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Potential of Biocontrol Agents in Plant Disease Control for Improving Food Safety

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ABSTRACT

Plant disease control is mainly based on extraneous application of pesticides to improve agriculture productivity. However, only a part of applied pesticides is used for killing of pathogens and pests. Large part of applied pesticides remains either as residual pesticide or gets volatilized or leached resulting in ecological and environmental problems, and human health hazards. The increased consumer demands for safe food have invigorated research on development of safe and ecofriendly biopesticides. The use of microorganisms for biological control of pests is considered as a pragmatic approach which can drastically lessen the adverse outcomes of agrochemicals in soil. Rhizospheric microorganisms isolated from various crops produce different antagnostic compounds and inhibit the growth of various phytopathogens and insect pests. Moreover, in several plants, hormones like salicylic acid, jasmonic acid and ethylene contribute towards induction of both, systemic acquired as well as induced systemic resitance. In this article, antagonistic rhizosphere microorganisms have been explored for control of phytopathogens. Further, recent advances in field of biopesticides using rhizosphere microorganisms under field conditions is discussed for improving crop productivity in sustainable agriculture

Keywords: Rhizosphere microorganisms; Plant diseases; Phytopathogens; Pesticides; Biocontrol agents; Biopesticides

1. INTRODUCTION

Diseases caused by plant pathogens adversely affect global crop productivity and account for 20-40 per cent yield losses annually in various cereal and legume crops[1]. In India, 57,000 metric tonnes of synthetic pesticides were used during the 2016-17 to control the plant pathogens and insect pests, whereas the amount of biopesticide consumption was only 6340 metric tonnes. The development of resistance due to continuous use of pesticides in modern farming and increased availability of pesticide residues in vegetables, cereals and grains has generated many problems. Moreover, the unregulated and indiscriminate use of chemical pesticides causes pollution of soil, water and air alongwith decrease in the soil microflora and fauna. Beneficial rhizosphere microorganisms could be exploited to provide sustainable solutions in reducing the application of pesticides for agricultural crop production[2]. Biopesticides offer several advantages including complete biodegradability and water solubility over traditional chemical/synthesised pesticides. Thus, microorganisms and plant-based biochemicals (Fig. 1) provide a safe alternative option for plant disease suppression in agriculture system[3].

Many beneficial microorganisms including bacteria, algae and fungii around plant root system constitute a complex microbial community termed as the rhizosphere microbiome[4]. These rhizosphere microorganisms interact with the plant roots and enhance the plant growth by improving acquisition of mineralised nutrients, vitamins, auxins and gibberllins, by inhibition of plant pathogens and also through stress tolerance under field conditions[5]. Recently, several bacteria, fungi, actinomycetes, protozoa and nematodes have shown the antagonistic activity which can be used in biocontrol of root and foliage related diseases of several crops[6,7]. The application of these specific antagonistic microorganisms and biopesticides in biological control of soilborne pathogens has been studied intensively in the last two decades. Furthermore, the understanding of the interactions of plants with beneficial microbial communities is increasingly relevant in the context for soil health and ecosystem functioning improved crop productivity to meet increased demand for food by an expanding human population and to

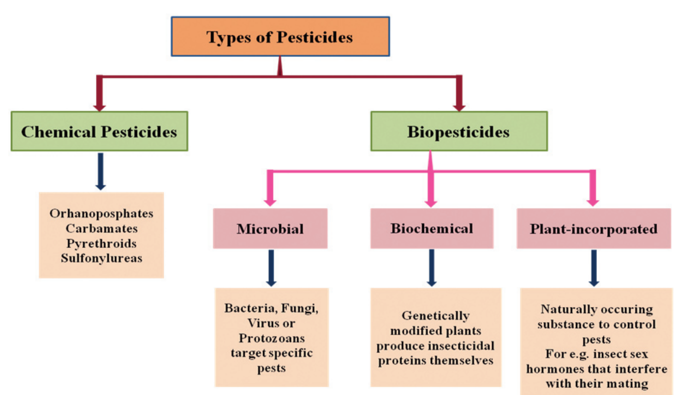

Figure 1. Categories of pesticides used for control of phytopathogens and insect pests.

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minimise the application of pesticides for the control of plant pathogens.

2. RHIZOSPHERE MICROBES INVOLVED IN BIOCONTROL OF PLANT DISEASES

Rhizophere harbours an extremely complex microbial community and includes saprophytes, epiphytes, endophytes, pathogens as well as many beneficial microorganisms[4,8]. Bacteria are the predominant component of the rhizosphere colonizing microbial population, however several fungi, acticomycetes, protozoa and algae (Fig. 2) are also found in the rhizosphere[9]. The bacterial population possessing the biocontrol as well as plant growth promoting activities[10,11] includes various species of *Azospirillum*, *Alcaligenes*, *Arthrobacter*, *Azotobacter*, *Bacillus,* *Burkholderia*, *Bradyrhizobium*, *Enterobacter*, *Flavobacterium*, *Klebsiella*, *Mesorhizobium*, *Pseudomonas*, *Rhizobium*, *Rhodococcus* and *Serratia*, *Streptomyces*, etc. Rhizosphere fungi viz. *Beauveria*, *Metarhizium, Lecanicillium*, *Talaromyces, Trichoderma* and *Verticillium* species also play prominent roles in antagonising pathogens and insects[12].

Several phytopathogens cause severe diseases in various crops and reduce the plant biomass and crop yields. Moreover, food quality is also compromised by infestation with phytopathogens resulting in the huge economic losses[13]. Rhizosphere microorganisms having antagonistic activities have agricultural implications in biocontrol of several plant diseases caused by pathogens and pests[14]. Various strains of rhizobacteria such as *Agrobacterium*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Klebsiella*, *Pseudomonas,* *Rhizobium* and *Serratia* have been used successfully as biocontrol agents for disease protection in several agriculturally important crops[15,16]. Some microorganisms also play a key role in crop protection by acting as biotic elicitors against different biotic and environmental factors[17] and may provide resistance to insects and control various plant diseases[18]. The microorganisms produce sevral compounds which help them to adapt in a new environment as well contribute towards plant growth promotion and disease suppression. Rhizosphere microorganisms suppress the growth of phytopathogens by production of antibiotics, hydrolytic enzymes, cyanide, bacteriocins, siderophores and by induction of systemic resistance[14,19].

Biopesticides can be categorised according to their source (structure) and mechanism by which they mitigate or kill the pathogens and pests. They control pests by different modes of action i.e. by producing pest specific toxic metabolites that prevent establishment of pathogenic microorganisms for causing disease[20]. For instance, heat and protease tolerant endotoxin is produced by *Moraxella osloensis* associated with *Phasmarhabditis hermaphrodita,* which control mollusk pests (slug parasitic nematode)[21]. Most widely used bacterium *Bacillus thuringinesis* produces an endotoxin during spore formation and causes lysis of gut cells when consumed by insects. *Agrobacterium radiobacter* is used to control crown gall. *Beauveria bassiana* or *Metarhizium anisopliae* are used against spittlebugs of sugarcane and grasslands. *Trichoderma harzianum* is another important fungal biocontrol agent used against *Fusarium*, *Pythium* and other soil borne pathogens[22]. Viral biopesticides are host-specific; infecting only one or a few closely related species. These bacteriophages can be used as pesticide if they can attack bacteria that cause plant disease[20]. Baculovirus are enveloped viruses and are insect specific. The viral biocontrol agents *Cydia pomonella* GV (CpGV) control the codling moth on fruit and crop plants[23].

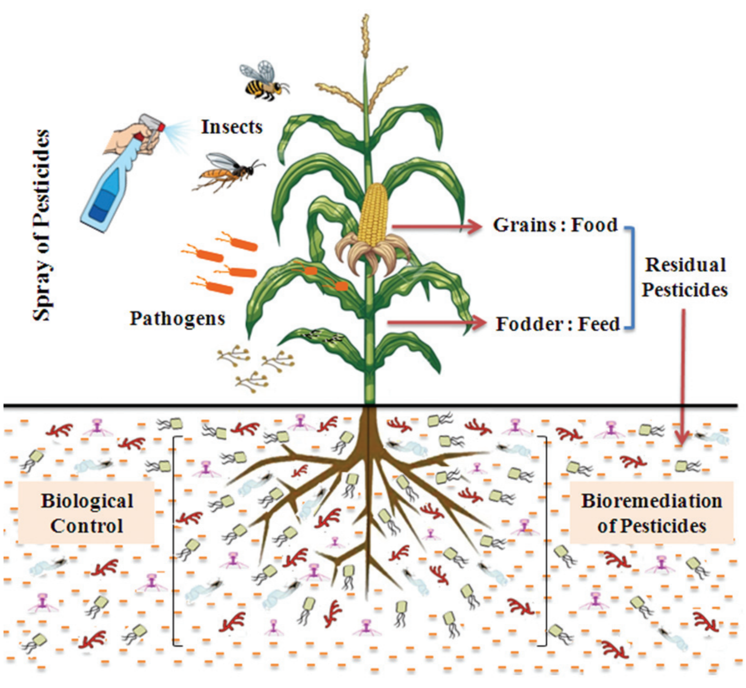

Figure 2. Role of microbes to minimise pesticide application and degradation of residual pesticides in soil.

3. MECHANISMS INVOLVED IN DISEASE CONTROL

Rhizobacteria exert their antagonistic effects by using diverse mechanisms involving production of antibiotics, bacteriocins and lytic enzymes, and by competing for nutrients[14,24]. Similarly, rhizobacteria chelate iron with siderophores and make it unavailable to the pathogens to eliminate them from the niche[25].

3.1 Root Colonisation

The antagonistic and biocontrol performance of the rhizospehere bacteria is determined by their root colonisation ability. Thus, root colonisation is the most important phenomenon of rhizospehere bacteria which involves adherence of bacteria on to roots or penetration into the endophyte roots, and subsequent dwelling around or into the roots. The root colonisation is established by a continuous communication between the plant and microbes. Plants release the root exudates, which are used by the rhizosphere bacteria to colonise themselves around the roots[4]. These root exudates are used by pathogens as well around the roots. However, due to strong competition, the beneficial rhizosphere microrganisms outcompete these pathogens from the ecological niche.

3.2 Competition

Rhizosphere microorganisms compete with the pathogens for available nutrients around the roots of the host plant and thus, create competitive environments for nutrient uptake. Thus, the rhisphere microorganisms compete with the phtopathogenic bacteria and fungi for nutrients and ultimately eliminate them from the root sphere. For example, *Bacillus* sp. inhibited the growth of pathogenic fungi *Botrytis cinerea* by creating competition for nutrients[26]. Similar, biocontrol activity of antagonistic bacteria was reported against fungal pathogen *Botrytis cinerea* by creating competitive environments[27]. Siderophore production by rhizobacteria is very important attribute to provide competitive environment for uptake of iron in the rhizosphere. Siderophores produced by rhizosphere microorganisms suppress the growth of phytopathogens by chelating the iron from the soil and make it unavailable to the phytopathogens[25,28].

3.3 Suppression of Pathogens by Secondary Metabolites

Production of secondary metabolites by antagonistic rhizosphere bacteria is most potent and broad-spectrum mechanism for biocontrol of phytopathogens[16] as shown in Fig. 3. Fan[29], *et al.* isolated *Bacillus subtilis* strain 9407 from healthy apples and it showed strong antifungal activity against apple ring rot disease caused by *Botryosphaeria. dothidea* by the production of fengycin. Similarly, lipopeptides produced by *Bacillus* XT1 CECT 8661 reduced the damage of grey mould disease caused by *B. cinerea* and also triggered the antioxidant activity in fruit[30]. Likewise, an extracellular lipopeptide having the antifungal and anticancer properties is produced by *B. velezensis* strain KLP2016. The lipopeptides fengycin, surfactin and mycosubtilin produced by different strains of *Bacillus subtilis* were found to inhibit the growth of the phytopathogenic fungi *Fusarium oxysporum* f. sp. *iridacearum*, which adversely affects the growth of ornamental bulb plants[31]. These lipopeptides, especially mycosubtilin exhibited a remarkable protection ability of bulbs from fusariosis.

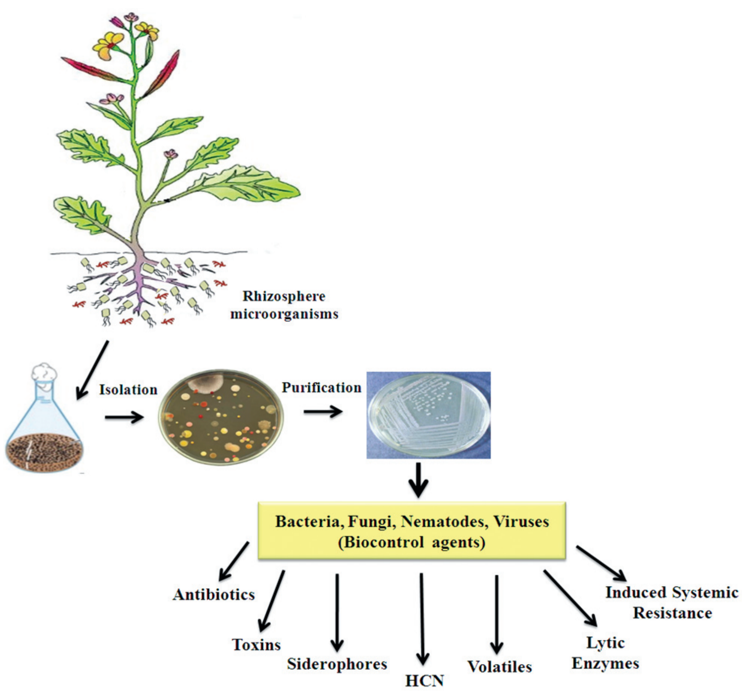

Figure 3. Mechanisms involved in the biological control of plant diseases and insect infestation.

Several antagonistic microbes secrete hydrolytic enzymes such as chitinases, glucanases, proteases and lipases that inhibit the growth of phytopathogens by causing lysis of their cell wall[15]. These extracellular enzymes cause the deformation or degradation of cell wall components of fungi and insects. Other biocontrol agents having either antibiotic or siderophore production could be used in combination with hydrolytic enzyme-producing bacteria, leading to a synergistic inhibitory effect against phytopathogens[32].

Iron (Fe) plays a vital role in cellular growth and metabolism. Siderophore makes a complex with iron making it soluble in the surrounding environment, which ultimately reaches to the cell surface through diffusion process. Siderophore production is an important mechanism of enhanced plant growth which eliminates the phytopathogens by creating a competition for iron in the rhizospehere[33]. Sahu and Sindhu[25] reported that siderophore-producing *Pseudomonas* sp. controlled the disease and promoted the growth of green gram. Some siderophores like pyoverdines inhibit the growth of fungi and bacteria under *in vitro* conditions. Another siderophore pseudobactin, produced by *P. putida* suppressed the growth of *Fusarium oxysporum* and *Rhizoctonia solani*.

Antibiotic production is the prominent mechanism to suppress the growth of phytopathogens[14]. Antibiotics produced by rhizobacteria include 2, 4-diacetylphloroglucinol (2. 4-DAPG), phenazine-1-carboxylic acid (PCA), pyrrolnitrin, kanosamine, oligomycin A, butyrolactones, xanthobaccin, zwittermycin A and viscosinamide[34]. Antibiotic 2, 4-DAPG is involved in the membrane destruction of *Pythium* sp. DAPG produced by *P. fluorescens* inhibit the growth of nematode (*Meloidogyne incognita*) and *Fusarium oxysporum*. Antibiotics fengycin and iturins were produced by *Bacillus subtilis*, which inhibited the growth of fungus *Podosphaera fusca*[30]. HCN is another secondary metabolite produced by rhizosphere-inhabiting species of *Pseudomonas* and *Bacillus*[35]. Release of HCN was reported in the rhizosphere of tobacco by *Pseudomonas* strains and these soils became suppressive to *Thielaviopsis basicola*, causal agent of black root rot of tobacco[36].

3.4 Induced Systemic Resistance and Systemic Acquired Resistance

Induced systemic resistance (ISR) and systemic acquired resistance (SAR) are important for chemical as well as physical defence mechanisms of the host plant[37]. PGPR strains trigger the ISR in the root system which gradually reaches to the other parts of plant. Various chemical molecules such as lipopolysaccharides, DAPG antibiotic, siderophores, cyclic lipopeptides, homoserine lactones, acetoin and 2, 3-butanediol produced by PGPR strains have been reported to induce systemic resistance[18,37]. In the plants, ethylene and jasmonic acid signaling pathway also elicit the ISR. Several rhizosphere microorganisms including *Pseudomonas*, *Bacillus*, *Serratia*, *Azospirillum*, *Trichoderma* and mycorrhiza have been reported as ISR inducers[37]. On the other hand, some PGPR strains produce salicylic acid (SA) that stimulates systemic acquired resistance. The role of SA in ISR elicited by PGPR was observed against blue mold of tobacco[38]. Similarly, *P. fluorescens* strain PF15 and *P. putida* strain PP27 protected tomato plants from *Fusarium* wilt by ISR[39]. However, mainly the necrotic pathogenic bacteria and the pathogenesis-related (PR) proteins are responsible for activation of SAR. These PR proteins include various enzymes which may either act directly to lyse invading cells, reinforce cell wall boundaries to resist infections or induce the death of localised cells[37].

3.5 Detoxification of Virulence Factors

Many PGPR strains used quorum sensing as mechanism to regulate production of virulence factor. Specific protein moleculaes are produced by the PGPR which bind with the toxin produced by pathogens to diminish its harmful effects. Albicidin toxin produced by *Xanthomonas albilineans* is detoxified by *Alcaligenes denitrificans* and *P. dispersa,* whereas *B. cepacia* and *Ralstonia solanacearum* strains are found to hydrolyse fusaric acid, a phytotoxin produced by *Fusarium* species[40]. These bacteria controlled the quorum sensing capacity of pathogens by impairing autoinducer signals and thus arresting the expression of virulence factor.

4. DEVELOPMENT OF BIOPESTICIDES

Globally approximately 20-40 per cent of the crop yield is lost annually due to various plant diseases caused by phytopathogens. Chemical pesticides are used to control the plant diseases, which exert several harmful impacts on the human and environment. Rhizosphere microorganisms offer a safe, efficacious and eco-friendly solution for use as biopesticides to control and various plant diseases[14]. These biopesticides are target specific and have been used for crop protection from diseases and insects in cereals, legumes, fruits, flowers and ornamentals plants. Microbial biopesticides contribute in global biopesticide market with 30 per cent share. Though several bacterial species have been exploited for use as biopesticides, yet *Bacillus* and *Pseudomonas* species are still the major part of biopesticides. *B. thuringiensis* (Bt) is a well known and commercially used biopesticide world over, which occupies about 95 per cent of total market of biocontrol products[41]

5. CONCLUSIONS

Biological control is the best eco-friendly approach for disease suppression with no environmental hazards. Rhizosphere microorganisms play important role in the control of plant diseases. Different mechanisms contribute towards disease control by rhizosphere microorganisms and many antagonistic microorganisms have recently been used as effective biocontrol agents for suppression of various plant diseases. In the present intensive agriculture crop production system, the potential of biocontrol agents is yet to be exploited fully, because the research in this area is still confined to the laboratory. Moreover, the commercially produced biocontrol products have not been used efficiently by the farmers owing to the lack of information regarding its use. In addition, the performance of biocontrol agents in the field is attributed to the physiological and ecological constraints, and some of the biocontrol agents fail to perform under field conditions. These microorganisms having disease control ability could be modified by genetic engineering technology for enhanced competitiveness and disease suppression in the agriculture field. Thus, there is an urgent need of in-depth study of the mechanisms used by different biocontrol agents for minimising the application of pesticides to provide safe food for ever-increasing human population.

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Contribution in the current study, he has compiled and organised the information in this review chapter.