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Protective Fusarium spp. in plant growth promotion: A novel approach

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Abstract

Among the soil fungi, *Fusarium* is emerging as a potential alternative for plant growth promotion by virtue of its several plant growth promoting traits in the recent times. The fungus shows enormous diversity in soil and can also successfully combat against several soil borne plant pathogenic microbes, especially the fungal pathogens. The role of *Fusarium* as an effective biocontrol agent in a number of plant diseases is well known. A lot of non-pathogenic *Fusarium* spp. have been blessed with several plant growth promoting traits *viz.*, phytohormones production, phosphate solubilization, siderophore production, ammonia production etc. In addition, control of certain plant diseases like vascular wilt has been made possible by protective *Fusarium* spp. in both green house and field trials. Hyphal parasitism, mycolysis, antibiosis and competition for food with the phytopathogens have been shown to be the effective mechanisms for controlling such diseases. Thus, these non-pathogenic and protective *Fusarium* spp. could turn out to be the best candidates for improving agricultural output in near future.

Keywords: Fusarium, plant growth, biocontrol, phytohormone, phosphate solubilization, siderophore, phytopathogen, plant disease, agriculture

Introduction

Tropical fungi are a major component of biodiversity, essential to the survival of other organisms, crucial in global ecological processes, a source of novel bioactive compounds, a source of biocontrol agents, a source of plant pathogens, a threat to human health, able to contribute to sustainable development, a part of human culture, and are there...' – [26]

Soil microorganisms including fungi are central to providing plants with minerals and other important metabolites. The saprophytic fungi could also augment the growth of other beneficial microbes in the soil and reduce the proliferation of the potential soil borne pathogens by way of secreting a number of antimicrobials in the immediate environment surrounding the rhizosphere. They may compete with the plant pathogens for the nutrients and space to get established in the environment and induce systemic resistance in plant. Similar to plant growth promoting rhizobacteria (PGPR), some non-pathogenic rhizospheric fungi were reported to promote plant growth upon root colonization and were functionally designated as 'plant growth promoting fungi' (PGPF) [30]. They were potentially applied in agriculture as biostimulator, biofertilizer and/or biocontrol agents. As biostimulator, they were reported to synthesize hormones such as indole acetic acid (IAA) and gibberellin (GA) and transport these in plants [41]. Some PGPF were reported to produce enzyme, 1-amino cyclopropane-1-carboxylate deaminase for lowering the action of ethylene in the plants. Arbuscular mycorrhizal fungi (eg., Glomus spp.) have a wide application in the agricultural fields as biofertilizer since they stimulate plant growth through increased uptake of nutrients. Several PGPF were reported to solubilize inorganic phosphates making these available to plant. They were also found to secrete siderophore under iron deficiency to meet their iron requirement and also supply iron to the plant. Siderophore was also found to be crucial in suppression of diseases caused by plant pathogens with

inefficient iron uptake system. Biocontrol agents exhibited plant growth promotion via indirect mechanisms. They protect plant health through their antagonistic activity to reduce the deleterious effects of plant pathogens on crop yield by producing antibiotics, cell wall degrading enzymes such as chitinase, and the ability to induce systemic resistance. Competition between pathogenic and biocontrol strains for space and/or nutrients on the rhizospheric region was also reported to be important for biocontrol activity [39]. The genus Fusarium has earned a lot of notoriety with regards to its pathogenecity. Some species, such as F. graminearum and F. verticillioides, have a narrow host range, infecting predominantly the cereals. By contrast, F. oxysporum has a remarkably broad host range, infecting both monocotyledonous and dicotyledonous plants. Common plant diseases caused by Fusarium spp. include vascular wilt, root rot, crown rot, head blight, scab, canker, abnormal growth and decay on vegetables, wood, herbs, and ornamental plants. Out of 101 economically important plants over 81 plants had a report of at least one associated Fusarium disease [42]. The most disastrous disease caused by Fusarium species in agricultural history throughout the world was the Panama disease of banana by F. oxysporum f. sp. cubense in 1960 at Panama causing serious loss in banana plantation and the industry. In addition, there are a number of species which are responsible for opportunistic infections of humans, especially the immuno-suppressed and other animals. The mortality rate for human patients with systemic Fusarium infections was reported as more than 70%. Many Fusarium species also produce secondary such as mycotoxins metabolites, (zearalenones, trichothecenes, fumonisins, and moniliformin), as well as phytotoxins (fusaric acid) problems. These mycotoxins may cause allergic or carcinogenic symptoms in long term consumption of mouldy feed on humans and animals, whereas fusaric acid leads to wilting and stunting of plants.

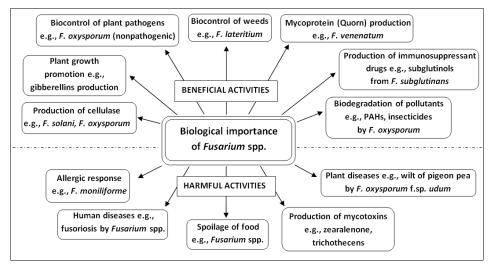


Fig 1: Biological activities of different Fusarium species

Besides this negative impact, many Fusarium species have been reported to be exploited for the benefit of plants and human beings (Fig. 1). They may be used as the source of cellulase, production of mycoprotein and immunesuppressant drugs, for mycoremediation, plant growth promotion and biological control of plant pathogens and weeds. The ability of the saprophytic Fusarium species to improve plant growth is comparatively underexplored. But of late, there are increasing number of evidences where Fusarium isolates have been found to be responsible for the overall growth promotion of the agricultural crops. Nonpathogenic Fusarium species have also been reported to produce a variety of plant growth promoting metabolites, viz., IAA, gibberellin, siderophore, ammonia etc. Few Fusarium species are also potential phosphate solubilizers. A number of Fusarium species have been documented which are effective metal scavengers and exploited for mycoremediation in metal polluted sites. There are also reports of controlling of Fusarium wilt by using nonpathogenic species of Fusarium [39].

Four plant associated Fusarium species viz., F. proliferatum, F. verticillioides, F. fujikuroi, and F. oxysporum were identified for their tryptophan dependant IAA biosynthesis [65]. Also, F. moniliforme was found most effective fungus for the production of gibberellin. GA₃ was also reported to be produced by F. oxysporum, F. avenaceum, F. graminearum, F. solani, F. semitectum, F sacchari, F. konzum and F. subglutinans [64]. An endophytic fungus, Fusarium verticillioides RK01 had been reported to increase phosphate solubilization and benefit the growth of soyabean plant. Fusarium isolates belonging F. roseum and F. oxysporum were found to potential in the production of iron chelator known as siderophore which was involved in pathogen suppression. Fusarium isolate JDF12 produced siderophore and exhibited antifungal activity against a number of phytopathogens [14]. It was observed that some nonpathogenic strains of Fusarium oxysporum can control Fusarium diseases responsible for severe damages in many crops and thus can act as potential biocontrol agent [19]. These non-pathogenic attributes of the Fusarium soil isolates which significantly influence plant growth and developments could be exploited to increase crop productivity. This will eventually reduce the rampant use of chemical fertilizers in agricultural fields and minimize their detrimental effects on our ecosystem.

Fusarium spp. as biological control agents against plant pathogens

The possible role of nonpathogenic Fusarium spp. for suppression of Fusarium wilt suggested that presence of a large population of Fusarium spp. resulted suppressive nature of the soil which might be disappeared after elimination of Fusarium by heat treatment and reappeared after reintroduction of the fungi in to the heat-treated soil. Later on, several studies have clearly showed that nonpathogenic Fusarium spp., especially F. oxysporum have some protective role for biological control of Fusarium wilt in numerous crops in green house and field trials in different area of the world [35]. Scientists preferred to use the term 'protective' rather than 'nonpathogenic' [3] since these protective strains were either 'true nonpathogenic' or 'pathogenic' to specific crop, but showed their protective role when applied to a nonhost plant for control against further infection by its specific pathogen (forma speciales). This phenomenon was first described in 1971 [47] and defined as 'cross-protection' or 'premunition'. Besides Fusarium wilt, protective F. oxysporum showed their efficacy against other fungal pathogens including Pythium ultimum, Phytophthora capsici and Verticillium dahlia [3]. Moreover, some endophytic strains of nonpathogenic F. oxysporum were reported to reduce damage caused by Meloidogyne incognita in tomato roots. F. oxysporum strain Fo47 recovered from Fusarium wilt suppressive soil in France has been studied extensively for its bioprotective activity. Sometimes use of nonpathogenic Fusarium isolates can be more effective than other biocontrol agents. For example, Fusarium wilt of tomato has been more effectively controlled by nonpathogenic isolates of F. solani than the biocontrol agents such as Burkholderia, Gliocladium, Pseudomonas and Trichoderma [39]. In hydroponic system non-pathogenic Fusarium oxysporum F221-B reduced incidence and severity of lettuce root rot and wilt (caused by F. oxysporum f.sp. lactucae F442-G), about 60-80% compared to the inoculated control and significantly promoted the growth of three lettuce varieties [62]. Preinoculation of Glomus mossaea and F. equiseti GF18-3 resulted effective control against cucumber mosaic virus [21]. Species of Fusarium used for biocontrol of plant pathogenic fungi is shown in the Table 1.

Fusarium spp.	Pathogens/ diseases	Reference	
F. solani	Phomopsis sclerotioides, Mucor spinosus, Fusarium wilt of tomato	[17]	
F. proliferatum	Plasmopara viticola	[22]	
F. sacchari	Fusarium wilt of pigeonpea	[10]	
F. chlamydosporum	Ground nut rust (Puccinia arachidis)	[45]	
F. lateritium	Sclerotinia sclerotiorum on lettuce, and Eutypa spp. on grapes and apricots	[46]	
F. oxysporum	Fusarium wilt of cucurbits	[23]	
F. equiseti	Fusarium crown and root rot of tomato, cucumber mosaic virus (CMV)	[29, 21]	

Table 1: Fusarium species for biocontrol of plant pathogenic fungi

Data in the table were obtained and updated from Leslie & Summerell, 2006

Factors affecting bioprotective activity of nonpathogenic Fusaria

- 1. Plant protection depends on the population density of the biocontrol agent or more precisely on the ratio of pathogen versus protective strain [39].
- 2. The protective strains are usually more effective when they are applied a few days before inoculation of the pathogen.
- The protection is often improved by associating the strains with rhizobacteria, especially fluorescent pseudomonads.
- 4. The efficacy of biological control conferred by protective strains of F. oxysporum depends on environmental conditions, especially soil type [40].

Mechanisms of action of non-pathogenic Fusarium spp. as biocontrol agents: The different mechanisms of antagonism occur across a spectrum of directionality related to the amount of interspecies contact and specificity of the interactions (Fig. 2). Direct antagonism results from physical contact and/or a high-degree of selectivity for the pathogen by the mechanism(s) expressed by the biocontrol agents whereas indirect antagonisms result from activities that do not involve sensing or targeting a pathogen by the biocontrol agents. It is also apparent that antagonism often involves the synergistic action of several mechanisms.

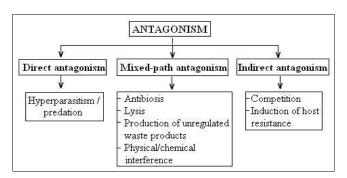


Fig 2: Types of interspecies antagonisms leading to biological control of plant pathogens [15]

The activation of systemic acquired resistance in host plants and competition between pathogenic and biocontrol strains for nutrients and/or space on the roots of the hosts are the two mechanisms reported to be associated with biocontrol activity of nonpathogenic *Fusarium* [23, 10]. Direct microbial antagonism for competition of same nutrients may lead to hyperparasitism, antibiosis and mycolysis [1].

Nonpathogenic *Fusarium* spp. colonize in the epidermal and cortical tissue of root and secrete some secondary metabolites (eg., fusaric acid) at a low concentration which may elicit a general cascade of nonspecific defense responses in plant. Typical defense reactions such as wall

appositions, intercellular plugging and intracellular osmiophilic deposits were frequently observed. Biocontrol strains of F. oxysporum increased peroxidase (POX) and phenylalanine ammonialyase (PAL) activities and lignin deposition in asparagus [27] and the content of ferulic, caffeic and vanillic acids in tomato. The protective strain Fo47 induced increased activities of chitinases, β-1,3-glucanase, β-1,4-glucosidase, peroxidase and PR1 in tomato [9, 24]. A thermolabile compound produced by nonpathogenic F. oxysporum 1012 was associated with the induction of resistance against Fusarial wilt of *Ipomea tricolor*. Vascular occlusion induced by a nonpathogenic F. oxysporum resulted suppression of Fusarium wilt of cotton due to limited movement and reduced population of the pathogen within vascular tissue [28]. The nonpathogenic Fusarium oxysporum, strain Fo47 was able to trigger plant defense reactions in pea [4].

Saprophytic competition for the carbon sources in soil and rhizosphere was reported to be one of the mechanisms for biocontrol activity of nonpathogenic F. oxysporum strains such as CS1, CS20, C5, C14 [39, 50]. In vitro growth inhibition of F. oxysporum f. sp. dianthi WCS-816 by the strain Fo47 was due to competition for carbon sources [38]. Parasitic competition for the infection sites at the root surface was evidenced by another mechanism for suppression of pathogen by nonpathogenic F. oxysporum strains. The strain Fo47 was reported as an effective competitor for the pathogen at the apex of flax root and reduced both colonization rate and activity of pathogen in the flax root tissue [18]. Competition for infection site at the cucumber rhizosphere with F. oxysporum f.sp. cucumerinum was demonstrated in soil infested with nonpathogenic strain C5 [44]. Whatever the mechanisms involved, these protective strains offer a unique opportunity to develop biological methods to control *Fusarium* induced diseases [3].

Formulations of protective Fusarium spp.: It involves production of final product by mixing the microbial component with different carriers and adjuvants for better protection from environmental conditions, greater survival of the biological agent, controlled rates, improved bioactivity, storage stability, easy to handle and deliver at the site of action. A formulation made of microgranules enriched with food base, provided better survival and efficacy than the traditional talc and charcoal based formulation. Regarding their physical state, biopesticide formulations can be divided into liquid and dry formulations. The formulation can be applied as seed treatment and soil dressing. The commercial formulations of nonpathogenic strains are available for the control of Fusarium wilt (Table 2).

Table 2: Specification of two commercial formulations based on protective *F. Oxysporum*

Trade name	Biofox C	Fusaclean
Target pathogens	F. oxysporum, F. Moliniformae	F. oxysporum,
Crops	Basil, carnation, cyclamen, tomato	Asparagus, basil, carnation, cyclamen, tomato, Gerbera
Formulations	Dust or alginate granules	Liquid formulation
Methods of	Seed treatment, soil	Soil drench as
application	drench	potting mixture
Country registered	Italy	France
Manufactures &	SIAPA	Natural Plant
suppliers	SIAFA	Protection

Antibiotic production by *Fusarium* **spp.:** Different *Fusarium* species were reported to produce antibiotics (Table 3) and this property of nonpathogenic Fusaria remains unexplored for their bioprotecive activity.

Table 3: Antibiotic production by *Fusarium* spp. [37]

Antibiotics	Fusarium spp.	Activity
Avenacin, Fructigenin Sambucinin	F. avenaceum, F. fructigenum, F. sambucinum	Antibacterial specially <i>Mycobacterium</i> spp.
Fusarubin	F. javonicum, F martii, F. solani	Gram negative bacteria
Fusarinic acid	F. fujikuroi, F. vasinfectum	Inhibits germination of spores of <i>Ustilago zeae</i>
Fusanin	F. oxysporum	Active against Gram-positive (fusanin A), Gram-negative (fusanin B) bacteria
Lycomarasmin	F. lycopersici	Inhibits growth of Lactobacillus casei
Poine	F. sporotrichiella var. poae	Active against streptococci, staphylococci
Enniatins ^a	F. tricinctum	Antibacterial, antiprotozoan

Production of Indole-3-acetic acid (IAA) by Fusarium Indole-3-acetic acid (IAA) is a major plant auxin that stimulates both rapid (e.g., increases in cell elongation) and long-term (e.g., cell division and differentiation) responses in plants. It has also role in root initiation and better development of roots, thus increasing the absorptive surface of plant roots for uptake of water and nutrients. The hormone can be synthesized by the plants themselves but also by diverse soil microorganisms including fungi. IAA was first detected in culture filtrates of Rhizopus suinus by K.V. Thimann in 1935 [16]. Tryptophan is the key precursor for biosynthesis of IAA in plants and microorganisms, and application of exogenous tryptophan increases IAA production. Root exudates are the main sources of tryptophan in soil. Several biosynthetic pathways for IAA production exist, sometimes in parallel in the same organism [16]. There are four intermediate pathways for production of IAA from tryptophan (Fig. 3): (i) via formation of indole-3pyruvic acid and indole-3-acetaldehyde which was reported in majority of microorganisms such as bacteria (Agrobacterium, Azospirillum, Pseudomonas), cyanobacteria (Nostoc), yeast (Sacchromyces uvarum), fungi (Fusarium, Rhizoctonia, Colletotichum); (ii) via tryptamine formation; (iii) via indole-3-acetamide (IAM); and (iv) via acetonitrile formation. The enzyme tryptophan-2-monooxygenase (IaaM) converts tryptophan into IAM which is further converted into IAA by the action of IAM

hydrolase (IaaH). Putative homologs of the bacterial (A. tumefaciens and Rhizobium spp.) IaaM and IaaH genes were identified from four Fusarium species (F. proliferatum, F. verticillioides, F. fujikuroi, and F. oxysporum). Tryptophan independent biosynthesis of IAA was reported in Azospirillum [11], aerobic methylobacteria, viz., Methylobacterium mesophilicum, Aminobacter aminovorans, Paracoccus kondratievae, Methylovorus mays [31], Anabaena.

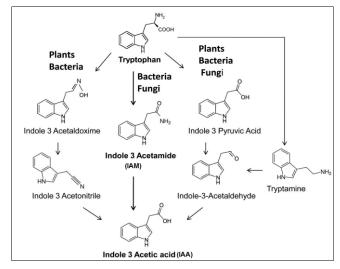


Fig 3: Tryptophan-dependent IAA biosynthetic pathways

Production of Gibberellin (GA) by Fusarium: GA controls many aspects of plant growth and development including seed germination, seedling emergence, stem and leaf growth, floral induction and sex expression in plants [13, ^{16]}. Historically, gibberellin was discovered in the culture filtrate of Gibberella fujikuroi (anamorph Fusarium moniliforme), the causal agent of 'bakanae' or 'foolish seedling' disease of rice. In 1926 Kurosawa identified the substance and Yabuta in 1931 partially elucidated its chemical structure and called it as gibberellin A. Chemically, all gibberellins are tetracyclic diterpenoid carboxylic acids, being defined by their chemical structure based on the ent-gibberellane carbon skeleton and assigned gibberellin 'numbers' depending on chronological order of their identification. There are, at least, 136 types of GAs identified from higher plants (128 species), 28 GAs from fungi (7 species), and only four GAs from bacteria (Joo et al., 2005). Nevertheless, only a small number of them, such as GA₁, GA₃, GA₄ and GA₇ are prominent bioactive. Detailed characterization at chemical. biochemical and genetic levels for GA_3 biosynthesis in F. fujikuroi has been reported. The pathway consists of two early cyclization reactions from geranyl geranyl diphosphate to ent-kaurene via ent-copalyl diphosphate, followed by multiple oxidative steps to give final 19-10 γ-lactone product GA₃. In fungi, the GA biosynthesis genes are found on one chromosome, but in plants, they are found randomly on multiple chromosomes [36]. Plants produce low amount of GA₃, therefore microorganisms have been utilized for commercial production of GA₃. The fungi are not dependent on GAs for their development but produce and secrete large quantities of the compounds to modify the behavior of their host or as signaling factors towards the host plant [6]. Besides F. fujikuroi, GA production had also been reported from other species of Fusarium such as, F. solani, F.

oxysporum, F. pallidoroseum F. proliferatum, F. sacchari, F. semitectum, F. subglutinans and F. verticillioides [5], other fungi including the genera of Aspergillus, Penicillium, Neurospora, Cladosporium and bacterial genera belonging to Azotobacter, Azospirillum, and Pseudomonas [33].

Siderophore production by Fusarium: Iron is essential for the growth of almost all organisms. It is required in metabolic processes such as TCA cycle, electron transport chain, oxidative phosphorylation and photosynthesis. Being a component of cell, iron deficiency can cause growth inhibition, decrease in RNA/DNA synthesis, inhibition of sporulation and change in cell morphology. Though it is the fourth most abundant element comprising 4.7 % of the earth's crust availability of iron in aerobic environment at biological pH is very low. Under such conditions it tends to precipitate forming oxyhydroxide polymers. The solubility product constant for ferric hydroxide is about 10⁻³⁸. At neutral pH the free available iron is at a concentration of less than 10-17M, which is far below that required for microbial growth (10⁻⁹M). Under conditions of iron starvation, microorganisms compete with other ironrequiring microorganisms through activation of high affinity iron uptake system. One component of the system is the siderophore (G. iron bearer) which is a relatively low molecular weight, ferric ion specific chelating agent released extracellularly to bind with Fe3+ [48]. Other component of the system is the specific membrane receptors that recognize and internalize the ferri-siderophore complex within the cell, and iron is released intracellularly from the complex by ferric reductase for the use of the cell [8]. Regulation of the siderophore production is based on the concentration of iron in the environment. So, siderophore production is shut off when iron is present at sufficient concentration and vice versa [12]. Although considerable structural variation exists among the several dozen siderophores chemically characterized, most can be classified as hydroxamates or catechols. Fungal siderophores are generally of the hydroxamate in nature which are typically composed of three hydroxamate groups linked by peptide or ester bonds to form an octahedral complex. They belong to either of three types: ferrichrome, coprogen, fusarinine. Fusarium roseum strain ATCC 12822 (synonym of F. graminearum) produced malonichrome, a ferrichrome siderophore, specifically, contains a cyclic hexapeptide with one alanine, two glycines and three AHOs, in which the hydroxylamino groups are acylated with malonic acid (Emery, 1980). F. oxysporum strain FGSC 9935 reported to produce three different ferrichrome

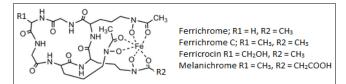


Fig 4: Basic structure of Ferrichrome

type siderophores: ferricrocin, ferrichrome C, and malonichrome [43] (Fig 4). Siderophore mediated suppression of soil borne plant diseases are one of the most studied mechanisms involved in biocontrol by fungi. Moreover, siderophore has been reported to induce the biosynthesis of other antimicrobial compounds by

increasing the bioavailability of iron and other minerals which would suppress the growth of pathogenic microorganisms ^[25]. Alternatively, it also stimulates plant growth by increasing the availability of iron in the soil surrounding the roots.

Solubilization of unavailable phosphate by Fusarium

Phosphorus (P) is one of the most essential macronutrients required for growth and development of plant. It exists in two forms in soil, as organic and inorganic phosphates. However, approximately 95–99% of soil phosphorus is present in the insoluble form and hence cannot be utilized by the plants. The problem of P deficiency is generally alleviated through the application of P fertilizers. But after application, a large proportion of fertilizer phosphorus is quickly bound to metal cations to from insoluble inorganic salts ^[49]. Moreover, repeated and injudicious applications of chemical P fertilizers lead to the loss of soil fertility by disturbing microbial diversity, and consequently reduces yield of crops.

Alternatively, many soil microorganisms are effective in releasing P from inorganic P through solubilization and from organic pools of total soil P by mineralization [7]. Several theories have been proposed to explain the mechanisms of microbial solubilization of P. Broadly these theories have been categorized into three groups: (i) the organic acid theory, (ii) the sink theory, and (iii) the acidification by H+ excretion theory [34]. In the well recognized and accepted organic acid theory, the insoluble sources of P are solubilized by P-solubilizing organisms either by: (a) lowering the pH, or (b) by enhancing chelation of the cations bound to P. A variety of organic acids such as lactic acid, maleic acid, malic acid, acetic acid, tartaric acid, citric acid, fumaric acid and gluconic acid have been reported to produce by a group of soil fungi including F. oxysporum to solubilize inorganic phosphate [2]. Other organic acids involved in the P solubilization are αketogluconic acid, glycolic acid, oxalic acid, succinic acid, and propionic acid. In the sink theory, P-solubilizing organisms remove and assimilate P from the aqueous medium and hence, activate the indirect dissolution of calcium phosphate compounds by consistent removal of P from broth culture medium. Mineralizations of most organic phosphorous compounds were carried out by production of non-specific phosphatases, enzymes: (i) dephosphorylate phospho-ester or phosphoanhydride bonds of organic matter, (ii) phytases, which specifically cause release of P from phytic acid, and (iii) phosphonatases and C-P lyases that cleave the C-P of organophosphonates. Scientist observed that increased phosphate solubilization by F. verticillioides RK01 was due to increasing activities of acid phosphatase, alkaline phosphatase and fungal biomass [52]. Developing fungal inoculants with high phosphatase and phytase activity would be of great practical interest for augmenting plant nutrition and reducing P pollution in soil.

Conclusion

The identity of *Fusarium* as a potential plant pathogenic fungus is well established. Still, it is one of the most diversified fungal microbes on earth which offers new opportunities to the researchers to unravel the unexplored traits of the fungus. There are a number of aspects of the fungus which have not been explored till date or rather

underexplored. The PGPF features of *Fusarium* are the emerging areas of interest to the plant pathologists of late. The findings of the present investigation highlighted that the *Fusarium* spp. have great potential to enhance soil fertility and plant growth promotion. These non-pathogenic, protective *Fusarium* soil isolates could be exploited as seed inoculants for improvement of crop yield in agricultural fields and also as potential bio control agents against soil borne phytopathogens. However, this assessment of plant growth promotion by the protective *Fusarium* isolates needs further study under field conditions to confirm the present findings and also to recommend the isolates as bioinoculants.

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