethal dose for 50% of the group (LD50) is 619 ppm.

Furthermore, as compared to the control, the purified chemical [Cyclo (Trp-Phe)] extended the larval period (10.3–11.1 days) and pupal (10.9–11.8 days) period. The existence and biological activity of Cyclo (Trp-Phe) isolated from the *Streptomyces* genus was first documented in an ICRISAT study.

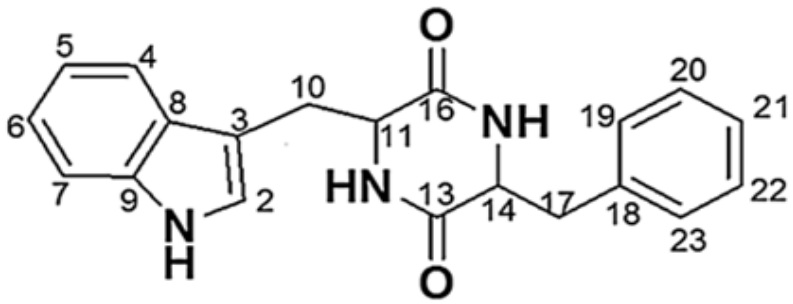


Figure 27. Structure of Cyclo (Trp-Phe) produced by Streptomyces.

Table 11. Anti larvicidal and Pupicidal activity of Cyclo (Trp-Phe) produced by *Streptomyces*				
Compounds (ppm)	**Dosage (ppm)**	**Antifeedant (%)**	**Larvicidal (%)**	**Pupicidal (%)**
Cyclo (Trp-Phe)	250	26.9 ± 0.8	23.3 ± 2.7	21.9 ± 0.7
	500	47.0 ± 1.2	39.4 ± 5.5	36.5 ± 3.6
	1000	70.9 ± 0.4	67.6 ± 3.8	59.3 ± 5.3
Standard Azadirachtin	250	58.0 ± 1.0	54.6 ± 4.1	48.7 ± 3.1
	500	69.2 ± 0.6	81.9 ± 4.3	78.9 ± 14.9
	1000	88.8 ± 0.3	97.8 ± 2.2	
Standard Phorbol ester	250	34.9 ± 0.9	33.9 ± 3.3	21.7 ± 2.9
	500	56.2 ± 1.1	59.0 ± 3.9	35.1 ± 4.8
	1000	76.2 ± 1.4	86.3 ±3.9	62.9 ± 12.6

The compound demonstrates significant insecticidal properties, viz., antifeedant, insecticidal, and pupicidal activities against *H. armigera* (Table 11). This underscores the critical role of microbes in the discovery of novel insecticidal compounds and their potential application in biopesticide formulations (Sathya et al. 2016).

15.2. ICRISAT study on *Helicoverpa armigera* Nucleopolyhedrovirus [HaNPV] collected from different locations in India

The legume pod borer, known as the cotton bollworm (*Helicoverpa armigera*), represents a significant challenge to global agricultural productivity. This polyphagous pest infests over 182 different plant species and poses control difficulties due to its resistance to numerous commonly used insecticides. Annual global crop losses attributed to *Helicoverpa* species surpass US$5 billion despite the expenditure of US$1 billion on pesticides. Nucleopolyhedrovirus (NPV), belonging to the Baculoviridae family, is a promising alternative for managing this versatile pest. The *Helicoverpa armigera* nucleopolyhedrovirus (HaNPV) is a naturally occurring insect pathogen that affects *H. armigera* and has a broad host range across Australia, Asia, and Africa.

Numerous strains of NPV have been utilized to formulate commercial biopesticides in countries such as the United States, Australia, India, China, and Thailand. HaNPV has demonstrated significant efficacy in managing *H. armigera* in legumes, oilseeds, cotton, and vegetables. The virulence of these strains were known to differ among species and geographical variants.

Methodology

- A 32 k Da protein, along with a few minor proteins of variable molecular weights, was present in purified HaNPV virus preparations (Sodium dodecyl sulphate-Polyacrylamide gel electrophoresis (SDS-PAGE) of HaNPV protein preparations), (Fig. 29).

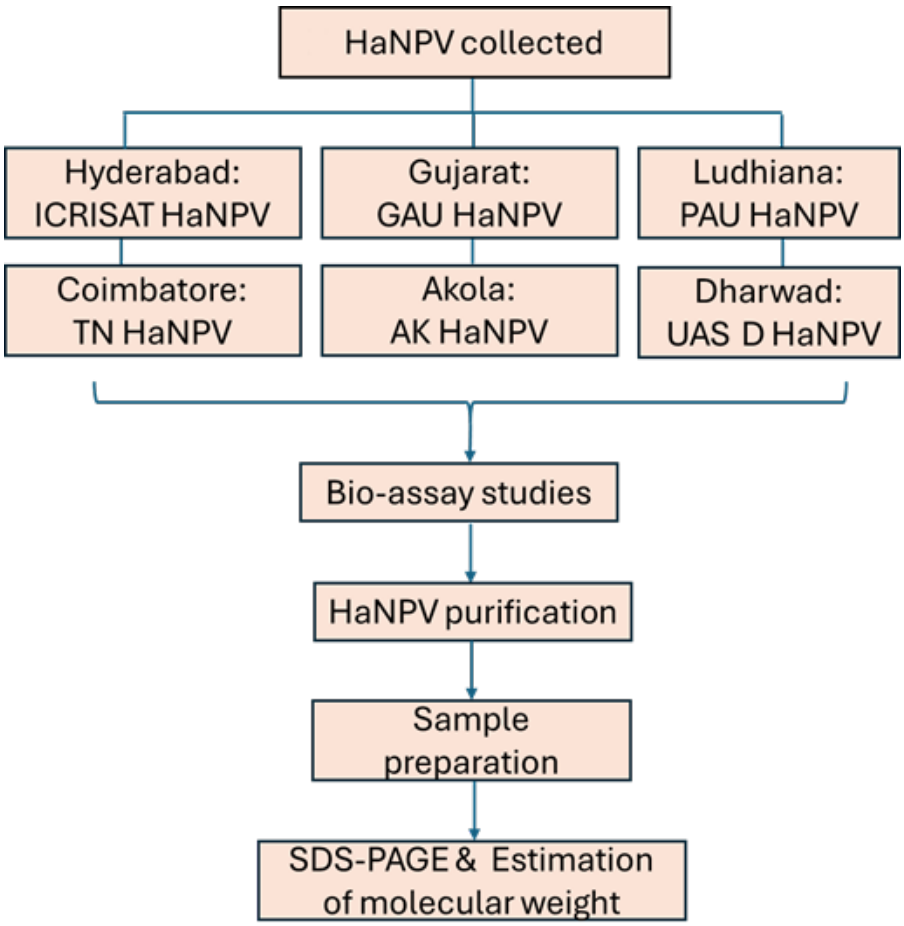
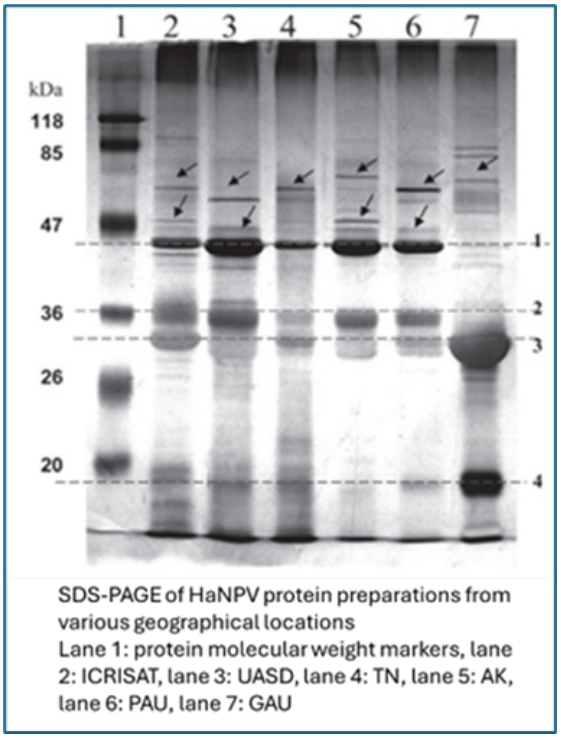


Figure 28. Schematic representation of methodology of sample collection (HaNPV) and preparation and molecular weight estimation.



SDS-PAGE of HaNPV protein preparations from various geographical locations
Lane 1: protein molecular weight markers, lane 2: ICRISAT, lane 3: UASD, lane 4: TN, lane 5: AK, lane 6: PAU, lane 7: GAU

- Fifteen duplicates of third, fourth, and fifth instar larvae were used in a lab environment to examine the impact of HaNPV on various Helicoverpa life stages.
- The ICRISAT HaNPV was found to be the most effective of the six HaNPV isolates obtained from six different regions of India, according to bioassays conducted against second and third instar larvae of *H. armigera* (*Sireesha et al. 2015*).

Figure 29. SDS-PAGE of HaNPV protein preparation (source: Sireesha et al. 2015).

Table 12. LT50 (h) values of NPV strain against second instar *H. armigera*						
Concentration (POB/ ml)	**LT50 (h) values of NPV strain against second instar *H. armigera***					
	ICRISAT	**GAU**	**PAU**	**TN**	**AKOLA**	**UAS D**
1.8×10^2	118.80	123.12	121.68	126.24	133.92	131.76
1.8×10^6	131.28	136.32	134.64	141.12	142.80	145.68
1.8×10^5	148.32	149.76	150.72	150.72	154.32	154.32
1.8×10^4	157.20	162.72	165.60	167.04	165.84	167.28
1.8×10^3	175.68	180.72	178.32	181.20	179.04	189.84
1.8×10^2	205.20	210.96	214.80	234.24	246.96	254.64

- The ICRISAT insectary provided the test insects. The larvae were fed treated chickpea leaves that had been air-dried for 30 minutes, pods were obtained from the glasshouse and treated with HNPV (dose: $6x10^9$ polyocular bodies (POBs)/ml diluted in one liter of water).
- Following a 24-hour feeding period, the larvae were fed fresh and untreated chickpea seeds that had been pre-soaked.
- Adult emergence, pupal abnormalities, and larval mortality were recorded. Pupae were taken from the larval population that received HaNPV treatment to ascertain the impact of the virus on fecundity and egg hatching.

Salient findings

- Larvae in their first and second instars that were exposed to HaNPV died in total, compared to 2.7% in the control group. With HNPV, the mortality rate decreased significantly in later stages, reaching 59.8% as opposed to nil in the control group.
- The hatching of eggs from the resulting population of fifth-instar larvae treated with HNPV was significantly reduced. Compared to healthy adults, an infected pair had a 20.4% decrease in oviposition and a 30.5% decrease in hatchability.

15.3. Use of *Metarhizium anisopilae*, *Streptomyces sp.*, and HaNPV on *Helicoverpa armigera* in chickpea

H. armigera is a pest in several crops including sunflower, lentil, chilies, chickpea, pigeonpea, and cotton, which creates key production constraints. Although using chemical pesticides for this polyphagous pest is effective, it is not considered sustainable due to cost, environmental impact, and safety. Accumulation of residues builds resistance in several pest species. Hence, an immediate need is to develop eco-friendly control measures for *H. armigera*.

Nucleopolyhedrosis virus (NPV) and plant-based products are viable substitutes for insecticides that can effectively be included in an integrated pest management (IPM) program in conjunction with other biocontrol agents such as *Streptomyces sp*. and *Metarhizium anisopilae* (Agale et al. 2017).

Bio-pesticide preparation and test against *Helicoverpa armigera*

- Biopesticides, such as *M. anisopliae*, *Streptomyces*, HaNPV, consortiums, neem seed powder, and Spinosad, were prepared in the laboratory.
- Three different dilutions of each bio-pesticide was prepared
  - I. *M. anisopliae*: 4.3×10^3, 3.9×10^4 and 2.9×10^5
  - *II.* *Streptomyces*: 12.6×10^4, 5.8×10^5, and 5×10^7
  - *III.* HaNPV: 10.10×10^6, 4×10^7 and 3×10^8
  - IV. Neem seed powder: 2.5 gm, 5 gm and 10 gm
- The consortia were made using the four treatments mentioned above in combination with concentrations of 0.1, 0.3, and 0.5 ml per liter for Spinosad against *H. armigera* larvae in their second instar.

Using three doses of each treatment, the effectiveness of the biopesticides, neem powder, and Spinosad against *H. armigera* larvae in their second instar was evaluated by recording the mortality rate of *H. armigera* larvae at 24, 48, and 72 hours after treatment.

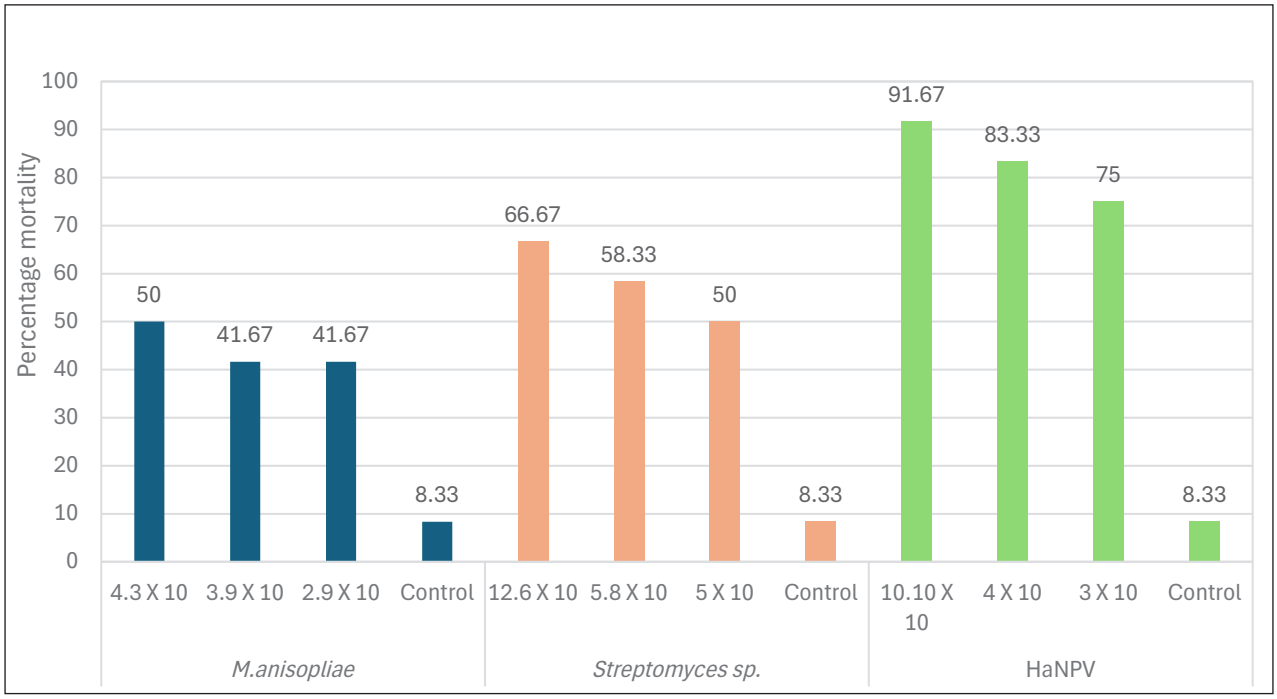


Figure 30. Effect of different bioagents concentrations against H. armigera (Source: Agale et al. 2017).

Salient findings

- Different concentrations (biopesticides) used resulted in variations in efficacy. When *M. anisopliae* was treated for 48 hours, its infectivity increased, and at the highest concentration (4.3×10^3 conidia / mL), the mortality rate was 50%. Larval mortality in the control was not observed.
- After 48 hours of treatment, *Streptomyces sp*. exhibited the highest percentage of mortality (66.67%) at concentrations of 12.6×10^4 colonies/mL and the lowest percentage of mortality (5×10^7 colonies/mL). After 72 hours of treatment, there was no larval mortality.

- The maximum mortality rate, 91.67%, was seen in HaNPV at a dilution of 10.10 X 10^6 POB/mL. Compared to *M. anisopliae* and *streptomyces* sp., HaNPV was demonstrated to be effective in terms of the mortality rate of *H. armigera larvae*.
- Until two days, neem seed powder failed to eliminate larvae; nevertheless, at concentrations of 10 gm, it caused 100% mortality after 72 hours of treatment. Mortality was lowest at the lowest concentration (2.5 gm), which was comparable to a 5 gm concentration.
- Following a 24-hour treatment period, the Spinosad experimental results showed 100% larval mortality at doses of 0.3 and 0.5 milliliters per liter.
- 91.67 % mortality was observed with consortia by 48 hours of treatment.

15.4. Entomopathogenic *Actinomycetes* for biological control of lepidopteran insects

Microorganisms are more diverse and found throughout nature than higher-order species. As a result, they provide an endless supply of novel metabolites and compounds of industrial, agricultural, and medical value. The production of fungicidal compounds (validamycin, blasticidin-S, natamycin, kasugamycin, etc.), bactericidal compounds (streptomycin, oxytetracycline, blasticidin-S, kasugamycin, etc.), and insecticidal compounds (avermectins, Spinosad) is one of the agriculturally advantageous traits that meet the need for biological control agents.

- Fifteen bacteria and 96 *Actinomycete* isolates were tested for entomopathogenic activity for control of lepidopteran insects (*Chilo partellus, Helicoverpa armigera*, *Spodoptera litura)* (Vijayabharathi et al. 2014).
- Using the food impregnation experiment, a total of 111 isolates (15 bacteria + 96 fungi) were examined for their intracellular and extracellular metabolites' potential to kill *H. armigera* larvae in their second instar. The effectiveness of promising isolates against 7-day-old *C. partellus* larvae and third-instar *H. armigera* and *S. litura* larvae was further examined.
- Biocontrol studies like Diet Impregnation Assay, Detached Leaf Bioassay, were conducted.
- The screened isolates were tested in greenhouse experiments.

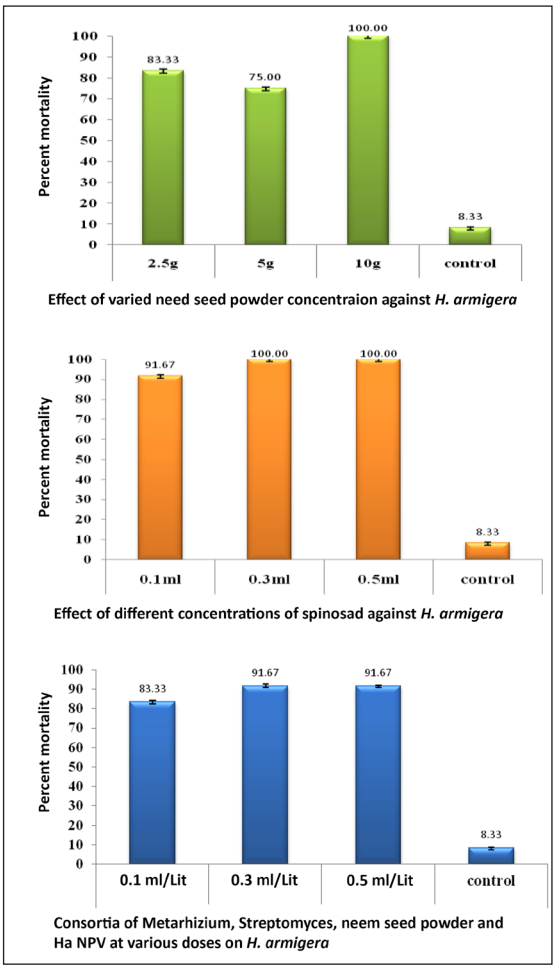
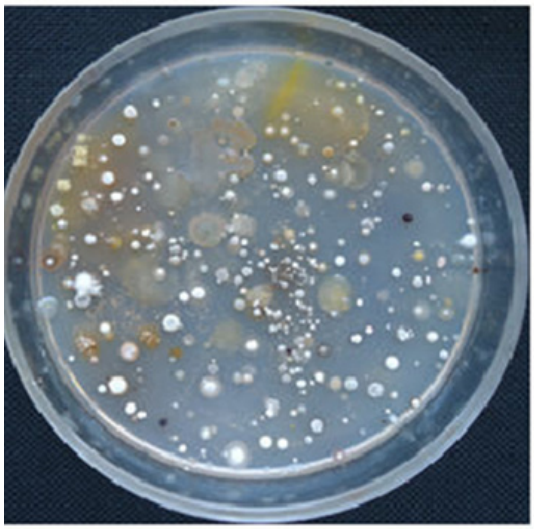


Figure 31. Effect of neem seed powder, Spinosad, and a consortium of Metarhizium, Streptomyces, and neem seed powder on H. armigera (Source: Agale et al. 2017)



Salient findings

Diet Impregnation Assay

- Fifteen *Actinomycetes* were found to be insecticidal and all 15 bacterial isolates did not act against *H. armigera*.
- The insecticidal activity of extracellular metabolites (ECM) and intra-cellular metabolites (ICM) of these 15 isolates was further assessed against three-instar *H. armigera*, three-instar *S. litura*, and seven-day-old *C. partellus*. ECM extract from seven isolates (BCA 508, BCA 546, BCA 659, BCA 667, BCA 698, CAI 85, and SAI 25) out of the 15 *Actinomycete* isolates caused 100% mortality on second instar *H. armigera*, while the other isolates had 55 to 99% mortality (Vijayabharathi et al. 2014).

Detached Leaf Bioassay

- The Extracellular metabolites-ECM (BCA 546, BCA 659, CAI 13, CAI 87, CAI 132, CAI 133, CAI 155, and SAI 25) and Intracellular metabolites-ICM (BCA 689, BCA 698, CAI 8, CAI 13, CAI 70, CAI 85, CAI 132, and SAI 25) of eight isolates showed 100% mortality when the 15 isolates were assessed for insecticidal activity using detached leaf bioassay.
- Fourteen isolates showed considerable (PB0.001) action against the second instar of *H. armigera*, except for isolate BCA 508 (Vijayabharathi et al. 2014)

Greenhouse Experiments

- 15 isolates exhibited 68 to 89% mortality by Extracellular metabolites-ECM and 59 to 71% mortality by Intracellular metabolites-ICM. The fact that every isolate exhibited statistically significant insecticidal activity is noteworthy.

Table 13. Details of the isolates (Vijayabharathi et al. 2014)

S.no	Isolate	Culture ID
1	*S. cyaneofuscatus*	BCA 546
2	*S. cavourensis subsp. cavourensis*	BCA 659
3	*S. albolongus*	BCA 667
4	*S. hydrogenans*	BCA 689
5	*S. albolongus*	BCA 698
6	*Streptomyces sp.*	CAI 8
7	*S. cavourensis subsp. cavourensis*	CAI 13
8	*S. antibioticus*	CAI 70
9	*S. antibioticus*	CAI 85
10	*S. cyaneofuscatus*	CAI 87
11	*S. antibioticus*	CAI 132
12	*S. carpaticus*	CAI 133
13	*S. bacillaris*	CAI 155
14	*S. griseoplanus*	SAI 25

15.5. The use of *Lecaniicillium lecanii* against rusts, early and late leafspots in groundnut

Groundnut plants (TMV 2 cultivar) maintained in pots at the ICRISAT glasshouse showed the symptoms of rusts (*Puccinia arachidis*), early (*Cercospora arachidicola* [*Mycosphaerella arachidis*]) and late leaf spot (*Phaeoisariopsis personata* [*M. berkeleyi*]). The legions were invaded by a whitish mycelium on both the upper and lower surfaces. The fungus actively grew on sporulating areas of the legions i.e in case of rusts and late leaf spots on the lower surface while in case of early leaf spot legions on the upper surface. The fungus was identified as *Verticillium lecanii* which was later isolated, grown and tested for its pathogenicity on rust, early and late spots in the lab. The congenial conditions for this biocontrol fungus were found to be in the temperature range of 20 – 25°C and RH exceeding 80%. In addition to the glasshouse plants, the biocontrol fungus was also found growing in the fields of ICRISAT and in farmers' fields in the Indian states of Andhra Pradesh, Karnataka and Tamil Nadu. In inoculation experiments, there was a significant reduction in the extent of rust and late leafspot development on leaves inoculated with *L. lecanii*. Receptivity and percentage leaf area damage of rust and late leafspot were reduced when inoculated with *L. lecanii* (Subramaniyam et al. 1990).

15.6. The use of entomopathogenic nematodes on pests

In the surveys conducted at the ICRISAT campus between June to December 1991, a total of 110 soil samples were collected in 5 vertisol and 6 alfisol fields. Ten soil cores were collected from each field and the technique employed by Bedding and Akhurst 1976 was used. This technique consisted of using the rice moth larvae as baits for the nematodes. 10 full grown larvae were positioned at the bottom of a 250 cubic cm plastic container and the soil from each field was placed on top of the larvae. Muslin cloth covered the containers and incubation was done at 25±1°C. Only *Steinernema* nematodes were found. The nematodes killed the rice moth larvae within 2 days. The nematodes also infected Spodoptera litura caterpillars (Singh et al. 1992).

15.7. ICRISAT study on the effect of biopesticides on natural enemies

Pigeonpea crop is known to be attacked by around 250 insect pests. Nine insects, including *Etiella zinckenella*, *Riptortus pedestris*, *Megalurothrips usitatus*, *Empoasca kerri*, *Clavigralla gibbosa*, *Exelastis atomosa*, *Melanagromyza obtuse*, *Cydia ptychora*, and *Maruca testulalis*, were recorded as moderate pests, causing damage ranging from 31 to 50%. Using a single insecticide repeatedly to control pod borers in pigeonpea could result in some major issues of development of resistance and residue in grain.

The pigeonpea variety ICPL-161 was planted in a 20x20 m area with a 120 cm row-to-row spacing and the following treatments were imposed. The study was conducted for two years.

1. *Streptomyces* sp. ($5.85x10^7$ colonies/ml)
2. HaNPV (500LE/ha)
3. *Metarhizium anisopliae* ($39.2x10^4$ spores/ml)
4. Neem fruit powder (15–20 kg ha^{-1})
5. Consortia (*Streptomyces* sp. - SAI-25) + HaNPV+ *Metarhizium anisopliae* + Neem fruit powder) @ ($5.85x10^7$ colonies ml^{-1} + 500LE ha^{-1} + $39.2x10^4$ spores ml^{-1} +15–20 kg ha^{-1})
6. Farmers' practices (mostly chemical)
7. Spinosad 45% SC
8. Untreated control

Effect of biopesticides on ladybird beetle

According to the pooled mean population for the two years, the population throughout the treatments was roughly the same. The first, second, and third sprays, which included different observation days, showed no variation in treatment throughout the spraying schedule periods. The neem fruit powder treatment, however, produced 0.31 beetles per seven plants in contrast to the other treatments. This demonstrated that none of the treatments had a discernible impact on the population of natural enemies.

Effect of biopesticides on spider *Araneus* sp. in pigeonpea

The pooled data results on *Araneus sp*. from the first, second, and third sprays on various days, showed no differences between any of the treatments. In contrast to the other treatments, the consortium treatment (0.29 spider/7 plants) had a lower fatal effect and a higher spider population. Spinosad (Tracer; 45, 56, 73, and 90 g) was investigated for toxicity against natural enemies linked to pigeonpea insect pests by Mittal and Ujagir (2005). The natural spider population was unaffected by the insecticides during crop growth.

Effect of biopesticides on dragonfly *C. servilia* in pigeonpea

There was no difference between any of the treatments during the spraying schedule times, according to the pooled data on the *C. servilia* population on the first, second, and third sprays at various observation days. All treatments, however, recorded a higher number of dragonflies and had less harmful effects than the others.

Effect of biopesticides on praying mantis, *M. religiosa* in pigeonpea

In field conditions, the pooled mean of the *M. religiosa* population in 2016 and 2017 on the first, second, and third sprays at various observation days revealed no differences between the treatments during the spraying schedule periods.

17. Aerobic composting using microbial consortium

Low soil organic carbon levels and macro and micronutrient deficiencies are major limiting factors for increasing food production in the semi-arid tropics (Chander et al. 2018). Soil organic carbon is the most crucial factor affecting soil health, influencing chemical, physical, and biological parameters and eventually impacting crop yields (Wani et al. 2003). Therefore, it becomes imperative to encourage methods that enrich soil with organic matter.

The process of composting turns large organic waste into a stable, low-volume product that is rich in nutrients (Kharrazi et al. 2014). The conventional composting method, which involves farmers piling dung and straw, is time-consuming and less effective. In this situation, adding half-decomposed compost, manure, or plant debris has a negative influence on plant health. One of the tested and validated methods for efficiently recycling agricultural waste and creating high-quality compost for crop production is vermicomposting (Wani et al. 2014), through the churning of the raw biomass and the mixing of a wide variety of bacteria as it passes through the earthworm's gut. Microorganisms that inhabit the earthworm gut are capable of breaking down organic materials and polysaccharides (Aira et al. 2007; Zhang et al. 2000). However, one of the challenges in handling a vermicompost unit and a key reason for on-farm failure in some cases is the difficulty in continuous upkeep of ambient dwelling conditions for earthworms. Adding a microbial consortium culture externally, is the alternative to speed up the organic matter decomposition (aerobic composting). According to Richardson and Simpson (2011), high microbial activity during composting can produce chemicals that function as plant growth hormones and indirectly improve the nutrient quality of the compost by nitrifiers, sulphur oxidizers, and nitrogen fixers (Tomati et al. 1988, Pizzeghello et al. 2001; and Ghosh et al. 2003). Lack of technical know-how on standard operating procedures and applications is a significant obstacle in scaling up compost production. To recycle farm wastes, the present ICRISAT study evaluates the effectiveness of microbial consortia (for aerobic composting), proving its efficacy in comparison to vermicomposting.

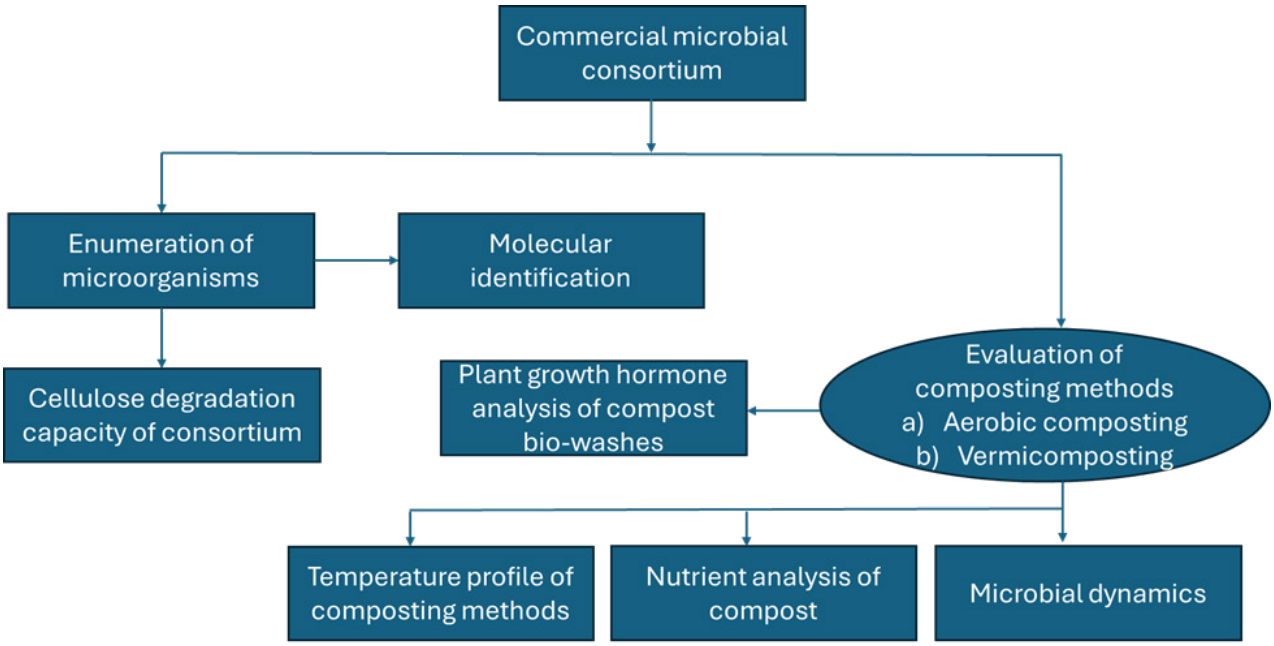
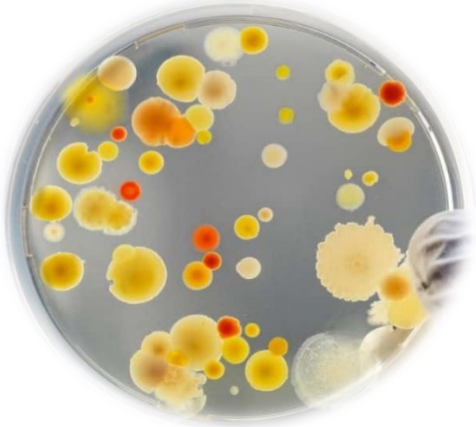


Figure 32. Methodology followed in the study.

Population of microorganisms in the microbial consortium

The commercial microbial consortium was subjected to serial dilution and spread plate to enumerate bacteria, fungi, and *Actinomycetes* population. The enumerated colonies were represented as colony-forming units (CFU) per gram of sample. The bacterial population was found to be 4.5 cfu ml^{-1} at 10^{-7} dilution. Based on the colony morphology, 21 single colonies, including *Actinomycetes* were screened and further cultured. The pure culture colonies were stored at 4°C until further tests.



Cellulose degradation capability of the isolated pure cultures

The cellulase production by the isolated pure cultures was evaluated by testing on carboxy methyl cellulose-Congo red agar medium. The halo zone surrounding the microbial colonies indicates cellulase production. A total of 16 isolates out of the 21 examined for cellulose degradation generated the cellulase enzyme. In the Congo Red Agar plates, a distinct halo zone was discovered surrounding the colonies. Two (MP 2 and MP 20) of the 21 microorganisms that were examined were shown to produce the highest amount of cellulase. The screened microorganism are as follows: *Staphylococcus simulans* (MP 1), *Staphylococcus arlettae* (MP 2), *Halobacillus sp.* (MP 3), *Bacillus aquimaris (*MP 4), *Halobacillus sp.* (MP 5), *Bacillus licheniformis (*MP 6), *Bacillus infantis* (MP 7), *Staphylococcus arlettae* (MP 8), *Bacillus fexus* (MP 9), *Bacillus infantis* (MP 10), *Bacillus sp.* (MP 14), *Micro bacterium resistens* (MP 17), *Bacillus marifavas* (MP 18), *Bacillus sp.* (MP 20), *Streptomyces sp.* (MA 1).

Comparison of composting methods

- Two composting methods, vermicomposting by earthworms (*Eisenia fetida*) and aerobic composting by a microbial consortium, were evaluated for their efficacy in decomposing size-reduced sorghum stalks.
- Vermicomposting was carried out by introducing earthworms into cement tanks layered with sorghum stalks and cow dung.
- Microbial consortium was applied at the rate of 1 kg per ton of sorghum biomass. The consortium was applied across layers of size-reduced sorghum biomass (0.30 m) and slurry made from cow dung. The temperature was recorded daily in both composting types.

The findings showed that similar to vermicomposting, aerobic composting is a useful approach for quickly converting farm wastes into valuable compost. Vermicompost was ready after about 60 days, and aerobic compost after around 50 days. The quick breakdown of biomass throughout the earthworm's digestive tract and the mixing of various microorganisms in the coelomic fluid appear to be the causes of the vermicomposting process. However, with aerobic composting, a large microbial population achieved by adding culture and turning the compost regularly at 10-day intervals appears to have sped up the decomposition process.

Microbial profiling during the composting process

Samples of vermi-and aerobic-compost were collected and subjected to a spread plate study on day 1, day 20, and day 55, to enumerate bacteria, fungi, and *Actinomycetes* (Fig. 33).

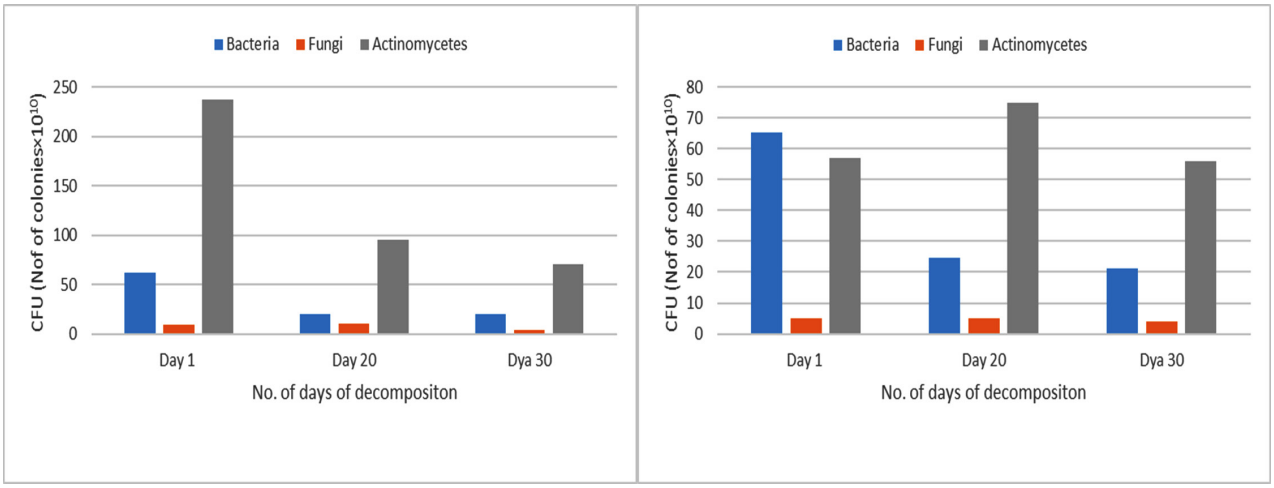


Figure 33. Microbial dynamics (population) during vermicomposting and aerobic composting.

- The study's findings showed that bacteria were the most prevalent microorganisms present, followed by *Actinomycetes*, with fungi being the least abundant.
- The higher population in aerobic composting was likely a result of the external introduction of microbial consortium (Fig. 33).

Temperature pattern during composting

- The daily mean temperature was relatively higher at 0.15 m depth during aerobic composting (38–67°C) compared to vermicompost (29–48°C) for up to 30 days.
- High microbial activity in the first 15 days led to a peak temperature of 67°C on the 9th day (Fig. 34) during aerobic composting, but turning on the 10th day brought it down to 52°C to prevent negative effects on microbial population and composting.
- In contrast, vermicomposting maintained a temperature below 40°C during the first week (Fig. 34).

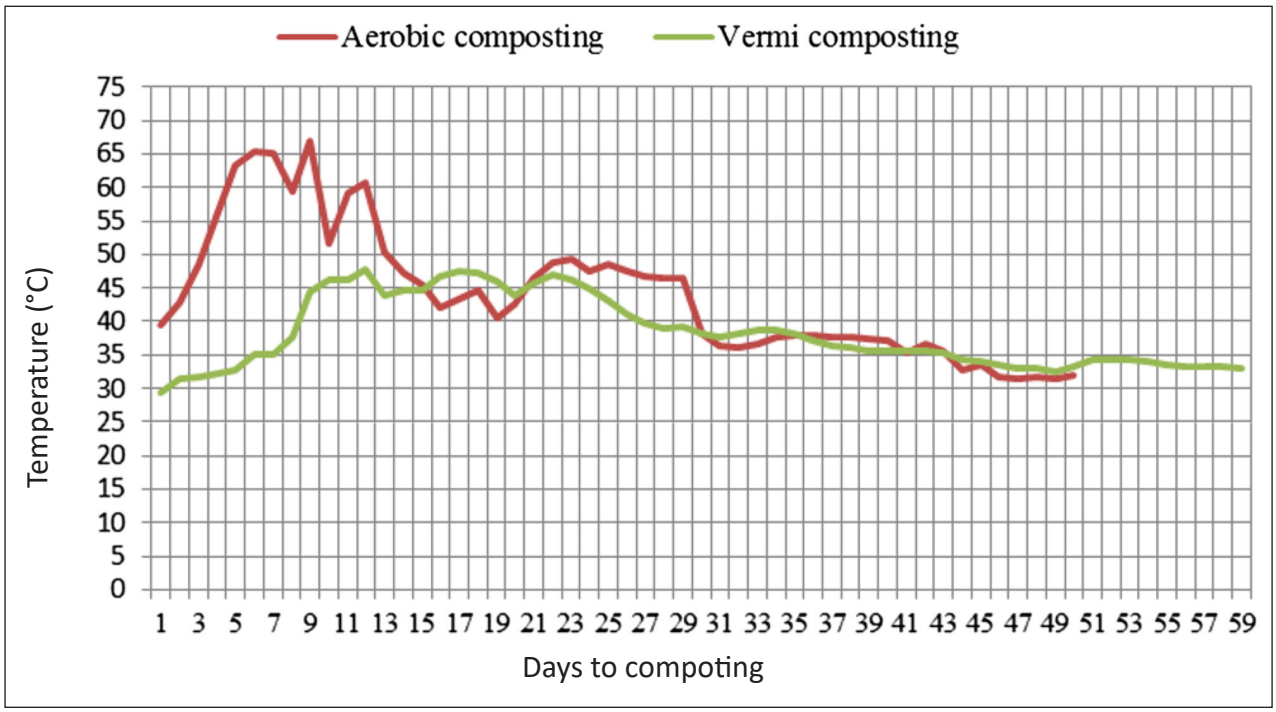


Figure 34. Temperature profile of aerobic composting and vermicomposting. (Source: Chander et al. 2018)

On-farm evaluation of composts

The composts were tested in on-farm studies in India (Sagar district and Kadapa district). This included vermicompost trials in Sagar district with chickpea and wheat during the 2011–2012 post-rainy season (6 trials with chickpea and 9 trials with wheat) and aerobic-compost trials in Kadapa district with paddy crop during the 2016 rainy season (40 trials with paddy crop).

- On-farm treatment plot-size: 2000 m^2 side by side.
- Two treatments assessed were as follows:
- 1. **Balanced nutrition** (BN), which involved adding N, P, and K based on soil tests along with deficient micro- and secondary nutrients (S, Zn, and B).
- 2. **Integrated nutrient management** (INM) consisted of 75% of BN inputs combined with compost. To replace 25% of the N need for non-legumes and 25% of the P requirement for legumes, vermicompost or aerobic compost was applied.

In both treatments, crop management practices were the same. As a basal application, composts and all necessary nutrients—apart from nitrogen for non-legumes—were supplied. For non-legumes, 50% N was added as a base, 25% N after one month, and the remaining 25% N after two months.

Salient findings

- Vermicompost in wheat and chickpea crops and aerobic compost in paddy crops were evaluated on farms. The results showed that using composts not only maintained or increased crop yields compared to applying chemical fertilizers alone for balanced nutrient management but also resulted in a 25% reduction in the use of chemical fertilizers.
- The combined application of vermicompost and chemical fertilizers increased yield by 9% in chickpea and 5% in wheat. Similarly, compared with applying only balanced chemical fertilizers, the use of aerobic compost in conjunction with chemical fertilizers increased paddy yield by roughly 7%.
- Therefore, vermicomposting and aerobic composting are both good scalable technologies for recycling trash on farms, but the technology's applicability depends more on each farm's specifics.

Conclusion

Regenerative agriculture and nature positive farming are increasingly advocated globally for transforming agri-food systems on a sustainable basis under the emerging global megatrends. For advancing regenerative agriculture and nature positive farming, harnessing the power of biologicals is crucial. Biologicals are revolutionary to improve soil and plant health, and reduce dependency on agrochemicals. These natural solutions enhance nutrient cycling, fortify plant resistance to environmental stressors, and support long-term ecosystem sustainability through the use of beneficial microbes, biofertilizers, biostimulants, and biopesticides. By utilizing biologicals, farmers can improve biodiversity, restore soil fertility, and create resilient and nutrient dense food systems that adhere to ecological principles. A route toward sustainability, productivity, and environmental stewardship is provided by incorporating biological solutions into farming systems, as agriculture confronts issues such as soil degradation, climate change, dwindling biodiversity, and environmental deterioration. Smallholder farmers and ecosystems in semi-arid areas would benefit from ICRISAT's research and innovations on biologicals to promote resilient and sustainable farming systems that co-exist with nature. Moreover, increased investments would be needed for further advancing the research on biologicals for transforming agri-food systems in the face of climate change, land degradation, water scarcity, and loss of biodiversity.

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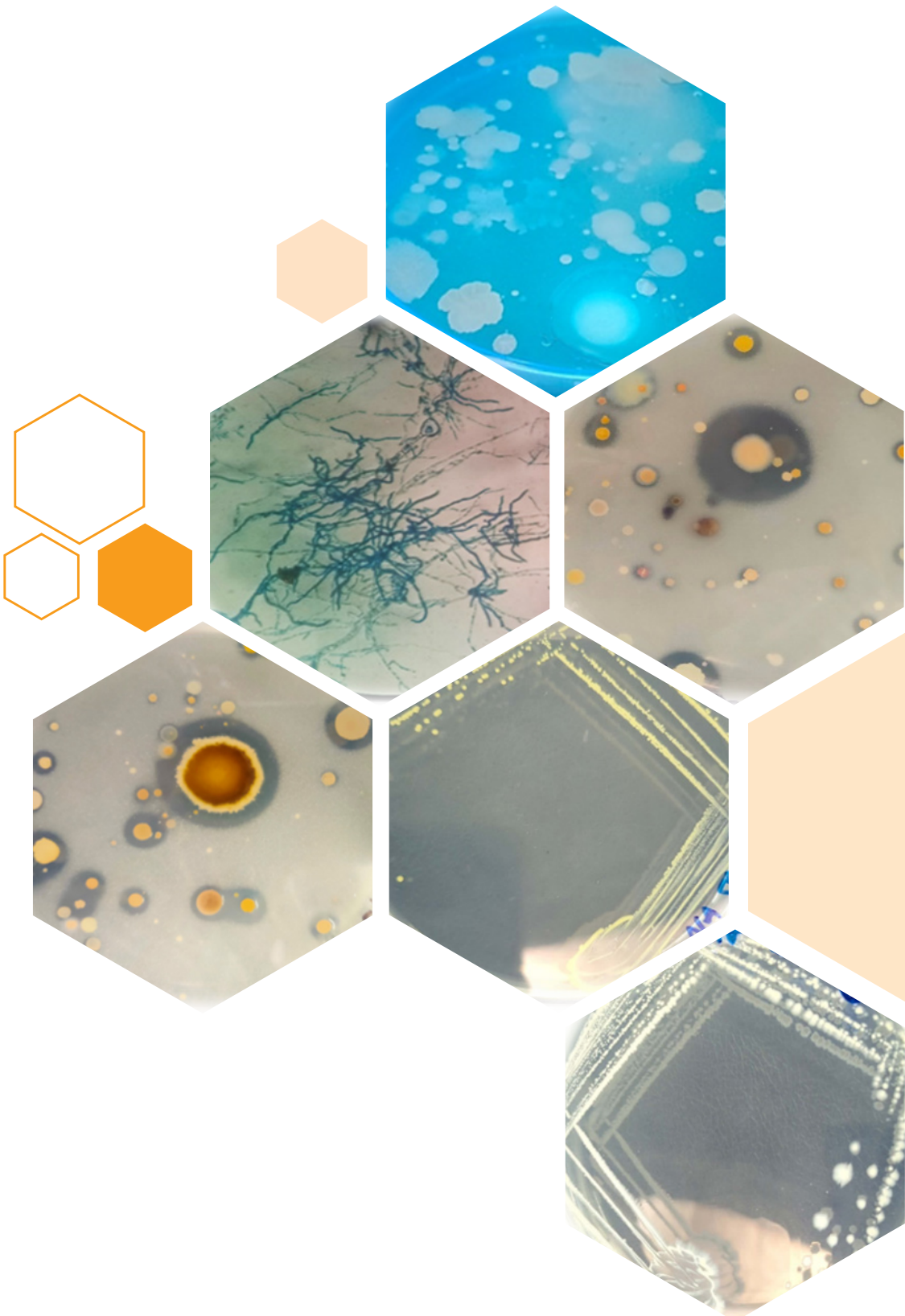

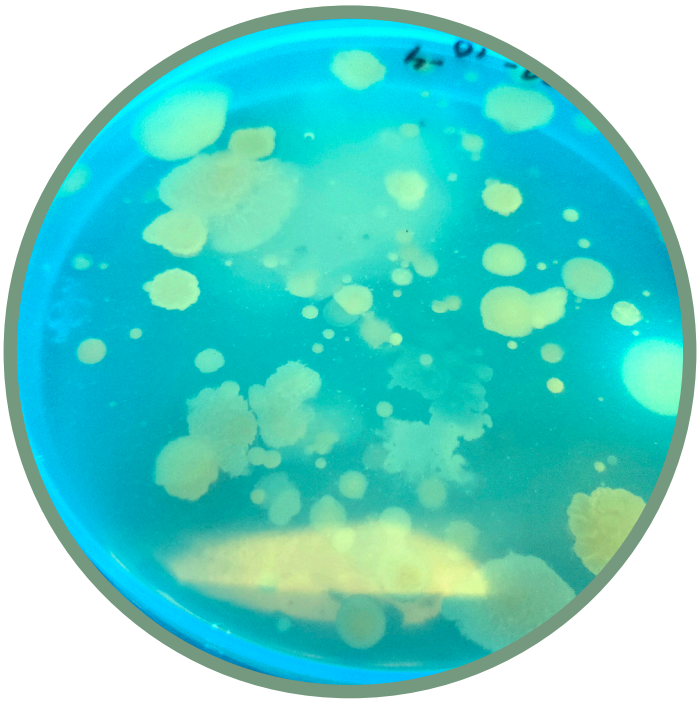

ICRISAT
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About

The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) is a pioneering non-profit organization focused on scientific research for development, committed to transforming dryland farming and agri-food systems. Working with global partners, ICRISAT develops innovative solutions to address hunger, poverty, and environmental degradation, benefiting 2.1 billion people across the drylands of Asia, Africa, and beyond.

ICRISAT was established under a Memorandum of Agreement between the Government of India and CGIAR, dated 28 March 1972. In accordance with the Headquarters Agreement, the Government of India has extended the status of a specified "International Organization" to ICRISAT under section 3 of the United Nations (Privileges and Immunities) Act, 1947 of the Republic of India through Extraordinary Gazette Notification No. UI/222(66)/71, dated 28 October 1972, issued by the Ministry of External Affairs, Government of India.

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