PLANT GROWTH PROMOTING FUNGI AS A POTENTIAL BIO-CONTROL AGENT AGAINST BACTERIAL LEAF BLIGHT IN RICE CROP

Abstract

A disease termed bacterial leaf blight has affected rice output, *Xanthomonas oryzae* pv.*oryzae* which is the causative agent damages the shoot portion of the rice crop. It results in a severe reduction in yield. Chemical treatments are now being used to combat this issue. However, in the long term, it causes greater harm to the environment. As a result, plant growth-promoting fungi extracted from the rice plant's rhizosphere are tested to determine if they have any antagonistic impact on the causal bacterium, *Xanthomonas oryzae* pv.*oryzae,* to utilize PGPF a simpler and safer approach of combating the BLB illness affecting rice.

Keywords: *Xanthomonas Oryzae Pv.Oryzae*, Rhizosphere, PGPF, BLB, Biocontrol Agent.

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I. INTRODUCTION

Rice is a variety of grass that is grown as an essential food in most Asian nations. It is loaded with carbohydrates and also includes fat, fiber, protein, vitamins, and fatty acids. Different types of rice, which are grown and consumed by farmers and consumers in over sixty percent of the world, include these nutritional components. When rice is grown constantly in the fields, a disease known as bacterial leaf blight disease, that spreads swiftly, takes place, especially during the wet season. Bacterial leaf blight, which affects almost every type of rice, is one of the most devastating diseases. In an infectiousness test for the bacterial leaf blight, *Xanthomonas oryzae* pv.*oryzae* was found as the causal bacterium [1]. The disease causes the leaves of a rice plant seedling to turn yellow to straw-colored before they wilt and die. The plant has light green to grayish green leaves with water-soaked streaks at first, but as the bacterial blight occurs, the lesions become larger and have edges that are irregular. The leaves also start turning yellow, droop, and die. When the milky bacterial slime from the very last phase of the bacterial blight dries, a white crust emerges on the leaves [2]. Plant extracts are used to treat agriculture plant ailments, and this approach is seen to be typically safe because plant products are inexpensive, rapidly biodegradable, and environmentally friendly in their effects. Over the last two decades, Plant growth-promoting fungi (PGPF) became known as an effective biological method for lowering BLB. PGPF are beneficial fungi that live on plant roots and stimulate plant development. The processes used to stimulate plant growth include auxins, cytokinins, gibberellic acids, jasmonic acid, ethylene and salicylic acid [3]. Furthermore, they can help to improve plant health by battling phytopathogens through a number of mechanisms such as competition, siderophores production, antagonism, and systemic resistance. The plant's latent defense is triggered by PGPF-induced systemic resistance, which triggers the activation of various defense-related chemicals and enzymes in areas other than the pathogen assault. Peroxidase (POD), a rapid-acting defense-related enzyme against plant infections, plays a role in lignification, suberification, polymerization of hydroxyproline-rich glycoproteins, cell wall elongation control, and plant defense against pathogens. The oxygen-scavenging enzyme catalase (CAT) protects cells from the damaging impacts of hydrogen peroxide through the transformation of it to water as well as molecular oxygen during growth [4]. Polyphenol oxidase (PPO) is essential in the initial stages of plant response when membrane damage results in the production of phenols such chlorogenic acid. PPO catalyzes the conversion of phenols to free radicals, which may interact with biological substances that inhibit pathogen growth. Phenylalanine ammonia-lyase (PAL) is the key enzyme responsible phenyl propanoid metabolism, is required for the production of a number of secondary chemicals involved in defense, including phenols and lignin [5].

II. *XANTHOMONAS ORYZAE* PV.*ORYZAE*

Xanthomonas oryza pathovar (pv) *oryzae* (*Xoo*) is a slime-producing gram-negative rod-shaped bacterium with round ends. Individual cells range in size from around 0.4m to 0.7m. Cells move via a single polar flagellum. When *Xanthomonas oryzae* pv.*oryzae* is cultivated for two days on Peptone Sucrose Agar (PSA) medium, yellow viscous bacterial colonies are visible. Spherical, convex, mucoid, and yellow colonies are formed [6]. This is a phytopathogen which leads the plant known as oryza sativa to develop bacterial leaf blight (BLB). Xoo thrives on dead plants and seeds and spreads through water in rice fields [7]. *Leersia sayanuka* is an alternate host that also serves as a main inoculum, allowing the bacteria to grow in the absence of the primary host. The bacterial leaf blight caused by *Xanthomonas oryzae* pv.*oryzae* drastically decreases production. The organism can survive in the soil for one to three months, depending on the moisture content and pH of the soil. *Xoo* spreads from plant to plant via irrigation or rainwater, and it feeds on dead plants and seeds [8]. The bacteria enters the host plant through water holes, growth ruptures on the roots, and perforations in the leaves and roots after being introduced. *Xoo* infects both the plant's xylem and leaf veins, causing nutrition restriction and plant withering. Bacteria spread from leaf wounds and is carried away by wind or rain. Symptoms include pale-green to grey-green water-soaked streaks around the leaf tip and margins on immature plant’s leaves [9]. These lesions gather and become yellowish-white with wavy edges. Finally, the entire leaf may be damaged turning pale or yellowish and withering. Leaf sheaths and culms of more sensitive types may be impacted. Wilting, leaf desiccation, and mortality are all symptoms of systemic infection, especially in young transplanted plants. Older plant's leaves become yellow and perish [10].

III. RHIZOSPHERE

A variety of abiotic and biotic factors influence plant growth in agricultural soils. The rhizosphere is the thin layer of soil that surrounds plant roots and is an important and active zone for root activity and metabolism [11]. The word 'rhizosphere' was coined by Hiltner to describe the limited region of soil around the roots where root action enhances microbial populations. The original concept has now been broadened to include the soil surrounding a root, where root development and activity have altered its physical, chemical, and biological properties [12]. Ecology is the study of the rhizosphere, which contains a vast number of microorganisms. The rhizosphere is host to a diverse range of microbes, including bacteria, fungus, protozoa, and algae. The rhizosphere is a micro ecosystem in which the rhizobiome, or local microorganisms, interacts with plant roots in a variety of ways. This study looks at how exudates—both diffuse and volatile—play a role in supporting a diverse rhizobiome, as well as the differences between the rhizobiome’s of domesticated plants and wild plants in order to gain insight into how plants and their rhizobiome’s interact [13]. Crop plants are affected by a range of biotic and environmental factors that are detrimental to growth and yield. In order to overcome these negative effects, plants work with the bacteria that reside in their rhizosphere (an area of the soil touched by root exudates) [14].

IV. PLANT GROWTH PROMOTING FUNGI(PGPF)

Plants collaborate with a wide range of microorganisms, including rhizosphere fungi and bacteria. This results in increased vigor in plant growth, and advancement, as well as changes in plant metabolism. Plant growth promoting fungi (PGPF) comprise rhizosphere fungi that enter plant roots and promote plant development. The PGPF are an assortment of non-harmful saprotrophic fungus. Endophytic PGPF dwell inside roots and constantly exchange metabolites with plants, whereas epiphytic PGPF live autonomously on the root surfaces and self-sustaining PGPF live outside of plant cells, suggesting that PGPF induce non-obligatory mutualism in the rhizosphere with a greater spectrum of host plants [15]. As a result, PGPF does not include symbiotic mycorrhizal fungi, which have been shown to benefit plant growth. Furthermore, PGPF is not a member of the same taxonomic group as mycorrhiza. They often interact with plants in a variety of complex ways, developing unique techniques to boost host plant production, growth, flowering, and seed germination [16]. In addition to mediating positive effects on development and growth of plants, PGPF has favorable effects on reducing the growth of phytopathogenic bacteria. Not every PGPF organism will assist the growth of plants in all settings or with all plant hosts. Not every PGPF organism will boost the growth of plants in all settings or with every kind of plant hosts [17]. *Trichoderma* spp. are common necrotrophic mycoparasites found in PGPF biocontrol inoculants, whereas *Sphaerodes mycoparasitica* is an uncommon biotrophic mycoparasite. Not every PGPF organism will assist the growth of plants in all settings or interact with all kinds of plant hosts. Not every PGPF organism will boost the growth of plants in all settings or with every kind of plant hosts [18].

V. *TRICHODERMA*

Trichoderma is a soil-borne fungal species that suppresses plant-pathogenic fungus. Among the several methods are antibiosis, parasitism, fostering host-plant resistance, and competitiveness [19]. *T.asperellum*, *T. harziamum*, *T. viride*, and *T. hamatum* species make up the majority of the biocontrol agents. The biocontrol agent often develops on the root surface in its natural habitat, where it has an impact on root infection but can also help with foliar diseases. *Trichoderma* is a fungi that has evolved to a variety of environmental situations [20]. This is owing to its very extensive metabolism, which can break down a wide variety of substrates and create a wide range of secondary metabolites (SM), the best studied of which comprise peptaibols, polyketides, pyrones, terpenes, and di-diketopiperazines-like compounds. The best examples of secondary metabolites producing *Trichoderma* spp. are *Trichoderma virens* and *Trichoderma viride* [21]. *Trichoderma* is the most thoroughly investigated biological control agent for plant diseases, and it is also widely marketed as a biopesticide, biofertilizer, and soil amendment in many locations. Certain *Trichoderma* strains are able to be utilized for integrated pest management, phytoremediation, and contamination-related bioremediation [22]. *Trichoderma harziamum* biocontrol is a complex process controlled by the synthesis of additional enzymes such as chitinase and protease, as well as secondary metabolites. *Trichoderma reesei* is a mycoparasitic fungus that destroys biomass. *Trichoderma reesei* belongs to the filamentous mesophilic fungus. *T.reesei* anamorph may produce massive amounts of enzymes that breakdown cells (cellulases and hemicellulases) [23].

VI. PGPF AS BIOCONTROL AGENTS

BCA includes naturally occurring compounds (biochemical pesticides), microbes (microbial pesticides), and bacteria (microbial pesticides), and plant-produced chemicals that include genetic material or plant-incorporated protectants in general. Examples of biochemical pesticides include organic acids, plant, and pest growth regulators, extracts of plants, pheromones, minerals, and other compounds [24].

VII. MECHANISM OF BIOCONTROL AGENT BY PGPF

The interconnections between the disease triangle's factors, namely infectious agent, susceptible host, and environment, affect plant disease epidemiology in plants. The correlations between each of these elements reveal the degree and occurrence of the condition. BCAs interact with each of the components of the disease triad. Interactions between BCA and pathogens have shown a diverse set of biological regulatory mechanisms [25].

VIII. ANTAGONISM

1. **Parasitism and Hyper Parasitism:** Parasitism is the interaction of two phylogenetically unrelated species in which one, the parasite, thrives and the other, the 'host,' suffers. Parasitic activity has been shown by *Rhizoctonia solani*, *Pythium* spp., *Sclerotium rolfsii*, and *Sclerotinia sclerotiorum* against a wide range of phytopathogens. *Rhizoctonia solani* causes a number of plant diseases; including rice blight and potato black scurf, and *Trichoderma* spp. is being investigated as a possible BCA for all of these diseases.

2. **Commensalism:** Commensalism is a symbiotic partnership in which one partner gains while the other suffers no consequences [25]. The advantageous organism is referred to as a commensal and receives nourishment and protection from its host species.

3. **Competition:** Competition is an indirect strategy that is crucial in pathogen biocontrol. Biocontrol via competition occurs when nonpathogenic microorganisms fight with pathogens for organic resources in order to multiply and grow in the plant acting as a host. BCA's are superior nutrient absorption systems over phytopathogens [26]. Controlling fusarium wilt by carbon competition. One example is rivalry between pathogenic and nonpathogenic *F.oxysporum* strains.

4. **Systemic Acquired Resistance:** Plants respond to biotic as well as abiotic stress by producing chemical compounds such as glutamate, which starts the plant immune systems. To counteract abiotic as well as biotic stresses, plants employ different types of active defense mechanisms. PGPR and PGPF generate chemical signals that can cause long-term changes in plants, increasing their capacity to endure infection caused by pathogens and increase systemic host defense mechanism against a wide range of pathogens, a process known as induced resistance. SAR and ISR are two types of induced resistance that indicate the plant's defense response against phytopathogens. SAR is a plant's innate resistance capacity that activates in response to elicitors of chemical nature produced from virulent, nonpathogenic microorganisms as well as chemical stimuli made by the plant. The ISR is naturally prevalent in plants and is frequently linked with activation by nonpathogenic plant-related rhizobacteria [27]. The SA-mediated mechanism is unrelated to the ISR and does not require PR proteins. It is specific to certain plants and determined by the genotype of the plant. SA-mediated process is independent to the ISR and does not necessitate the presence of P

5. **Antibiosis:** The interactions between one bacteria and a relatively lesser molecular-weight chemical or by the action of antibiotic, that is harmful to other microorganisms are referred to as antibiosis. Antibiosis substantially aids in the management of plant diseases and disorders.

6. **Siderophores:** All living plants require 14 essential elements, including iron, in along with water, carbon dioxide, as well as oxygen. To completely collect ferric ion in iron-lacking circumstances, the PGPF generates siderophores, which are low-molecular-weight (500-1500 Da) organic compounds. Because of their unique capacity to acquire

iron from their environment, siderophores generating PGPRs are garnering interest. They absorb iron from their surroundings. Producing a iron-siderophores complex that travels by diffusion and reverts to the surface of the cell. Based on their iron-coordinating functional groups, bacterial siderophores are divided into four families: carboxylates, pyoverdines, hydroxamates and phenol catecholates.

7. **Volatile Substances:** Microorganisms in the soil, including PGPR, generate a variety of organic and inorganic chemicals that are volatile which are present. The volatile compounds generated by PGPR inhibited a wide spectrum of phytopathogens, implicating a role in soil-borne disease biocontrol. *Pseudomonas*, *Bacillus*, and *Arthrobacter* volatile compounds, for example, provide greater disease resistance, abiotic stress tolerance, and increased biomass output directly or indirectly. Acetoin and 2, 3-butanediol, both antifungal drugs, are produced by *Bacillus sp*. *Bacillus megaterium* has been identified to produce ammonia, which restricts the development of *Fusarium oxysporum*. Several more investigations on *Pseudomonas* spp. indicated the formation of ammonia with hydrocyanic acid for PGP and biocontrol action.

8. **Lytic Enzyme Synthesis:** The PGPR/PGPF might be able to decrease phytopathogen development and activity by producing various lytic enzymes. PGPR enzymes implicated in the degradation of fungal cell walls which include ACC-deaminase, cellulases, chitinase, lipases, proteases, and -1,3-glucanase. Because the fungal cell wall is largely made up of chitin, glucans, and polysaccharides, bacteria that generate -1, 3-glucanase and chitinase are very effective at inhibiting fungal development. PGPR-mediated lytic enzyme expression can enhance phytopathogen suppression. Chitinase from *S.plymuthica* strain C48,for example, *Botrytis cinerea* germ-tube elongation and spore germination are inhibited. *Botrytis cinerea*, *Sclerotium rolfsii*, and *Fusarium oxysporum* sp. have been found to be inhibited by chitinase generated by *Paenibacillus* sp., *Streptomyces* sp., and *Serratia marcescens.*

IX. ADVANTAGES OF PGPF/PGPR AS BCA'S

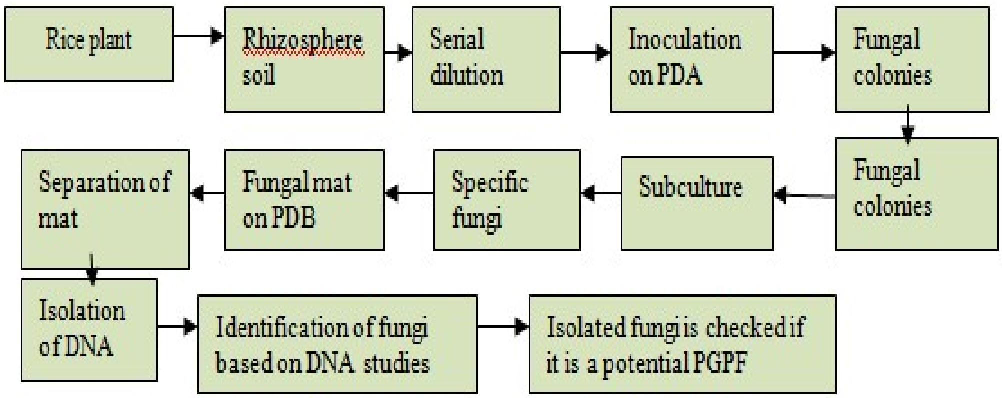
Although agrochemicals and genetic approaches for plant disease management are used as tools, they are not always successful. Furthermore, some agrochemicals are non-biodegradable and have a harmful influence on the ecosystem. Overuse of pesticides for plant disease control has resulted in a rise of pathogen-resistant populations. PGPR has been seen as a promising approach and a long-term means of treating soil-borne diseases and illnesses in this scenario. In numerous places, the use of PGPR and PGPF in environmentally friendly agriculture is growing. As a result of their rhizosphere ability, PGPR with biocontrol the effectiveness often provides long-term defense against soil-borne phytopathogens. In particular the capacity to swiftly penetrate the rhizosphere. For carbon, the PGPR/PGPF mainly relies on the plant's rhizobium stores. The PGPF create antibiotics to protect plants from dangerous microbes, while others act as parasites, pathogens fight with them for room and nourishment. Through ISR, they also protect plants from dangerous bacteria, fungus, viruses, and nematodes. Arbuscular mycorrhiza fungi (AMF) additionally help plants with procurement of resources, disease control, as well as soil contamination along with growth tolerance. AMF has been shown in several studies to be an effective BCA against phytopathogens and nematodes. Using PGPR/PGPF as BCAs minimizes the impact of crop chemicals (fertilizers and insecticides) upon agricultural ecosystems, hence avoiding pollution [28].

X. INTERACTION OF PGPF WITH *XOO*

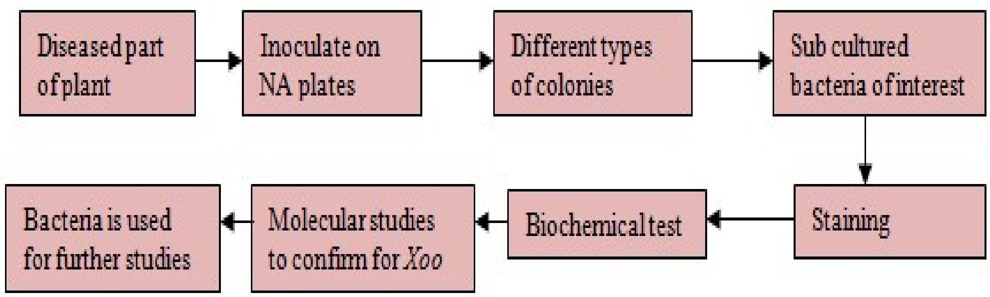
There are several *Trichoderma* strains known to be effective against *X. oryzae* pv.*oryzae* as biocontrol agents in greenhouse and field conditions. *T. atroviridae* increases rice plant development while suppressing Growth *of Xanthomonas oryzae* pv. *oryzae* in vitro, mentioned in a few recent study. *Trichoderma* causes resistance to biotic and abiotic pressures either directly or indirectly through the secretion of specific chemicals, antibiotics, lytic enzymes, and hormones competing for a niche. For instance, *Trichoderma* produces ISR activity produced by strains in the leaves, which has been shown to be effective against several illnesses in tomato plants. *Bacillus* sp. treated rice plants showed resistance to bacterial leaf blight as well as eliciting systemic resistance (ISR). For field testing, talc-based *Trichoderma* powder formulations were used and shown a substantial result in suppressing bacterial blight of rice [29]. *Xanthomonas oryzae* pv.*oryzae* pathogenicity, including systemic signals like as salicylic jasmonic acid (JA) and acid (SA) in both vivo and in vitro. *Trichoderma* Spp. generates systemic acquired resistance (SAR) via SA and induced systemic resistance (ISR) via JA and ethylene (ET) signal pathways, which have to do with the way plant defense mechanisms are activated [30]. Changes in biochemistry, activation of numerous defense-related enzymes, and induction of pathogenesis-related (IR) proteins are examples of these pathways. Peroxidase (PCD) is a fast-responding defense-related enzyme against plant diseases that is specifically engaged in lignification, suberification, polymerization of hydroxyproline-rich glycoproteins, control of cell wall elongation, and pathogen resistance in plants. Catalase (CAT) is an oxygen scavenging enzyme that converts water and molecular oxygen to shield cells from the damaging effects of hydrogen peroxide during development. Polyphenol oxidase (PP) is a plant enzyme that controls nourishment and development and is an essential component of plant protection towards biotic and abiotic stresses. Phenyl alanineammonia-lyase (PAL) shows up to be a critical component of ISR, as it is involved in the biosynthesis of salicylic acid (SA), an important signal that contributes to systemic resistance, as well as the synthesis of several defense-related secondary compounds such as phenols and lignin. *Trichoderma* spp. can thus induce plant resistance via induced systemic resistance (ISR), which boosts activity of plant peroxidase (POD), polyphenol oxidase (PPO), catalase (CAT), and phenyl alanineammonia-lyase (PAL) enzymes in rice leaves, thus improving rice plant growth [31].

XI. METHODOLOGY

A simplified flowchart below depicts the process of obtaining fungus from the rhizosphere of soil-borne plants.



Similarly a simplified flow-chart to show how *Xoo* is isolated and identification is carried out is given below-



1. **Collection and Isolation of Microorganisms from an Infected Rice Leaf:** Sampling of diseased leaves were gathered from a variety of locations across Karnataka and treated to *Xoo* isolation. The sick leaf parts were cut into little pieces. 1% sodium hypochlorite was applied to the surface to sterilize it for one minute, rinsed with distilled water three or four times, then dried with a cloth. The leaf portions were put on nutritional agar and incubated at 35°C for 24-36 hours. Convex, mucoid, and yellow colonies were found on the plates.

2. **Characterization of Isolated Bacteria Biochemically and Molecular Basis:** Morphological, microscopic, biochemical, and molecular tests were used to identify the bacterial isolates. Biochemical testing is performed. Gram's staining, catalase, oxidase, and KOH tests, in addition to, starch hydrolysis, gelatin liquefaction and pectin hydrolysis, were all used. The microbes were identified by molecular characterization. For PCR amplification, universal 16s rRNA primers have been utilized. The sequences were documented, NCBI Gen bank is used to given a unique accession number. The NCBI Genbank is used to validate the *Xoo.*

3. ***Trichoderma* spp. Collection and Separation from Rhizosphere Soil:** Soil samples from rice crop rhizosphere were collected from numerous paddy fields. By raising the plant up whilst the dirt was still adhered to the roots, a total of five of rhizosphere soil were collected. Samples of soil were kept in sealed containers at 4°C until they could be

used. The soil samples from the rhizosphere were further diluted onto various concentration solutions, agitated, and 0.1ml of the the supernatants were transferred onto PDA media plates and cultivated at 28°C for 7 days. Colonies were separated from the plates and reinoculated on new PDA petriplates. After 7 days of subculturing, individual spore colonies were formed by incubating the media plates at 28°C, Fungi colonies were also studied.

4. ***Trichoderma* Spp. Visual and Biological Characteristics:** Fungi were identified using standard guidelines based on cultural and microscopic characteristics. The microbial samples containing genomic DNA genomic isolation kit was used to isolate the DNA. Standard primers of 18s long were used for PCR amplification. The sequences were entered into the NCBI Genbank and assigned an accession number. The NCBI program was used to identify *Trichoderma* spp.

5. **Plant Growth Boosting Fungal Strain *Trichoderma* Spp. were Tested in Vitro. for Antibacterial Activity Towards *Xanthomonas Oryzae* Pv.*Oryzae* using the Agar Plug Technique:** The agar plug method is used to test microbial antagonism. The interaction of antagonism involving *Trichoderma* spp. and *Xoo* is explored here. Trichoderma spp. plant growth stimulating fungal strains were cultivated on PDA petriplates for 5 to 7 days at the ambient temperature with alternate light and dark phases. A sterilized 5 mm cork borer was utilized to puncture the fungal discs taken from a seven-day-old culture. The fungi were grown on a Muller Hinton culture plate previously swabbed with the bacterium *Xoo.* If there are clear zones in the MHA plate, this indicates that an adversarial interaction has occurred.

XII. CONCLUSION

The use of efficient microbes as inoculants for biocontrol and biofertilization is expanding right now as a result of the ongoing need to create sustainable agriculture and conserve the environment while decreasing the use of fungicides and fertilizers in the field of agriculture. Seed or seedling preparations utilizing combination bio-fertilizers and biocontrol techniques have been employed to boost crop development while reducing negative environmental consequences. *Trichoderma* spp. are beneficial to the environment because they stimulate plant development by improving nutrient delivery, decreasing plant diseases, and increasing plant defense. Biogenic volatile organic materials are a diverse group of biochemical substances produced by fungi and other bacteria. Only lately has the beneficial impact of VOCs in promoting plant growth and biocontrol been acknowledged. VOCs, or volatile organic compounds, generated by common fungi are important in protecting ginseng plants from mycopathogens. Their discoveries might aid in the creation of bio-pesticides for the ecologically friendly management of crop diseases, as well as the potential application of *Trichoderma*'s antibacterial components. They offered real-world instances of biodegradable, naturally existing antifungal chemicals derived from *Trichoderma* that may be employed right away. As biocontrol agents, PGPF might be an essential part of an integrated agriculture system for management. The survival of microorganisms during storage, on the other hand, is a critical issue in inoculant technology, and several factors must be considered, such as the growth medium, the physiological condition that exists for the microorganisms when extracted, the procedure for dehydration, the pace of drying, the temperature of storage, and the water activity of the inoculums. All of these conditions reduce the usability of inoculants to three to six months in standard storage conditions. Consequently, research into extending the shelf life of inoculants or developing novel carrier inoculant compositions is becoming more and more significant. Particularly, biotic and abiotic variables can impact a variety of processes and inhibit interactions among plants and helpful fungus, resulting in suboptimal performance in terms of boosting plant development and disease prevention. More research on plant-microbe interactions and their effects in various ecologies and geographies is required to understand the relationships between PGPF and plants, especially in the case of using it in novel habitats. Native micro flora are chosen for boosting crop yield since they adapt better to their surroundings than imported strains. The majority of scientists want to create rhizosphere microbial strains which are more adaptable to diverse crops and can stimulate plant development in a range of settings.

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