



Prof. S. Kannaiyan Memorial Oration-Series:11

# **Prof. S. Kannaiyan Memorial Oration**

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Topic of Oration:Agriculturally important Microorganisms for<br/>improvement of Crop health status andinduction of systemic resistance against Phytopathogens

#### Prof. S. Kannaiyan Memorial Oration- 2023

## Agriculturally Important Microorganisms for Crop Health Improvement and Induction of Resistance towards Biotic and Abiotic stresses

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Soil is an essential part of the terrestrial ecosystem because it is a living, dynamic matrix that is believed to be a storehouse for microbiological activity. Soil microorganisms play a crucial role in various soil processes that impact plant productivity, making them an integral part of the ecosystem. Numerous microorganisms are necessary for a sustainable biosphere, and it is now understood that rhizosphere microbial populations play a key role in sustaining stress tolerance, nutrient uptake, and root health. In both naturally occurring and managed agricultural soils, microorganisms play a crucial role in the breakdown of organic matter, the removal of toxins, the development of soil structure, and the cycling of carbon, nitrogen, phosphorus, and sulfur. In recent years, the use of biological fertilizers has gained attention because to the growing global preference for natural or "organic" products. Both organic farming and nature heavily rely on a microbial inoculant that is composed of a range of beneficial bacteria that occur naturally and are referred to as "effective microorganisms." According to studies, there are numerous applications for effective microorganisms in agriculture, cattle, gardening, landscaping, composting, bioremediation, cleaning septic tanks, algae control, and home settings. When these cultures are placed in their natural habitat, their individual beneficial effects are greatly enhanced in a synergistic manner.

When put into production systems, beneficial microorganisms can help balance out microbial populations and establish a population structure that is better for healthier and more productive plants. Microbes are also necessary for plant growth, the maintenance of soil-borne plant diseases, and the encouragement of changes in vegetation. Consequently, a wide range of agro settings use agriculturally important microorganisms (AIMs) for a number of reasons, such as nutrient delivery, biocontrol, bioremediation, and the restoration of damaged soils through both natural and artificial inoculation (Chakraborty and Chakraborty,2013). Soil fungus and bacteria, which are vital components of many biochemical cycles, aid in the cycling of organic compounds.

#### **DIVERSITY OF BENEFICIAL SOIL MICROORGANISMS**

The microbial community in bulk soil is far more diverse than that in the rhizosphere. This is because the rhizosphere's friendly environment fosters fierce competition that suppresses less competitive bacteria, while the bulk soil's lack of favorable conditions lessens competition among microbes. Consequently, faster multiplication is possible for microorganisms that manage to establish themselves in the rhizosphere. Many secondary and primary metabolites, such as phenolics, terpenoids, and alkaloids, as well as primary metabolites, such as amino acids, organic acids, and carbohydrates, are released by the roots of many plants. The rhizosphere's diverse and abundant microbial life is propelled by these rhizo-deposits. A diverse range of microorganisms, including fungus, bacteria, viruses, nematodes, and protozoa, make up the rhizomicrobiome. It is possible to divide the many groups of organisms inside the rhizobiome according to the different roles they play. These groups include bacterial feeders, shredders, parasites, pathogens, root feeders, fungal feeders, and top predators. Grazers, decomposers, predators, mutualists, and photosynthesizers are examples of other species.

An incredibly complex microbial population, comprising pathogens, beneficial bacteria, saprophytes, epiphytes, and endophytes, is present in the rhizosphere. These microbial communities usually coexist peacefully in natural systems, with all populations balancing one another out in their competition for resources and available space. A disruption in this natural equilibrium can occur in "artificial" systems, such as agriculture, which can significantly change the microbial community, cause the loss of helpful bacteria, or allow plant diseases to infiltrate and have a disastrous impact on plant productivity. In these situations, adding advantageous microorganisms to production systems can help tip the microbial community's balance in favor of a population structure that promotes better plant health and productivity. They are divided into two main categories based on the most well-known beneficial effects they have on plants: (a) Plant growth promoting microorganisms (PGPM), which directly promote plant growth, and (b) biological control agents (BCA), which indirectly increase plant productivity by controlling plant pathogens. More precisely, PGPM has demonstrated biocontrol effects for soil-borne diseases. On the other hand, BCA has shown characteristics that directly encourage plant growth (Chakraborty *et.al.*, 2014a).

The quantity of bacteria in the rhizosphere that fix nitrogen and solubilize phosphate is significant from an agronomic perspective. Because of the decreased oxygen levels brought on by root respiration, there are comparatively less aerobic bacteria in the rhizosphere. They are found in large quantities in the root hair region and infrequently at the root tips, making up around 4–10% of the entire root area. In the rhizosphere, the majority of amino acids and growth factors that bacteria need are easily obtained from the root exudates. Unlike their impact on bacteria, plant roots do not change or increase the overall number of fungus in the rhizosphere. In order to isolate and screen beneficial agriculturally important microorganisms for their desirable characters (Fig.1 and Fig.2), select efficient strains, create an inoculum, and prepare a carrier-based formulation, a great deal of work was done in the rhizosphere of agricultural fields, forests, and riverine soil of North Bengal (Chakraborty, 2016).



Fig.1. Microbial populations (Fungal and Bacterial isolates) of rhizopsphere of agricultural fields, forests and riverine soil of North Bengal



Fig.2. Phosphate solubilizers, chitin degraders and cellulalse producers (%) from agricultural soil, riverine soil and forest soil of North Bengal

#### **BENEFICIARY RHIZOMICROBIOME.**

#### Plant Growth-Promoting Rhizobacteria (PGPR)

A variety of advantageous, free-living bacteria that support plant root growth can be found in the rhizospheric zone. The rhizobacteria *Pseudomonas*, *Rhizobium*, *Bacillus*, and *Acinetobacter* contribute to the growth of plants. These bacteria are vital because they generate growth regulators and phosphorus solubilizers, which increase an organism's resistance to biotic and abiotic stress. PGPR not only functions as a bio-control agent but also boosts fertilizer efficacy, encourages rhizoremediation, and effectively controls phytopathogens. Based on the findings of

scientists, it can be stated that PGPRs can provide protection, especially against soil-borne diseases, to seedlings that have undergone bacterial inoculation. The increasing acceptance of organic farming methods has led to a reevaluation of PGPR as biofertilizers that require little to no additional inputs. Biofertilizers such as *Rhizobium*, *Pseudomonas fluorescens*, and *Allorhizobium* spp. are beneficial to plant growth and development. The beneficial effects of PGPRs on various plant systems have been well-documented (Table 1) by a plethora of investigations carried out in historical and modern situations (Chakraborty *et al*, 2014b).

A noteworthy 33% increase in Arabidopsis growth promotion was noted. In a controlled pot experiment, the use of Bacillus spp., Pantoea agglomerans, and Pseudomonas taiwanensis enhanced plant health measures and increased the production of siderophores and IAA (indole-3acetic acid). It was possible to use Bacillus spp. as a bioinoculant to increase plant health metrics, increase the population of beneficial bacteria in the soil, and increase the yield of maize crops. During infection, PGPRs emit signal molecules that are essential for the promotion of plant development. These signal molecules attract beneficial microbes, which encourage the growth of plants. Higher levels of malic acid were found in the rhizosphere, acting as a signal to draw beneficial Bacillus subtilis and encourage the formation of biofilms on Arabidopsis roots. A broad-spectrum resistance response against pathogenic bacteria can be triggered by PGPRs. Iturin A and surfactin are two instances of antibiotic metabolites that are known to be produced by a variety of bacterial species. These substances work as antiseptics for plant roots, effectively keeping harmful fungus and bacteria at bay. By directly synthesizing hormones, antibiotics, and other secondary metabolites, PGPR have been shown to influence soil security, improve crop growth, development, yield, and quality, increase nutrient availability by solubilizing phosphate and potassium; and induce tolerance to biotic and abiotic stresses by regulating plant-related gene expressions.

#### Plant Growth Promoting Fungi (PGPF)

Plant growth promoting fungi (PGPF) are rhizosphere fungi that have the ability to stimulate plant development after root colonization. These saprophytes, which are non-pathogenic soil dwellers, have been shown to benefit a number of crop plants by shielding them from disease and encouraging their growth. A few isolates from this group of PFPF are particularly good at making plants resistant to phytopathogens on a systemic level. Plant illnesses have been found to decrease in correlation with the development and yields of plants enhanced by the fungus *Trichoderma*. Another fungus, *Talaromyces flavus* showed far more potentiality in solubilising phosphate and inhibiting various fungal pathogens. Apart from these, several species of *Aspergillus* have also been reported to influence plant growth and development by mobilizing soil phosphate

Сгор	PGPR
Rice	Bacillus megaterium, Aeromonas veronii, Enereobater cloacae,
	Rhizobium leguminosarum, Pseudomonas sp., Burkholderia sp.,
	Herbaspirillum sp. Azospirillum sp., Bacillus sp., Paenibacillus
	sp., Brevundimonas sp.
Wheat	Azotobacter sp., Azospirillum brasilense, Pseudomonas
	fluorescens Bacillus circulans, Cladosporium herbarum
Barley	Bacillus licheniformis, Pseudomonas putida, Paenibacillus
	polymyxa, Rhodobacter capsulatus
Maize	Bacillus megaterium, Azospirillum lipoferum, Burkholderia
	cepacia, Azospirillum brasilense, Azotobacter sp.,
	Herbaspirillum seropedicae, Pseudomonas aurantiaca, Bacillus
	amyloliquefaciens, Microbacterium oleovorans
Sorghum	Bacillus cereus, Seratia marcescens
Soybean	Bradyrhizobium japonicum, Azotobacter chroococum Bacillus
	subtilis, Bacillus thurigiensis, Azospirillum brasiliense,
	Pseudomonas aureofaciens Serratia liquefaciens, , Serratia
	proteamaculans,
Groundnut	Pseudomonas fluorescens
Chilli	Bacillus simplex, Bacillus cereus
Lettuce	Agrobacterium sp., Alcaligenes piechaudii, Bacillus
	megaterium, Pseudomonas fluorescens
Tea	Bacillus pumilus, Bacillus megaterium, Ochrobacterum
	anthropi, Serratia marcescens
Mandarin	Pseudomonas poae

Table 1. Beneficial PGPR used in growth improvement of various crops

Another fungal symbionts - Arbuscular mycorrhizal fungi (AMF), are gaining popularity due to the growing range of applications they have in sustainable agriculture and ecosystem management (Chakraborty, 2019). They establish mutualistic relationships with almost 80% of the major vascular plant groups. AMF root colonization is a dynamic process that is impacted by a number of edaphic variables. Mycorrhizae are a vital component of the soil's structure, serving as a link between plants and the soil. They also directly improve soil fertility and quality by increasing the intake of immobile nutrients, particularly phosphorus, which the fungus mobilizes.

Despite unfavorable environmental circumstances, AMF is essential for the vigorous development of host plants because of a variety of complex interactions between the fungus and the plant. The plants' overall health is facilitated by these interactions, which lead to higher rates of photosynthesis, greater gas exchange, and improved water absorption. Diversity of AMF association with roots of *Citrus* spp., Tea, Arecanut, Coffee and Rubber (Fig.3) have been explored and immunologial formats have been developed for detection of two dominant AMF (*Glomus* sp. and *Gigaspora* sp.) from soil and plant roots. The effective development of a mutualistic relationship between plants and AMF is a tactic to enhance each partner's nutritional condition. Plant response and advantages from mycorrhizae can be influenced by a number of factors in agriculture, including the host crop's dependence on mycorrhizal colonization, the tillage system, fertilizer input, and the potential of the mycorrhizal fungus inoculum.

As a biocontrol agent against important phytopathogens, Trichoderma spp., a filamentous ascomycetous fungus that have been extensively investigated (Sharma and Sharma, 2020). They include several commercially significant species, including T. virens, T. harzianum, T. asperellum, T. viride, T. atroviride, T. erinacium and T. reesei. They are a common mycoflora that have colonized many soils across all ecosystems and agroclimatic zones. These species are well-known for their capacity to colonize cellulosic materials and for inducing systemic resistance against plant diseases in the rhizosphere of plants. These fungi are distinguished by their rapid development, vivid green conidia, and conidiophores that branch out frequently some Trichoderma strains have the capacity to inhibit plant pathogens, primarily those found in the soil or on plant roots, hence lessening the severity of plant diseases due to their strong antagonistic and mycoparasitic capabilities. They do manage a broad spectrum of plant pathogens by inducing either localized or induced systemic resistance in bacteria, fungi, oomycetes, and even viral diseases. They exhibit antagonistic properties against both foliar and soilborne fungal plant diseases, including ascomycetes, deuteromycetes, and basidiomycetes. Because of their high rate of reproduction, capacity to endure extremely harsh conditions, efficiency in utilizing nutrients, capacity to modify the rhizosphere, robust aggressiveness against plant pathogens, and effectiveness in promoting plant growth, they are widely recognized as effective biocontrol agents. Due to the availability of fungal prey and nutrients derived from roots, *Trichoderma* thrives in the rhizosphere and promotes the development of good interactions with plants.



Fig.3 . Root colonization of plantation crops with arbuscular mycorhhizal fungi (AMF) Biocontrol Agents (BCA)

Additionally, it has been demonstrated that certain rhizosphere-competent *Trichoderma* strains directly affect plants, boosting their capacity for growth and nutrient uptake, as well as the efficiency with which they use fertilizer, the percentage and rate of seed germination, and the stimulation of their defensive mechanisms against biotic and abiotic stresses.

Screening for exo and endo chitinase activity (Fig 4) of more than fifty isolates of *Trichoderma* species were done. Potential isolates of *Trichoderma harzianum*, *T. asperellum* and *T. erinaceum* obtained from forest soil, agricultural soil and riverine soil were evaluated both *in vitro* and *in vivo* using various crops such as cereal, pulses, plantation, horticulture as well as sericulture plant. These potential isolates of *Trichoderma* species have been deposited in cultural collection of National Bureau of Agriculturally important microorganism and submitted in NCBI data base. (Table 2).



Fig. 4. Screening for Exo- and Endo- chitinase activity of Trichoderma isolates

Soil types	Isolates	NBAIM Acc. No.	NCBI Acc. No
Forest Soil	<i>T. erinaceum</i> (FS/L-20)	NAIMCC-F-0949	HM107419
	T. harzianum (FS/C-90)	NAIMCC-F-0167	GU187914
	<i>T. harzianum</i> (FS/S455)	NAIMCC-F-01968	HM107420
	<i>T. harzianum</i> (FS/S-458)	NAIMCC-F-01969	HM107421
	<i>T. erinaceum</i> (FS/S-474)	NAIMCC-F-01960	GU187915
	<i>T. erinaceum</i> (FS/S-475)	NAIMCC-F-01953	GU191829
	<i>T. erinaceum</i> (FS/S-478)	NAIMCC-F-01954	HM117841
Agricultural Soil	T. harzianum (Ag/S476)	NAIMCC-F-01956	GQ454925
	T.harzianum (Ag/S471)	NAIMCC-F-01950	GU564469
	T. harzianum (Ag/S479)	NAIMCC-F-01955	GU564470
Rhizosphere Soil	T. harzianum (RHS/T- 460)	NAIMCC-F-01952	GU564471
	T. harzianum (RHS/T-477)	NAIMCC-F-01962	HM117840
	T. harzianum (RHS/M511)	NAIMCC-F-01961	GQ995194
	<i>T. asperellum</i> (RHS/M512)	NAIMCC-F-01963	HQ265418
	T. harzianum (RHS/M 501)	NAIMCC-F-01964	HQ334993
	<i>T. asperellum</i> (RHS/M 517)	NAIMCC-F-01965	HQ334994
	T. harzianum (RHS/S 560)	NAIMCC-F-01966	HQ334995
	<i>T. asperellum</i> (RHS/S 561)	NAIMCC-F-01967	HQ334996
	<i>T. asperellum</i> (RHS/S 559)	NAIMCC-F-01968	HQ334997

Table 2. Potential isolates of Trichoderma species se	lected for field evaluation after
screening their chitinase activities	

#### **MECHANISMS INVOLVED IN CROP IMPROVEMENT**

#### **BIOFERTILIZATION**

Biofertilizers are rhizobacteria that increase plant uptake of nutrients, thereby promoting plant development. These bacteria contribute to the host plants' improved nutritional status by means of nitrogen fixation, increasing the availability of nutrients in the rhizosphere, promoting the root surface area, or enhancing beneficial symbiosis of the host. One of the primary plant nutrients, nitrogen (N), can be assimilated by plants from the soil as nitrite, nitrate, or ammonia. The majority of soils lack these kinds of nitrogen, and the chemical nitrogen fertilizers used in agriculture often lose their potency due to rainfall or mineral leaching. On the roots of leguminous plants like soybean, pea, peanut, and alfalfa, atmospheric N<sub>2</sub>-fixing bacteria like Rhizobium and Bradyrhizobium can create symbiosis by forming nodules. In these nodules, the bacteria convert N<sub>2</sub> into ammonia, which the plant can use as a source of nitrogen. Besides, several potential non-symbiotic bacteria (Azospirillum, Azoarcus, Azotobacter, Bacillus polymyxa, Burkholderia, Gluconoacetobacter, Herbaspirillum) identified as free-living N2-fixers can fertilize several important agronomic plants such as wheat, sorghum, maize, rice and sugarcane. Phosphorus (P), another crucial nutrient for plants, is primarily found in soil as insoluble molecules that are inaccessible to plants and hence restrict their ability to grow. These P forms can be solubilized by some PGPR (Azospirillum, Bacillus, Burkholderia, Erwinia, Pseudomonas, Rhizobium) by enzymatic means, chelation, or acidity.

#### PHYTOSTIMULATION

Modification of root architecture and development of plants are evident by diverse PGPR due to their capacity to synthesis and secrete plant hormones such as indole-3-acetic acid (IAA), gibberellins (GAs), cytokinins, and specific volatiles known as phytostimulators. It has been shown that the biosynthesis of IAA by different PGPR increases root proliferation and that IAA may potentially function as a bacterial signaling molecule with direct effects on the physiology of bacteria. There are evidences where GA production by *Azospirillum* or *Bacillus* sp. induces growth promotion in plants. Through improved lateral and adventitious root formation, PGPR (*Azotobacter* spp., *Rhizobium* spp., *Pantoea agglomerans, Rhodospirillum rubrum, Pseudomonas fluorescens, Bacillus subtilis* and *Paenibacillus polymyxa*) reported to produce

cytokinins have been shown to have a role in crop plant root initiation, cell division, enlargement, and surface area expansion. One significant way by which rhizobacteria induce plant development is the finding of volatile organic compounds (VOCs) such as 2,3-butanediol, acetoin, terpenes, jasmonates that they create.

#### BIOCONTROL

Microorganisms that have been extensively researched and shown to have a measurable impact on plant health include members of the bacterial genera *Bacillus, Pseudomonas, Stenotrophomonas* and *Streptomyces* as well as the fungal genera *Ampelomyces, Coniothyrium,* and *Trichoderma. In vitro* tests of microbial isolates from habitats connected with plants revealed that 1–35% of them had the antagonistic ability to prevent disease growth. Antagonistic activity is caused by a variety of mechanisms, such as the pathogen's inhibition by antibiotics, toxins, and surface active compounds (biosurfactants); competition for nutrients, minerals, and colonization sites; and the development of extracellular cell wall-degrading enzymes like chitinase and  $\beta$ -1,3glucanase. In order to implement successful biological control using plant-associated antagonists, it is necessary to have a deeper understanding of the intricate ways in which antagonists regulate disease suppression in response to both biotic and abiotic stimuli, but also knowledge of the dynamics and composition of plant-associated bacterial communities and what triggers plant colonization.

Beneficial microorganisms, PGPR (*Bacillus megaterium*, *B. pumilus*, *B. altitudinus*, *Ochrobacterium anthropi*, *Pseudomonas poae*) (Table 3), BCA (*Trichoderma harzianum*, *T. asperellum*, *T. hamatum* and *Talaromyces flavus*) and AMF (*Rhizophagus fasciculatum*, *Glomus mosseae*, *G. intraradices*, *Gigaspora margarita*) were used (Fig 5) to boost plant health status by causing systemic resistance that gave broad-spectrum resistance to fungal pathogens (Table 4). Bioformulations of PGPR and BCA (Fig 6) and methods of their field application (Fig.7) singly or as a consortium, has been reported to induce resistance in plants against phytopathogens (Table 5). Field evaluation of *T. harzianum*, *Talaromyces flavus* and *Bacillus altitudinis* for management of sclerotial blight and root rot diseases of *Vigna radiata* have been worked out (Fig. 8).



Fig.5. Pot	ential P	'GPF an	d PGPR fro	om microb	ial resour	ces of North	Bengal
sele	cted for	biocont	rol of root d	liseases			

PGPR isolates	NBAIM Acc. No.	NCBI Acc. No.
Bacillus pumilus	NAIMCC-B 01483	JF836847
(BRHS/C1)		
Bacillus altitudinis	NAIMCC-B 01484	HQ849482
(BRHS/P 22)		
Bacillus altitudinis	NAIMCC-B 01485	JF899300
(BRHS/S 73)		
Bacillus pumilus	NAIMCC- B 01487	JQ765579
(BRHS/T382)		
Bacillus pumilus	NAIMCC-B 01488	JQ765580
(BRHS/T384)		
Enterobacter cloacae	NAIMCC-B 01486	KC703974
(BRHS/R-71)		
Burkholderia symbiont	NAIMCC-B 01489	JQ765578
(BHRS/P 92)		
Bacillus aerophilus	NAIMCC-B 01490	KC603894
(BRHS/B-104)		
Paenibacillus polymyxa	NAIMCC-B 01491	KC703775
(BRHS/R-72)		
Bacillus methylotrophicus	NAIMCC-B 01492	JQ765577
(BRHS/P-91)		

Table 3. Beneficial	plant growth	promoting	rhizobacteria
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Fig.6. Bioformulations of PGPR and BCA for field application



Fig. 7. Methods of application of bioformulation

AM Fungi	Crop disease Management	Fungal pathogen
Glomus mosseae	Wilt of tomato	Fusarium oxysporum
	Wilt of alfalfa	Verticillium albo-atrum
	Wilt of cotton	Verticillium dahliae
	Wilt of pigeon pea	Fusarium udum
	Wilt of chickpea and peanut	Fusarium solani
	Sclerotial rot of tea	Sclerotium rolfsii
	Charcoal stump rot of tea	Ustulina zonata
Rhizophagus fasciculatum	Root rot of pea	Aphanomyces euteiches
	Root rot of cowpea	Macrophomina phaseolina
	Wilt of chickpea	F. oxysporum f.sp. ciceris
	Root rot of mandarin	Fusarium solani
Glomus intraradices	Root rot of tomato	F. oxysporum f.sp. lycopersici
	Wilt of chickpea	Macrophomina phaseolina
	Root rot of pea	Aphanomyces euteiches
Gigaspora margarita	Wilt of pigeon pea	Fusarium udum
	Root rot of mandarin	Fusarium solani, F. oxysporum

Table 4: Crop disease management using arbuscular mycorrhizal fungi



Fig.8. Effects of *Trichoderma harzianum*, *Talaromyces flavus* and *Bacillus altitudinis* on sclerotial blight and root rot diseases of *Vigna radiata* 

Crop	Biocontrol agents	Pathogen
Rice	Bacillus altitudinus	Macrophomina phaseolina
	Pseudomonas plecoglossicida	
	Brevibacterium antiquum	
	P. fluorescens Aur6	Magnaporthe grisea
	Chryseobacterium balustinum Aur9	
	Trichoderma harzianum	Drechslera oryzae
	Glomus mossea	
Wheat	Trichoderma harzianum	Fusarium oxysporum
	Chaetomium globosum	
	Pirifolrmospora indica	Gaeumannomyces graminis var. tritici
	Trichoderma asperellum	Bipolaris sorokiniana
	Bacillus safensis	
Chickpea	Trichoderma harzianum	Fusarium oxysporum
	Gomus mosseae	Macrophomina phaseolina
	Rhizophagus fasciculatus	Sclerotium rolfsii
	Gigaspora gilmorei	
Mustard	Bacillus pumilus	Thanatophorus cucumeris
	Bacillus altitudinis	

Table 5. Management of fungal diseases of various crops using biocontrol agents

Moreover, growth, flowering, and fruiting of *Citrus reticulata* have all been successfully enhanced by these bioformulations, which have also created resistance against wilt pathogens (Chakraborty *et.al.*, 2019) and enhanced the quantity and quality of *Persea bombycina* foliage, all of which have improved the quality and yield of muga silkworm cocoons (Chakraborty *et.al.*, 2024). Cutting-edge technologies that have the ability to turn trash into profit, like value-added vermi-composting, tricho-composting (Fig.9) have emerged as some of the most reliable sources of income for the next generation of workers.



Fig.9. Rapid Composting methods of Trichoderma

#### INDUCTION OF IMMUNITY IN HOST AGAINST PHYTOPATHOGENS

Induced resistance is the term for the resistance mechanism that plants possess that can be triggered and employed to manage illnesses. The initial step in the development of immunity in plants is the recognition of the microbial molecules by the plants, which are subsequently transduced through several pathways that result in responses, which in turn activate defense mechanisms that suppress the bacteria. Plants can develop this immunity through chemical or biological elicitors, which keeps the plant ready to fend off future disease and pest attacks. When a disease attacks, this primed condition is marked by increased defense responses. When a plant encounters an elicitor, also known as a pathogen-associated molecular pattern (PAMP), such as lipopolysaccharides, peptidoglycan,  $\beta$ -glucans, chitin, or flagellin, it will typically respond defensively by fortifying its cell walls, increasing the amount of PR proteins it accumulates, and

biosynthesising phytoalexin. Additional receptors found in plants include resistance proteins and pathogen recognition receptors (PRRs), which are involved in pathogen detection. The majority of the R proteins interact with pathogen effector proteins through their role as NB-LRR (nucleotide binding leucine rich repeat) proteins. Plant sections expressing induced systemic resistance (ISR) have a higher level of induced immunity against a wider range of pathogens. The specific defense pathway that has to be triggered is frequently determined by the type of contact and proteins involved. Plant hormones create induced resistance by controlling a complex web of linked signaling pathways. Phytohormones such as SA and JA are thought to be important players in controlling signaling during plant defense. Due of SA's tremendous potential, systemic acquired resistance (SAR) has received a lot of attention in study using all available cutting-edge methodologies. The ability of SAR to be activated by spraying SA or its synthetic functional equivalents, such as 1,2,3-benzothiadiazole-7-carbothiocic acid S-methyl ester (BTH) or bioinoculants, even in the absence of pathogens, is crucial for the use of this technique in crop protection.

The induction of PR proteins in plants, such as chitinases, glucanases, and the antifungal protein osmotin, which have antipathogen activity and may break down fungal cell walls, lysozymal activity, which suggests antibacterial functions, and nuclease activity that is effective against pathogens, is one of the main components of SAR (Chakraborty and Sharma 2008). According to microarray research, hundreds of genes express themselves differently or more frequently when SAR develops. Significant impacts of these changed gene expressions include the creation of ROS and the strengthening of cell walls. In contrast to SAR, which is recognized for causing broad-spectrum resistance in plant tissues above ground in response to non-pathogenic microbes that might stimulate growth, JA and ethylene are the main regulators of ISR. However, signals from other plant hormones including auxin, ethylene, gibberellins, and abscisic acid (ABA) frequently overlap with signals from SA and JA. The utilization of microorganisms in agriculture can reduce the usage of toxic pesticides because of their potential to create systemic resistance and stimulate growth.

*Trichoderma* spp. have been utilized for the biocontrol of plant diseases; as a result, they have been the focus of much study, which has resulted in the creation of commercial goods and bioformulations that are currently sold throughout the world. *T. harzianum, T. asperellum, T. viride, T. virens,* and *T. aureoviride* are being used as microbial inducers of plant immunity

( Chakraborty *et al.*, 2020) . Sm1/Ep11, an immunity-inducing protein of the cerato-platanin family, is an elicitor produced by *Trichoderma* and this protein increases the expression of genes involved in defence, which in turn induces immunity (Table 6 ). The synthesis of defensive enzymes such chitinases, proteases, glucanases, and antifungal substances is one of the defense genes' many roles. Numerous genes possessing similar roles have been identified in many Trichoderma species. These include *chit 42, chit 3, exc1,exc 2, bgn 13.1, prb1, ThPG1, Th-Chit 42* from *T. harzianum, tac1, Tvsp1, TgaA,* and *TgaB* from *T.virens.* The majority of these *Trichoderma* species work against soil pathogens, including *Pythium ultimum, Fusarium oxysporum, Sclerotinia sclerotiorum*, and *Rhizoctonia solani.* 

Trichoderma species	Genes	<b>Biotic / abiotic stress</b>
T. harzianum	Sm1	Biotic and abiotic stress
	tri5	Soilborne pathogens
	ThPg1	Rhizoctonia solani
	Thkell	Osmotic and salt stress
	Th-Chit42	Sclerotinia sclerotiorum
	exc1, exc2, chit42,	Fusarium oxysporum
	cht3,bgn13.1,prb1	
T.virens	tac1	Pythium ultimum
	Tvsp1	Rhizoctonia solani
	TgaA, TgaB	Sclerotium rolfsii
T. asperellum	Chit42, chit3	Fusarium gramineraum,
		<i>F. oxysporum</i> f.sp. <i>cucumerinum</i>
T. brevicompactum	tri5	Candida albicans
T. longibrachiatum	egl1	Pythium ultimum

Table 6. Defence related genes of *Trichoderma* sp.

Elicitors/MAMPs generated by *Trichoderma* spp. which trigger a signaling cascade in the plant which involves signalling molecules such JA (jasmonate), as SA (salicylic acid) and the gaseous signaling molecule, ethylene (ET) are linked to plant immunity induction against phytopathogens. Downstream to the signaling by these molecules, greater accumulation of PR proteins occurs, which is one among the many defense responses triggered by *Trichoderma* sp and PGPR. In addition to the buildup of phytoalexins and terpenoids, the other factors include elevated activity of enzymes like lipoxygenase (LOX), phenylalanine ammonia lyase (PAL), peroxidase (POX), and polyphenol oxidase (PPO). Thus, cropprotection is achieved through induction of resistance due to activation of an array of defense responses (Chakraborty 2020).

Bioinoculants		Plant	Plant Pa		thogen		
Trichoderma sp.	Strain				-		
T. harzianum	T-39	Tomato		Bo	trytis cinerea		
		Cucumber		<i>B</i> .	cinerea		
		Bean		<i>B</i> .	cinerea		
		Straw	berry	Po	dosphaera xanthi		
		Grape	evine	Pla	Plasmopara viticola		
	T-22	Bean		<i>B</i> .	cinerea		
		Toma	to	Alt	ernaria solani		
	T-1	Peppe	r	Ph	ytophthora capsici		
T. virens	G-6	Cotto	n	Rh	izoctonia solani		
	G-6-5	Bean		Co	lletotrichum lindemuthianum		
	G-11	Tobac	co	<i>B</i> .	cinerea		
T. atroviride	P-1	Toma	to	Alt	ernaria solani		
T. asperellum	T-203	Cucur	nber	Pse	Pseudomonas syringae pv. lachrymans		
	GDF1009	Corn		Fu,	Fusarium gramineraum		
		Cucur	nber	Fu,	sarium oxysporum f.sp. cucumerinum		
	Ta-34	Mandarin		Fu	sarium solani		
Plant growth promoting rhizobacteria		acteria					
Bacillus megateriu	m		Tea		Fomes lamaoensis		
Ochrobacterium a	nthropi						
Bacillus pumilus			Soybear	n	Thanatophorus cucumeris		
Bacillus altitudinu	\$		Mustard				
Bacillus safensis			Wheat		Bipoaris sorokiniana		
Ochrobacterum ps	eudogrignor	nense					
AMFungi							
Glomus mosseae		Maize		Rhizoctonia solani			
		Tea		Ustulina zonata			
Glomus sp.		Mandar	in	Fusarium solani			
Gigaspora sp.			Bean		Rhizoctonia solani		
Gigaspora margar	rita		Mandar	in	Fusarium solani		

Table 7. Induced immunity in plants against phytopathogens elicited by bioinoculants

Among many others, Acinetobacter, Arthrobacter, Bacillus, Burkholderia, Enterobacter, Ochrobactrum, Paenibacillus and Pseudomonas are some of the PGPR genera, have been reported to induce systemic resistance in plants, as do several beneficial fungi including mycorrhiza such as *Rhizophagus fasciculatus* and *Gigaspora gigantea* in mandarin ( Chakraborty and Allay, 2022) and bioinoculants ( *Trichoderma asperellum, Rhizophagus*  fasciculatus and Bacillus pumilus) in Persea bombycina (Chakraborty et al, 2024). Systemic induction of resistance by AMF has been proved by the fact that the defense responses are not restricted to the roots alone but is also evident in the shoots. Expression of some defense genes, especially those of JA pathway, were much enhanced in the leaves of infected, AMF colonized plants. Transcript profiling of tomato leaves after exogenous application of JA revealed that JA- responsive genes were expressed earlier as well as to a greater extent in mycorrhiza, especially G. mosseae colonized, than the non-mycorrhizal ones, confirming the involvement of JA- related defense. In order to further confirm that Mycorrhizal induced resistance (MIR) involves JA- related defense in tomato against Botrytis cinerea, mutants impaired in JA signaling were tested and it was observed that resistance could not be induced efficiently in these mutants. This is similar to the involvement of JA in ISR provided by PGPR in Arabidopsis and tomato, as well as during induction of resistance by beneficial fungi such as Trichoderma and Piriformospora. The resistance induced by AMF in rice against Magnaporthe grisea, the causal organism of blast, was dependent both on the activation of genes involved in regulatory roles during host immunity, as well as greater expression, during pathogen attack, of defense effector genes. Interestingly, it has been reported that other than the systemic resistance induced by AMF in above ground parts, the induction was also evident in neighbouring plants, probably due to the AMF hyphal network which can travel long distances and pass on signals, thus acting as a plant to plant communication system. Thus it is clear that immunity is induced in different plants by bioinoculants (BCA, AMF and PGPR) against phytopathogens (Table 7). PGPR have been proven to exert influence on soil security, improve crop growth, development, yield (Table 8), and quality through directly synthesizing hormones, antibiotics, and other secondary metabolites; enhance nutrient availability through solubilization of phosphate, potassium, and induce tolerance to biotic and abiotic stresses by regulating plant-related gene expressions.

Transmission electron micrographs of wheat leaves probed with PAb of chitinase and labeled with gold conjugate following induction of resistance against *Bipolaris sorokiniana* using bioinoculants (*Bacillus methylotrophicus* and *Trichoderma asperellum*) showed distribution of intense gold particles throughout the cell structure (Fig. 10A). Increased activities of defense enzymes (Chitinase and  $\beta$ ,1-3 glucanase) were also observed during induced

Microorganism	Strain	Сгор	Increase in grain yield (%)
Azospirillum brasilense	Ab V5, AbV6	Maize	27
		Wheat	31
	Sp246		14.7
Bacillus polymyxa	Bp 4317		13.6–19.5
Bradyrhizobium japonicum	SEMIA 5079,	Soybean	8.4
	5080		
	532C,USDA110		12-19
	BR3267	Cowpea	38.1
Bradyrhizobium liaoningense	VIBA 1		54.8
Bradyrhizobium yuanmingense	VIBA 2		38.3
Burkholderia vietnamiensis	TVV75	Rice	22
	MGK3		12.1
Pseudomonas fluorescens	SS5	Tomato	57
Rhizobium leguminosarum	HB429	Common	48
sv. phaseoli		beans	
Rhizobium tropici	SEMIA 4080		36
	CPAO 12.5 L2		66
Rhizobium leguminosarim	NGB-FR126	Faba beans	46.8-81.4
sv. v <i>iciae</i>	NSFBR-30,		5-17
	HUFBR 15		

Table 8. Effects of seed inoculation with PGPR on grain yield of various crops

resistance by bioinoculants against spot blotch pathogen. Cellular localization of chitinase was demonstrated by indirect immunofluorescence tests using PAb of chitinase followed by labelling with FITC conjugates, as indicated by a strong bright apple green fluorescence in the mesophyll tissues of wheat and tea leaves, while epidermal cells and cortical tissues in mandarin roots. Treatment of mandarin roots with both *Trichoderma harzianum* and *Glomus mosseae* could induce immunity against Fusarium root rot disease. Similarly, *Gigaspora gigantea* colonized mandarin roots, treated with a BCA (*T. asperellum*) and PGPR (*Pseudomonas poae*) showed heavy deposition of gold particles near the cell wall of the inoculated roots when probed with PAb of chitinase and labeled with gold conjugate (Fig. 10B) also led to the development of immunity against *Fusarium solani* after treatment with bioinoculants was indicated by enhancement of chitinase not only in roots but also in epidermal cells and mesophyll tissues of leaves. Heavy deposition of gold particles were also evident in tea leaf tissue following induction of resistance using bioinoculants (Fig 10 C).



Fig. 10. TEM of (A)Wheat leaf, (B)Mandarin root and (C)Tea leaf probed with PAb of chitinase labeled with gold conjugates following application of bioinoculants and challenge inoculaton with phtopathogen

By upregulating defense activities, the application of bioinoculants protected wheat plants against *Bipolaris sorokiniana* and enhanced growth, as demonstrated by microarray analysis (Fig. 11). Defense responses are not directly activated by PGPR and other beneficial microorganisms, but the whole plant becomes primed for a faster and stronger response to pathogen attack when the defense responses become activated (Fig. 5). Thus, disease suppression and improvement of plant health status have been correlated with inhibition of the pathogen, increased availability of nutrients and immunity developed in the host. Besides interactions among the microorganisms, environmental factors such as extreme weather changes, soil characteristics or other such factors also may affect the growth and functioning of the PGPR. Studies on interactions between plants and *Trichoderma*, a PGPF with multiple beneficial traits have focused on the intracellular signaling responses that interpret the fungal perception into metabolic, genetic and developmental processes (Sharma and Sharma 2020).



## Fig. 11. Analysis of relative expression of defense genes by RT-PCR in wheat plants following induced immunity against phytopathogen using bioinoculants

It is thus clear that the bioinoculants in the soil activate the plant immune systems in such a way that they ward off the enemies on the one hand and accommodate the mutualists on the other. Molecular dialog between roots and ISR-eliciting microbes involve a complex array of signaling (Fig.12). It is probable that long distance signaling molecules may be generated and/or modified in the outermost root cell layer, as indicated by the expression pattern of *MYB72*, which is required for the onset of ISR in the roots. While the beneficial microbes influence plant immune system, plants also shape the composition of the root microbiome to their own benefit. What are the plant traits and corresponding genes that enable plants to maximize profitable and protective functions from their root microbiota? The signals between the plant roots and the surrounding microbiota are no doubt the key factors here. This signaling is genetically controlled and activated as per the environmental conditions. Studies combining metagenomic analysis and quantitative plant genetics have revealed that each plant has a core root microbiome.



**Fig 12.** Schematic illustration of molecular components and mechanisms involved during induced systemic resistance (ISR) in plants triggered by beneficial rhizosphere microorganisms. Abbreviations: MAMP- microbe associated molecular pattern; DAMP – damage associated molecular pattern; PAMP- pathogen associated molecular pattern; PGPR- plant growth promoting rhizobacteria; LPs- lipopolysaccharide; MAPK- Mitogen activated protein kinase; ROS – reactive oxygen species; PTI – pathogen triggered immunity; ETI- effector triggered immunity; SAR- systemic acquired resistance; HR – hypersensitive response; SA- salicylic acid; JA- jasmonic acid; ETH-ethylene; ABA- abscisic acid

#### ALLEVIATION OF ABIOTIC STRESSES USING BENEFICIAL MICROORGANISMS

Abiotic stresses that affect crops naturally are numerous and include high temperatures, droughts, flooding, salt, heavy metals, low soil nutrition, acidity, and so on (Chakraborty and Chakraborty, 2015). It is widely acknowledged that the primary factor limiting agricultural productivity is soil moisture. Drought is the primary abiotic stressor affecting crop output, since freshwater resources shrink in both quality and quantity. This is especially true in underdeveloped nations where irrigation infrastructure is severely lacking. Water scarcity also affects soil nutrient availability and transport. It is also known that drought stress alters the balance of plant hormones by decreasing the amounts of endogenous cytokinin and raising ABA levels in the leaves, which causes stomatal closure.Water scarcity also affects the diffusion of nutrients in the soil over short distances, as water carries the nutrients to the roots. Numerous pressures have an impact on a plant's metabolic processes, which lowers crop health and, consequently, productivity.

Various strategies to cope with abiotic stresses include

- Shifting of crop calendars
- Development of drought and heat tolerant varieties
- Modification of resource management practices
- Development of stress-tolerant plants through manipulation of stress-tolerant genes.

Besides microorganisms, specially PGPR for induction of tolerance against abiotic stresses have been considered ( Chakraborty and Chakraborty, 2022). The host may experience physical and chemical changes as a result of these PGPR, which could result in systemic tolerance to various abiotic stimuli. One of the most cost-effective sustainable agricultural techniques for increasing plants' resistance to multiple stresses is seed priming. In this technique, seeds are hydrated with particular chemicals (chemical priming), biotic stimuli (bio priming), or abiotic stimuli to encourage specific biochemical changes in response to stress events. These changes become "stress memory" and serve as a cue to withstand particular stress factors more effectively. Water stress can change the structure of the soil, rendering it unsuitable for microbial activity and crop growth. Nonetheless, certain PGPR can create exopolysaccharides (EPS) that shield soil microorganisms from harsh environments and promote their survival. Additionally, they facilitate the irreversible attachment and colonization of microorganisms on the roots by means of a fibrillar material network that binds the bacteria to the root surface.

It has been discovered that biopriming with PGPR from the genera *Rhizobium, Bacillus, Burkholdaria, Ochrobactrum, Paenibacillus*, and *Pseudomonas* stimulates plant growth and abiotic stress tolerance in a variety of crops. In addition to biopriming, the application of PGPR as a soil drench has demonstrated very positive outcomes in reducing abiotic stressors. It has been shown that PGPR can help many agricultural plants under stress from salinity, water, and temperature (Table 9).

While it is clearly established that PGPR can protect plants against stresses the exact mechanisms of tolerance enhancement by them still remain largely speculative. The possible explanations include:

(i) Production of ACC deaminase leading to the reduced level of ET in the root of developing plants

(ii) Production of phytohormones

(iii) Induced systemic tolerance (IST) by the bacteria and

(iv) Formation of bacterial biofilm, i.e., extracellular matrix

The bacterial biofilm that forms the extracellular matrix contains a variety of macromolecules that are good for plant growth and development, including sugars and oligo- and polysaccharides. The *Pseudomonas putida* strain GAP-P45, which produces EPS, creates biofilm on the root surface of sunflower seedlings and gives the plants resistance to drought stress. It has also been demonstrated that, in the majority of cases, tolerance appeared to be produced by a series of reactions that included high transcript levels of stress-responsive genes during times of stress, accumulation of osmolytes and phenolics, and improvement of the antioxidative response. Studies employing *Bacillus safensis* demonstrated that this PGPR could boost wheat plants' height, yield, and chlorophyll content in addition to increasing RWC and elevating antioxidant responses. *B.safensis* could reduce heat stress in wheat plant (Sarkar *et.al*, 2021) which was linked to less ROS generation and membrane damage and express a variety of redox enzymes and accumulate osmolytes by which improved overall thermotolerance (Fig.13)

Abiotic stresses	Crop	PGPR
Water stress	Wheat	Bacillus safensis
		Ochrobactrum pseudogregnonense
		Azospirillum sp.
		Pantoea agglomerans
	Maize	Azospirillum brasilense
	Chickpea	Pseudomonas putida
	Pea	Pseudomonas sp
	Mung	Paenibacillus polymyxa
	bean	Rhizobium tropici
	Clover	Bacillus megaterium
	Tomato	Enterobacter cloacae
		Pseudomonas putida
	Sunflower	<i>Rhizobium</i> sp
		Pseudomonas putida P45
Temperature stress	Wheat	Bacillus amyloliquefaciens
		Bacillus safensis
Heat		Ochrobactrum pseudogregnonense
	Canola	Pseudomonas putida GR12-2
	Sorghum	Pseudomonas sp. AMK-P6
	Chickpea	Pseudomonas putida NBR 10987
	Wheat	Pseudomonas sp PPERs23
Cold	Rape seed	Pseudomonas putida
	Grape vine	Burkholderia phytofirmansPsJN
Salinity stress	Wheat	Pseudomonas putida
		Enerobacter cloacae
		Serratia ficaria
		Pseudomonas fluorescens
		Bacillus insolitus
		Bacillus safensis
		Ochrobactrum pseudogregnonense
	Rice	Pseudomonas pseudocaligenes
		Bacillus pumilus
	Maize	Pseudomonas syringae ,P. fluorescens
		Eneterobacter aerogenes
	Groundnut	Brachybacterium saurashtrense
		Brevibacterium casei JG-08
		Pseudomonas fluorescens
	Tomato	Achrobacter piechaudii, Streptomyces sp.PGPA39
	Lettuce	Pseudomonas mendocina
	Cotton	Raoultella planticola RS-2

## Table 9. Amelioration of abiotic stresses in plants using PGPR



Fig. 13. Comaprison of differential expression of HSP genes in wheat plants under high temperature stress and primed with *Bacillus safensis* 

*Ochrobactrum pseudogrognonense* and *B. safensis* have also the capacity to increase growth and reduce the effects of salt and drought primarily because they accumulate osmolytes, maintain membrane stability by inhibiting lipid peroxidation, and upregulate antioxidant activity. A gene-*Thkel1* from *T. harzianum* which could regulate glucosidase activity in *Arabidopsis thaliana* against osmotic and salt stress. AM fungus like *Glomus intraradices* and *G. claroideum* through their symbiotic relationship with the plants lessen the effects of drought. Through a variety of methods, these relationships are essential in improving the drought tolerance of host plants. Enhancing the production of non-enzymatic and enzymatic antioxidants in host plants is one such strategy. Plant resilience is increased when antioxidant defenses are strengthened to better withstand the oxidative stress brought on by drought. Furthermore, AM fungi affect the plant's water transport routes, namely the transition between apoplastic and cell-to-cell water transport. The plant can respond to water scarcity with more flexibility because to this modulation, which also makes it possible for it to distribute water resources more effectively—particularly to the shoot where they are most needed.

#### WAY FORWARD

The main goal facing agricultural experts worldwide is to boost crop yields while utilizing less pesticide and fertilizer inputs. Since it is evident that plants have developed in the natural world alongside intricate microbial communities that are crucial to the fulfillment of plant functions like growth, vigor, and defense, the relevance of the root microbiome in this context cannot be understated. In light of contemporary methods like next-generation sequencing-based root microbiome research, it is imperative to have a thorough understanding of the intricate and dynamic interactions taking place at the microbial community level. If the basic ideas behind the formation of binary plant-microbe and microbe-microbe interactions are thoroughly understood, then this is feasible. Using the knowledge of root microbiome processes to increase crop stress tolerance, yield, and reduce pesticide input in a sustainable and environmentally friendly manner is critical. Nevertheless, plant improvement initiatives have not made considerable efforts to increase these features provided by the microbiome of the plant's second genome. The development of sustainable future crops that are better able to maximize the beneficial and protective functions from beneficial microbes in their root microbiome will be made possible by the ongoing advancement of our knowledge regarding the molecular and genetic basis of plantbeneficial microbe communication in the context of its evolutionary and ecological relevance.

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