

Current Status and Future Perspective on Enzyme Involving in Biocontrol of Plant Pathogen

Ami Chaudhari¹ and Jesal Patel²

¹C. G. Bhakta Institute of Biotechnology, Uka Tarsadia University, Bardoli, Surat394 350 Gujarat, INDIA.

²C. G. Bhakta Institute of Biotechnology, Uka Tarsadia University, Bardoli, Surat394 350 Gujarat, INDIA.

¹Corresponding Author: chaudhariami0@gmail.com

ABSTRACT

To sustain the quality and abundance of fruit, feed and fiber provided by farmers all over the world, plant diseases must be regulated. Plant diseases may be prevented, mitigated, or regulated using a variety of methods. Growers also rely on chemical fertilizers and pesticides for good agronomic and horticultural practices. Such agricultural inputs have taken a vital part in spectacular increases in crop yield and quality over the last 100 years. Microbial enzymes function as biocatalysts for key biochemical reactions and also assist microbes reproduce in a particular niche. The ability of rhizosphere microorganism to increase the growth of plant and control phytopathogens has long been known. Rhizosphere microbes may aid plants in several ways in their fight against phytopathogens. Of all recognized biocontrol pathways, the excretion of lytic enzymes is known as an important way to prevent phytopathogens from living in the region of the rhizosphere. Rhizosphere microorganism produces chitinases, cellulases, proteases, and glucanases in reaction to phytopathogen assault. For assessing antagonist-pathogen interactions, ecological characteristics of antagonists in the rhizosphere, and optimizing the effectiveness of bacterial, fungal, and viral biocontrol agents, new molecular approaches have become available. Given the experience of fungicides in near future, biological management would be another method to control diseases of plant. Since agro-ecosystem is a flexible and functioning structure that involves many variables that affect disease and production of crop, other IPM methods to control diseases of crop are also important in different surrounding conditions. As result, to successfully minimize disease production and crop yield loss in various crop systems, other IPM management mechanisms other than biological control should be considered and implemented.

Keywords- Biocontrol, Biofumigation, Disease, Enzymes, Pathogen

shifts in public perceptions of pesticide use in agriculture. Chemical pesticide use is still strictly controlled, and there is legislative support to withdraw majority of toxic chemicals from industry [1]. Furthermore, because of size at which such applications will have to be applied, proliferation of plant diseases in natural environments might prevent effective chemical application. As result, several pest management experts have based their attention on improving other pest and disease control inputs to conventional chemicals. Biological controls are form of alternative that can be used. Biological controls come in number of forms, but further advancement and successful implementation would necessitate better understanding of dynamic relationships that exist between plants, humans, and ecosystem. At that end, this article serves as comprehensive overview of nature and application of biological regulation to control the diseases of plant. This observation will i) define biocontrol and key mechanisms, ii) investigate relationship linking microbial diversity and biological control, iii) describe actual situation of biological control analysis and implementation, and iv) future aspects that will help to evolution of various and efficient biological controls for plant diseases. Use of organism species to suppress pathogen and mitigate disease is known as bio-control of plant diseases. Biological regulation has many meanings, but general concept is that it is mechanism for decreasing disease occurrence or intensity by manipulating microorganisms directly or indirectly [2]. As result, we can change the soil environment to create conditions favourable to effective to bio-control or develop bio-control techniques by understanding mechanisms of biological control of plant diseases through interaction between bio-control agents and pathogens. Control of plant pathogens with chemicals results in deposition of toxic chemical contaminants, which may create severe ecological issues. In recent years, Bio-logical control of plant diseases has been proposed as feasible control mechanism. Currently, industrial chemicals are used to effectively treat plant pests and microbial contamination in variety of farm crops. Persistent and indiscriminate use of these chemical fungicides, on other hand, has resulted in health risks in animals and humans due to residual toxicity. In the Western world, synthetic fungicides have been outlawed in large numbers due to their

I. INTRODUCTION

In response to phytopathogen attack, rhizosphere microbe produces chitinases, cellulases, proteases, and β -glucanases. The bio control enzymes use various mechanisms involved in phytopathogen elimination to indirectly support plant growth and survival. Environmental contamination caused by improper usage of agrochemicals and also manipulates by few pesticide critics, have resulted in significant

unfavourable characteristics, such as elevated and acute toxicity. Chemical fungicide resistance has evolved in many pathogenic microorganisms. This makes it difficult to treat grain and farm plant diseases. Alternative agents for treatment of pathogenic microorganisms are urgently needed, given negative effects of conventional fungicides on life-sustaining organisms structures. In agriculture, there is a significant need to minimise or eliminate the usage of synthetic pesticides [3]. Plant disease biological control has long been seen to be a viable alternative to chemical control. Biological regulation is the deliberate use of introduced or resident living species, rather than disease-tolerant host plants, to restrict plant disease behaviour and populations, or to replicate one organism using another organism. Biological controls can be used in number of ways, but continued advancement and successful implementation would necessitate better understanding of dynamic relationships that exist between plants, humans, and environment. Biocontrol action of enzymes such as lipase, protease, laccase/ligninase, cellulose, glucanase, and chitinase against pathogenic bacteria and fungi has also been studied. Microbial enzymes have lot of promise for biocontrol. These enzymes can provide defence to plants from variety of phytopathogens. Application and effectiveness of bio pesticide products could be improved by preparing them with biocontrol enzyme-producing microbial strains or by adding extracellular crude enzyme. This method also has lot of biotechnological promise in terms of avoiding phytopathogen-caused crop degradation. However, field implementation and performance of microbial enzyme-based biocontrol products are still being studied and are highly dependent on application techniques, formulation methods, and strain types used. Role of certain possible microbial enzymes in phytopathogen biocontrol is discussed in this Review [4].

II. MICROBIAL ENZYMES

Microbial enzymes have a lot of biocatalytic promise in a variety of industries. Microbial enzymes have been used in the manufacture of flour, wine, vinegar, pickles, and curd since the dawn of time. Because of their durability, ease of processing, and high bio-catalytic activity, microbial enzymes have piqued the industry's interest. Fermentation technology advancements have ensured a steady supply of microbial enzymes for the industries. Pharmaceuticals, baking, dairy, beverage, feed, biopolymer, paper and pulp, fibre, textile, cosmetics, detergents, organic synthesis, and waste treatment are only a few of sectors where microbial enzymes are used. Enzymes are classified into six classes by International Union of Biochemistry (IUB): (1) oxidoreductase, (2) transferase, (3) hydrolase, (4) lyase, (5) isomerase, and (6) ligase, and microbes can generate enzymes from all six groups. Global enzyme

demand reached \$5.5 billion in 2018 and is expected to hit \$7 billion by 2023. Amylase, arylsulfatase, β -glycosidase, cellulase, chitinase, dehydrogenase, phosphatase, protease, lipase, laccase, pectinase, xylanase, phytase, ureases, and others are examples of industrially developed microbial enzymes. Many of them play important role in ecosystem functioning, where they help with organic matter decomposition, biotransformation of complex organic molecules, and phytopathogen regulation [5].

III. CHITINASE

Chitinase is a hydrolytic enzyme that has the ability to degrade the chitin of pathogen such as insects, fungi, and insect larvae. Chitinase are naturally produced by a diverse range of organisms, including fungi, bacteria, yeasts, plants, actinomycetes, arthropods, and human. Chitinases are classified into two types based on how they work: Endo chitinases and exochitinases. Endo chitinase cleaves internal points at random along the length of the chitinase molecule, producing dimer diacetyl-chitobiose and N-acetyl glucosamine multimers like chitotriose and chitotetraose. Exochitinases are classified into two types: (1) chitobiosidases, which create diacetylchitobiose by cleaving non-reducing ends of chitin in a stepwise way, and (2) -1,4-glucosaminidases, which convert oligomers produced by Endo chitinases into monomers of N-acetyl glucosamine [6].

Bacteria generate chitinases largely to breakdown chitin for use as an energy source, although certain bacterial chitinases have showed promise as biological control agents against a range of phytopathogenic fungi-caused plant diseases. Bacteria with the ability to produce chitinases include *Serratia marcescens*, *Aeromonas punctata* and *A. hydrophila*, *Bacillus pumilus*, *B. thuringiensis*, *B. licheniformis*, and others. In addition, the fungi *Humicola grisea*, *Rhizomucor miehei*, and *A. flavus* have been identified as candidates with the potential to produce high chitinase titres.

The most promising options for maintenance of plant disease have been chitinolytic enzymes, such as chitinases, like Chitinase hydrolysis, prevalent in plant fungal infections. Chitinases not only contribute to the immunity of plants, but also contribute to plant growth and development. In order to increase disease resistance in plants, the current plant pathogenesis scenario focuses on the development of disease-resistant transgenic plants by incorporating chitinases encoding genes from any species into any plant. Another study discovered that *Corallococcus* sp. produces the chitin hydrolase CcCtII, which hydrolyzed chitin into N-acetylated chitohexose and inhibited the growth of the phytopathogen *Magnaporthe oryzae* in a dose-dependent manner [7].

IV. CELLULASES

Cellulase is a cellular enzyme produced with fungi, bacteria, and/or protozoa's that supports the breakdown into monosaccharides or simple sugars (e.g., Beta-glucose), shorter polysaccharides or oligosaccharides by hydrolysing 1,4-beta-D-glycoside linkages of cellulose or other related polysaccharides (e.g. hemicellulose, lichenin and cereal beta-D-glucanes). Cellulase is divided into three types based on the type of reaction it performs. Endocellulases, exocellulases (cellobiohydrolases), and beta-glucosidases are the three categories of cellulases depending on the sort of reaction they catalyse [8]. The host plant's primary and secondary cellular barriers assault and disintegrate cellulolytic enzymes separated by *Fusarium oxysporum*, a pathogenic fungus. The degraded products might enter the sweat stream, block capillaries and cause discomfort. *Fusariosis* is a disease that affects many cultures of economic importance (cucurbits, sweet potatoes, and tomatoes). Lignin and complex carbon hydrates linked with soil detritus breakdown by exo-cellulases by saprophytes of this fungus. As a result, fungal cellulases are favoured over bacterial cellulases and are frequently utilised in biotechnology applications due to their increased capability of penetration into celluloses. In biocontrol of the soilborne phytopathogen *Phytophthora parasitica*, the extracellular cellulolytic enzyme produced by this fungus was successfully utilised. In recent years, it has been discovered that some yeast strains can biocontrol phytopathogens by generating cellulases. Biologic activity against the *B. cinerea* and *Penicillium digitatum* in vitro and in vivo pathogens has been shown, for example, by the *Wickerhamomyces yeast*, as well as by the bacteria and fungus Actinomycetes cellulase. In biocontrol, plant pathogens have been documented. For example, streptomycetes *rubrolavendulae* S4 have been reported to exhibit antagonistic action causing diseased damping of plants in fungal pathogens *P. aphanidermatum* [9, 10]

V. PROTEASES

Proteases are omnipresent enzymes that are necessary for life. Peptide bonds in proteins are hydrolyzed, peptide or amino acid released. Thus, proteolytic cleavage has a significant effect on the behaviour of proteins as an irreversible post-translation amendment. Proteases may degrade, stop and remove proteins from the cells. Proteases are classified according to the reaction type: (1) endopeptidases, which cleave the amino acids internally; and, (2) exopeptidases, that remove amino acids from the amino terminal; or carboxy-terminal protein ends. Proteases are classified into two types based upon a catalytical reaction. A large-scale research of the biocontrol traits of *T. harzianum* has been undertaken among fungi. The two main

Trichoderma enzymes in charge of plant pathogens biocontrol were proteases and chitinases. Extracellular proteases against *Fusarium sp.*, *Colletotrichum sp.*, *Gloeocercospora sp.* and *Botrytis sp* have been reported in several Trichoderma strains. Insect control is also being investigated for entomopathogenic fungal proteases. Entomopathogenic fungal extracellular proteases are easily hydrolyzed by protein insect cuticle and are thus widely used as potential bioagents for prevention of crop loss due to insect attacks. In the last years, recombinant proteases with enhanced antifungal activity against *Penicillium expansum*, *B. cinerea*, *Monilinia fructicola*, and *A. alternata* have been investigated [11,12,13].

β -1,3-Glucanase

β -1,3-Glucanases are glycoside hydrolases found in plants, fungi, and bacteria that cleave long chains of -1,3-glucan. There are two kinds of β -1,3-glucanases: (1) exo β -1,3-glucanases that act randomly within a glucan chain, and (2) endo β -1,3-glucanases [14]. The role of β -1,3-glucanase in biological control of soilborne plant pathogens is also being investigated, and β -1,3-glucanase or other glucanase-producing microbes are now being used as efficient BCA. The ability of β -1,3-glucanases to modify fungal cell-wall β -(1,3)-glucan polymer can be successfully used in the development of BCA. *Pseudomonas cepacia* β -1,3-glucanases have been reported to be effective in the of plant pathogenic *S. rolfisii*, *R. solani*, and *P. ultimum* [15]. It has recently been reported that β -1,3-glucanases from *Paenibacillus terrae* have the potential to biocontrol the fungi that cause rice blast, *Exserohilum turcicum*, *X. campestris pv. glycines*, and *R. solani*, cause rice blast, corn spot disease, soybean bacterial spot disease, and rice sheath blight disease [16].

Major Microbial Enzymes in Biocontrol

Phytopathogens' cell walls may be degraded or lyzed by such enzymes. This effect is common in rhizosphere, where PGPM repels or destroys phytopathogens via secretion of lytic enzymes while also assisting plant growth and production indirectly. Thorough investigation of microbial hydrolases and other lytic enzymes has shown that they have biocontrol function against variety of phytopathogens. Biocontrol enzymes are group of fungal and bacterial enzymes that may prevent or alter cell wall synthesis, perforate cell membrane, or destroy cell wall of host or plant pathogens. Microbial biocontrol enzymes are defined in terms of their function and mechanisms [16].

VI. METHOD OF BIOLOGICAL CONTROL OF PLANT DISEASE

1. Suppressive soil

Many soil pathogens, including *Fusarium oxysporum*, *Gaeumannomyces graminis*, *Phytophthora cinnamomi*, *Pytium spp*, and *Heterodera avenae* (the oat cyst nematode), are well-growing and cause severe soil

conditions, referred to as conducive soils, whereas in other soils they develop and cause many less and much more milder diseases. Referred mechanisms by which the soils suppress different pathogenic agents are not necessarily simple, but can include biotic, abiotic and pathogen-dependent factors. However, in most cases they seem to work largely due to involvement of one or more pathogen-fighting microorganisms in those soils. Antibiotics, lytic enzymes, dietary competition, and overt parasitisation of pathogen are all examples of antagonists that prevent pathogen from reaching large enough populations to cause extreme disease. Pathogen and Fungi such as *Trichoderma*, *Penicillium* and *Sporidesmium* have proven to be the cause of disease suppressions well as bacteria from genera *Pseudomonas*, *Bacillus*, and *Streptomyces*, in suppressive soils. Through adding microorganisms that are antagonistic to pathogen, Suppressive soil may decrease disease amounts, as introduced in conducive soil. Planting of papaya seedlings in suppressive soil in orchard soil, infesting root-red oomycete holes *Phytophthora Palmivora*, for example, was used to manage *Phytophthora* root rot of papaya. After several years of serious disease, however, persistent cultivation in conducive soil, by the increasing concentrations of the microorganisms antagonistic to the pathogen, contributes gradually to a reduction in the disease. Continuous production of wheat or cucumber causes decreases in the use of wheat and cucumber, respectively, as damped *Rhizoctonia*. Similarly, continuous growth of the 'Crimson Sweet' watermelon variety causes *Fusarium* antagonistic species like those which cause the growth of *Fusarium wilt* watermelon to decrease instead of to increase. The production of future diseases in such soils is stifled. Pasteurization of soil for 30 minutes at 60°C reduces total suppression, showing antagonistic microflora. When appropriate crops are plucked into the soil as additional additives, the soil is a kind of suppression [17].

2. Biofumigation or Biodisinfection

Biological soil disinfection, which is more suited to colder climates, is dependent on plastic ground taping after fresh organic matter is absorbed. mechanisms behind this recently evolved methodology remain unknown. Organic soil fermentation resulting in toxic compounds and anaerobic conditions, all of which lead to pathogenic fungi inactivation or death. Biofumigation relates to the application of particular plant species with hazardous compounds discovered and is based on the prevailing mechanisms. "Bio-disinfection" is the utilisation of vast quantities of organic matter in the creation of anaerobic environments, which are primarily responsible for pathogen destruction. The chemical molecule family of glucosinolates, which can be degraded in hazardous compounds such as isothiocyanates by group of related enzymes, are found in many Brassicaceae plants. These chemicals, which are similar to certain organic

fumigants, function as biocides to combat variety of soilborne plant pathogens. Plant producers have traditionally used varieties with lower glucosinolates, as brassicas are used as animal feed, in order to prevent complications. Alliaceae family of plants also contains chemicals that have direct or indirect impact on pests and pathogens. During decomposition of garlic, onion, and leek tissue, volatiles such as thiosulfins and zwiebelanes are produced and translated into disulfides with biocidal effects of fungi, nematodes and arthropods. In addition to toxic effects of these chemicals, high concentrations of organic substances absorption in soil accompanied anaerobic conditions which are created by using plastic tarps, hazardous for many pesticides and microorganisms requiring aerobic survival conditions. [17,18]

3. Biopesticides

Plant diseases have major impact on crop production and storage. To avoid or regulate these diseases, farmers also depend heavily on chemical pesticides. Environmental pollution and pesticide residues on food, as well as social and economic issues, may arise from these chemicals' high efficacy and ease of use. As result, citizens and government leaders are increasingly calling for reduction in use of chemical pesticides. In this regard, biological control by natural hostile microorganisms has proven to be a potential option. The two most prevalent categories of biopesticides are microorganisms (bacteria, fungus, oomycetes, and viruses) and biochemicals (including plant products such as essential oils and other synthetic substances such as chitin and Chitosan), these biopesticides provide many benefits in terms of sustainability, mode of operation, and toxicity [17].

4. Microbial control of plant pathogens

A wide range of microorganisms, including fungi, bacteria, and viruses, naturally regulate plant pathogens to some extent. Any of these are used in biological management mechanisms such as augmentation, classical, and conservation. Fungi, oomycetes, bacteria, viruses, and plant parasitic nematodes are all targets for plant pathogens. Increased removal of conventional fungicides after government studies of their protection is driving development of microbial biopesticides of plant pathogens, however, the worldwide prohibition of methyl bromide is equally important, previously employed as a soil sterilant but gradually eliminated due to its connection to environmental ozone depletion. The marketing of biopesticides as plant pathogenic parasitic nematode control agents is relatively new. Since the mid-1990s, only to some extent useful control medicinal products have been publicly marketed. Around 80 drugs were on market or near to it in 2000. Microorganisms used for biocontrol of plant diseases have broad variety of MOA. Microbial antagonists live in same ecological niche as plant pathogen they are fighting and communicate with it directly. Interaction mechanisms include parasitic

interaction, competition for space, water, or food, as well as "chemical warfare" involving antibiotics or other secondary metabolites that harm the target pathogen. The second type involves an indirect effect in which the control agent induces the plant to build a resistance response that protects it against aggressive plant diseases. A low-virulent plant pathogen strain could act as an "inducer" for this type of control, new species of microbe, or natural product, in addition to plant itself. This is in stark contrast to new microbial insect management strategy, which is entirely focused on the use of virulent parasites to eliminate insect pests. The microbial antagonists of many plant pathogens have a

variety of ways for inhibiting the growth of the target pest. For example, soilborne plant pathogenic fungi are controlled by variety of species of fungal control agent *Trichoderma*. *Trichoderma* species can parasitize soil-borne plant pathogenic fungi, develop antibiotics and fungal cell-wall-degrading enzymes, compete for carbon, nitrogen, and other nutrients with soil-borne pathogens, and promote plant growth, likely through development of auxin like compounds. *Trichoderma* fungus is widespread soil fungus that thrives in rhizosphere. Since *Trichoderma* has effective control in variety of cases, in terms of disease control, its several mechanisms of action provide several advantages (Table 1) [17].

Table 1: Types of interspecies antagonisms to biological control of plant pathogens.

Type	Mechanism	Examples
Direct antagonism	Hyper parasitism/predation	Lytic/some non lytic myco viruses <i>Ampelomyces quisqualis</i> <i>Lysobacter enzymogenes</i> <i>Pasteuria penetrans</i> <i>Trichoderma virens</i>
Mixed-path antagonism	Antibiotics	2,4-diacetylphloroglucinol Phenazines and Cyclic lipopeptides
	Lytic enzymes	Chitinases, Glucanases and Proteases
	Unregulated waste products	Ammonia, Carbon dioxide and Hydrogen cyanide
	Physical/chemical interference	Blockage of soil pores Germination signals consumption Molecular crosstalk confused
Indirect antagonism	Competition	Exudates/leachates consumption Siderophore scavenging Physical niche occupation
	Induction of the host resistance	Contact with fungal cell walls Detection of pathogen-associated Molecular patterns Phytohormone-mediated induction

5. Plant Growth Promoting Rhizobacteria (PGPR)

PGPR bioinoculants are available commercially in a variety of forms. They go by various names and work in variety of ways: i) bioprotectants, which suppress plant disease; (ii) biofertilizers, which increase nutrient acquisition; and (iii) biostimulants, which produce phytohormones. *Bacillus*, *Paenibacillus*, *Streptomyces*, *Pseudomonas*, *Burkholderia*, and *Agrobacterium* are examples of bioinoculants that are commonly used as BCAs at commercial stage. Plant disease is suppressed by inducing systemic resistance, producing siderophores, or using antibiotics. Increase seed nitrogen uptake from nitrogen-fixing bacteria (*Azospirillum*) and iron uptake from siderophore-producing bacteria with biofertilizers (*Pseudomonas*). *Pseudomonas* and *Bacillus* species may develop phytohormones or growth regulators that induce extensive root growth, raising absorptive surface of plant roots, which have yet to be identified. Indole-acetic acid, cytokinins, gibberellins, and inhibitors of ethylene

synthesis are among phytohormones generated by these PGPR, which are referred to as biostimulants. Peat, granular, oil, and wettable powder formulations are currently used to distribute inoculants. Extent of their ability to colonise rhizosphere is significant determinant of growth promotion. Several recent studies have aided in production of new biofertilizers that make use of natural antimicrobial compounds formed by variety of antagonists [17].

VII. BIOCONTROL RESEARCH, DEVELOPMENT AND ADOPTION

Biological control emerged as academic field in 1970s, and it is now mature science with public and private sector funding. Biological control research appears in variety of scientific publications, including those devoted to plant pathology and entomology. In addition, three scholarly journals are dedicated solely to subject. Several USDA projects provide funding for

discipline's studies in United States. Section 406 programmes, provincial IPM awards, Integrated Organic Program, IR-4, and other National Research Initiative programmes are among them. Small business innovation research (SBIR) schemes also provide funds to encourage growth of entrepreneurial projects [19]. These businesses are meant to be conduits for scholarly study that can be used to start new businesses. Over last four decades, biological control analysis has yielded wealth of information. However, in addition to learning from past, biocontrol researchers must look to future to identify current and different problems, answers to which will aid in development of new biocontrol innovations and applications. At chemical, cellular, organismal, and ecological stages, fundamental developments in computation, molecular genetics, analytical chemistry, and statistics have contributed to recent studies aimed at characterising nature and roles of biocontrol agents, pathogens, and host plants. Some of study questions that will help us learn more about biological controls and environments under which they can be most effective [19]. Most pathogens would be vulnerable to one or more biocontrol techniques, but commercial adoption has been hampered by variety of factors. Biological controls' cost, comfort, effectiveness, and efficiency are all essential factors to consider, but only in comparison to other disease prevention options. Since cultural practises (such as good hygiene, soil planning, and water management) and host resistance will go long way toward controlling many diseases, biocontrol can be used only where these agronomic practises are inadequate for disease control. Chemical pesticides would be impossible to beat in terms of expense and ease as long as gasoline is inexpensive and plentiful. Ability of living organism to replicate will significantly reduce implementation costs if infection court or target pathogen can be efficiently colonised using inoculation. Key economic forces encouraging implementation of biological protection techniques in urban and rural landscapes, however, are regulatory and cultural issues regarding health and safety of various groups of pesticides. Disease management in forested and rangeland habitats, where high deployment rates over wider land areas are not commercially viable, requires self-perpetuating biological controls (e.g., hypovirulence of chestnut blight pathogen). Greatest achievements in biological control, in terms of effectiveness and efficiency, have been made in cases where environmental environments are most regulated or predictable, and where biocontrol agents may colonise infection court until it becomes infected. Biological control agents that function as bioprotectants have been effective in controlling monocyclic, soilborne, and postharvest diseases. Relevant applications for high-value crops that target specific diseases (such as fireblight, downy mildew, and variety of nematode diseases) have also been introduced. Use of BCAs in IPM systems is expected to rise in coming years as

research uncovers various conditions needed for effective biocontrol of various diseases [19].

VIII. FUTURE ASPECTS

Biocontrol enzymes are crucial products to prevent hazardous phytopathogens from being utilized by plants. Although biocontrol enzymes are not widely recognized for their production and commercial application as regards industrial enzymes, their usage in future, particularly in the creation of the biocontrol products, can be expanded. Some problems faced in the processing of biocontrol enzymes are lack of effective strains, expensive development costs, inadequate formulation design and instability under different conditions. Researchers recently attempted to address flaws in their production, and it was discovered that using agro-waste and animal material reduced cost of hydrolytic enzymes. Genetic engineering strategies are more effective than physical and chemical methods for enhancing enzyme output efficiency. In another study discovered that introducing recombinant gene P2 into *S. griseorubens* E44G strain increased its chitinolytic activity by 1.39-fold. Efficacy of fungal biopesticides can be increased by improving genetics of emopathogenic fungal enzymes. Any of study guidelines that will help us learn more about biological regulation and environments under which it is most effective. Biocontrol-active microorganisms' performance and behaviour are influenced by variety of environmental factors. However, there are still a few aspects that need to be researched and improved in order to increase biocontrol microorganism performance [20]. New strains and pathways of fungal/bacterial plant pathogens are quite diverse and their pathogenic nature varies on host plants and the hunt for new and new biocontrol microorganisms with various mechanisms is therefore crucial [21]. Finally, plant defence against phytopathogens is greatly enhanced by microbial enzymes having biocontrol characteristics. In addition, given some regulatory constraints such as certification and safety review, the use of microbial enzymes in the manufacturing of next-generation biocontrol agents with host-specific and broad-spectrum action might be extended.

IX. CONCLUSION

Biological disease management is appealing potential method for plant disease control. Meanwhile, it promotes activities that are in line with intention of long-term agricultural system. Thorough understanding of cropping method, disease epidemiology, biocontrol organism biology, ecology, and population dynamics, as well as relationships between these variables, is needed for effective biocontrol. One of most critical steps will be to understand causes or behaviours of antagonist-pathogen interactions, as this will provide rational

framework for selection and development of more efficient biocontrol agents. Novel uses of molecular approaches have broadened our understanding of basis of biological regulation of plant diseases in recent years. New molecular methods have been developed for evaluating antagonist-pathogen interactions, antagonist ecological characteristics in rhizosphere, and optimising effectiveness of bacterial, fungal, and viral biocontrol agents. As result, number of biological disease prevention agent's licence or on market worldwide has increased dramatically in recent years. Given experience of fungicides in near future, biological management would be alternative method for control of plant diseases. Since agro-ecosystem is variable and functioning structure that involves many variables that affect disease and crop production, other IPM methods for crop disease control are also important in different environmental conditions. As result, in order to successfully minimise disease production and crop yield loss in various crop systems, other IPM management mechanisms other than biological control should be considered and implemented.

Conflict of Interest

The authors declared that they have no conflict of interest.

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