Review Article

BIOLOGICAL CONTROL STRATEGIES OF PLANT PATHOGENS
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ABSTRACT

Food security has become a major concern worldwide in recent years due to ever increasing population. Providing food for the growing billions without disturbing environmental balance is incessantly required in the current scenario. In view of this, sustainable modes of agricultural practices offer better promise and hence are gaining prominence recently. Moreover, these methods have taken precedence currently over chemical-based methods of pest restriction and pathogen control. Adoption of Biological Control is one such crucial technique that is currently in the forefront. Over a period of time, various biocontrol strategies have been experimented with and some have exhibited great success and promise. This review highlights the different methods of plant-pathogen control, types of plant pathogens, their modus operandi and various biocontrol approaches employing a range of microorganisms and their byproducts. The study lays emphasis on the use of upcoming methodologies like microbiome management and engineering, phage cocktails, genetically modified biocontrol agents and microbial volatilome as available strategies to sustainable agricultural practices. More importantly, a critical analysis of the various methods enumerated in the paper indicates the need to amalgamate these techniques in order to improve the degree of biocontrol offered by them.

Keywords: Plant Pathogen; Biocontrol; Microbes; AMF; Bacteriophages; Microbiome; Sustainable strategies
INTRODUCTION
A large amount of crop loss occurs each year during both pre and post-harvest stages due to pathogen infestation that involves a wide variety of pathogens ranging from viroids and viruses to prokaryotic bacteria, eukaryotic fungi, oomycetes, and nematodes. These plant pathogens are highly persistent in their attack and induce direct and indirect losses to the tune of 40 billion dollars worldwide (Jamiołkowska 2020). Over the last decade some very important aspects of microbial applications in crop disease mitigation have been discussed (Mishra et al., 2018; Ellouze et al., 2018; Ellouze et al., 2020) as methods of sustainable agriculture. However, their field application is still inadequately worked out.

Given the paramount importance of the methods for controlling plant pathogens and diseases caused by them to improve productivity not only in terms of food but also for other materials obtained from plants like fibre, timber, oils, medicines, etc. and to meet the food demands of the exponentially growing world population, food production needs to increase by 70% until the year 2050 to address the internationally growing food security concerns (https://sustainabledevelopment.un.org/post2015/transformingourworld). It is high time when we need to shift to sustainable methods of agriculture so as to reduce biodiversity loss and greenhouse gas emissions that are currently placed at 60% and 25%, respectively (Jamiołkowska 2020). At present, most of the methods employed for plant protection from pathogens primarily involve the use of antibiotics and chemicals (Ab Rahman et al. 2018). Even though these shotgun methods deliver immediate protection, they ultimately lead to resistance and bioaccumulation of harmful chemicals in the crop systems. It is these drawbacks that emphasize the importance of sustainable and environment-friendly crop management practices to control diseases (Dy et al. 2018). Such practices help to improve the quality and quantity of agricultural produce that also includes organic crops. The organic systems exploit various naturally occurring plant protection resources like micro- and macro-flora and fauna found in the soils, protective products made from plant extracts, use of physical methods like weeding, mulching, and choice of cultivars, etc. to support organic produce (Stoleru and Sellitto 2016). Hence ingredients of organic agricultural practices can serve as model tools in establishing sustainable methods of agriculture otherwise. Overall, due to the growing concerns of environmental pollution and ecological toxicity resulting from the indiscriminate use of chemical formulations, there is an immediate need to base modern plant protection strategies on natural resources (Jamiołkowska 2020).

The terms biological control or biocontrol used extensively in scientific literature, cause tremendous confusion. Biological control, in its most basic form, is the employment of any living organism to combat a specific plant disease or pest through parasitism, antibiosis, or competition for resources or space (Eilenberg et al. 2001). In order for a disease or pest to thrive on a plant, three important criteria need to be fulfilled. These include the invader (the plant pathogen or pest), the environment, and the plant itself. Therefore, there are complicated processes at several levels that not only produce the diseases and pests but also modulate them (Pelczar et al. 2020). As a result, a larger definition of biological control is necessary, one that encompasses all levels, to realize its full potential in disease and pest management. This broad phrase refers to the use of living beings and their byproducts to manage pests and diseases in crops, either via hostile reactions or through the development of immunity against them (Köhler et al. 2019). Despite extensive studies devoted to field trial effectiveness of biocontrol agents (BCAs), this area is restricted due to changes in ecological characteristics, such as the host’s physiological and genetic state, climatological circumstances, and other factors that enhance the
variability of the desired BCA impact (Kagot et al. 2019; Del Martínez–Diz et al. 2020; Marian and Shimizu 2019). As a result, most biocontrol applications are limited to greenhouse crops, where environmental conditions are monitored and supervised (Buccellato et al. 2019). It is suggested here that combinations of BCAs and fungicides might be a better combination to be used in the field to provide more reliable disease control (Ons et al. 2020). However, this area is entirely barren and extensive research studies need to be conducted to come out with meaningful conclusions. In the current study, we examine several biocontrol approaches against plant diseases and upcoming strategies that offer improved biocontrol potential against a diverse population of pathogens that might possibly assist in the attainment of long-term sustainability goals.

PLANT DISEASE MANAGEMENT

Chemical control
There has been high dependence on chemicals to control diseases and pests in agriculture and even today, they continue to remain the main component of Integrated Pest Management (IPM) as demonstrated by the ever-increasing use of fungicides since the 1960s (Rampersad 2020). These chemical formulations, even though crucial to prevent large scale losses and spread of diseases in crops, come with several drawbacks such as, ecotoxicity, bioaccumulation, adverse effects on nontarget plants and animals, and human health. Exposure to these chemical-based pesticides, fungicides, etc. is known to cause various types of cancers, respiratory disorders, and hormonal imbalances in humans (Ons et al. 2020). Apart from these, data from FAO-WHO and US Food and Drug Administration shows that persistent organic pollutants (POPs) do not degrade easily and remain deposited on fruits and vegetables, ultimately entering animal-based food sources like dairy products, poultry, and meat (Ab Rahman et al. 2018). Furthermore, the use of chemical pesticides has led to the continual rise of resistant pathogens resulting into reduced efficacy of most chemical control methods (Ons et al. 2020).

Resistant varieties
The process of crop selection and plant breeding are well known and proven criteria that are applied in agriculture to improve crop varieties and produce disease-resistant cultivars. These practices are used even today and have proven to be beneficial in the fight against various types of disease-causing plant pathogens (Ab Rahman et al., 2018). The genetic route is one of the most favored biotechnological applications in our never-ending strive to increase food production. Genetically modified (GM) varieties are not only disease resistant but also produce better quality crops and greatly reduce the need for external inputs of costly chemicals, thereby making their production economically viable. Despite these advantages, GM crops require approval from regulatory agencies at a high cost and are not readily accepted by the consumers. Moreover, these crops can also exhibit susceptibility to pathogens within a few years of their cultivation due to a number of causes like mutations occurring in the targeted pathogens, reduction in field resistance due to various recombination events, and lack of genetic uniformity within the GM crops (Miah et al. 2017). Many crops have shown indications of resistance breakdown, including rice blast resistance, cotton leaf curl disease, grapevine downy mildew, and yellow wheat rust (Miah et al. 2017). Nevertheless, encouraging results are being achieved in the labs by using genome editing by CRISPER/Cas9 and insertion of gene cassettes using intragenic technologies and it is expected that in the near future, these approaches may be the way forward and can be used at par with conventional plant breeding technologies (Ab Rahman et al., 2018). Other breeding methods involving gene pyramiding, gene rotation, and multiline
varieties also offer advantages in controlling resistance. It is imperative that newer and better biotechnological tools are developed and applied in order to accelerate the production of improved disease-resistant cultivars so as to manage the newer aggressive pathogens (Miah et al. 2017).

Biological Control
Among the non-chemical methods of pest and pathogen control, biological control or biocontrol seems to be the most suited for organic cultivation. It is environmentally safe, sustainable, economically viable, and highly specific (Table 1). A number of such methods are currently being employed like the use of naturally occurring soil microbes against various pests and pathogens (Mishra et al., 2018). A deeper understanding of the relationships between plants and pathogens along with the environmental factors prevalent in a particular area needs to be understood prior to biocontrol implementation particularly under widespread disease conditions. In plant pathology, biocontrol is defined as the interaction of numerous environmental elements with the goal of reducing the negative impacts of harmful species while promoting the growth of beneficial crops, helpful insects, and microbes (Pal and Gardener 2006). Biological control is dependent on numerous agonistic and antagonistic interconnections between plants and microbes living in the rhizosphere and phyllosphere (Mishra et al. 2015) and their application to minimize disease and subdue pests. Organisms from the rhizosphere can be harnessed from the surrounding environment (the black box approach) or can be introduced into the field from external sources (the silver bullet approach). It is beneficial to apply a consortium of microbes with collaborative properties rather than relying on a single organism since microbial consortia make up a stable rhizosphere that offers more effective control against pathogens (Ram et al. 2018). Apart from microbial applications, the utilization of other plant products like extracts, biofertilizers, and biopesticides, natural enemies of pests and pathogens, and gene products also aid in carrying out biological control (Ab Rahman et al. 2018).

TYPES OF PLANT PATHOGENS
Plant pathogens are divided into three categories namely necrotrophs, hemibiotrophs, and biotrophs depending on the way they obtain energy from the plants (Agrios 2005). These interconnections in turn influence the way the plant responds to the pathogens (Ab Rahman et al. 2018; Narayanan et al. 2020).

Biotrophic pathogens
Biotrophic plant pathogens obtain their nourishment from living cells of the host plant with the help of complex mechanisms to access plant resources. They share a close relationship with the plants’ living tissue to the extent that some of the biotrophs have lost the ability to grow on non-living artificial media and have coevolved as obligate biotrophs. Examples include Uromyces fabae that causes rusts and Blumeria (Erysiphe) graminis that causes powdery mildews (Ellis et al. 2006; Latijnhouwers 2003). The non-obligate biotrophs on the other hand can be grown on artificial media, are not saprophytic, and restrict injury only to the host cells. Biotrophs form hyphae/haustoria that penetrate the host cell wall but not its plasma membrane. The plasma membrane at these points invaginates and gives rise to a perihaustorial/peri arbuscular membrane where nutrient exchange takes place (Spanu and Panstruga 2017). Effector molecules are released by the pathogen that further helps in the invasion of the host genotype (Latijnhouwers 2003; Walters and McRoberts 2006; Mang et al. 2009; Wiermer et al. 2005). Other examples include Ustilago maydis, which causes corn smut and Cladosporium fulvum that
causes tomato leaf mold, do not form haustoria and nutrient exchange between the plant and the microbes is carried out via apoplast (Spanu and Panstruga 2017).

**Necrotrophic pathogens**

Unlike biotrophs, the necrotrophic microbes are opportunistic, unspecialized pathogens that kill the host rapidly and sustain on its remains (Lewis 1973; Laluk and Mengiste 2010). They do not form haustoria and enter the plant via naturally found openings or wounds and secrete lytic enzymes and phytotoxins. They can be easily grown on artificial media. Necrotrophic pathogens include bacteria, fungi, and oomycetes that mainly attack young, weak, and damaged plants and are capable of a saprotrophic mode of existence (Laluk and Mengiste 2010; Trigiano 2007). Both bacterial and fungal necrotrophs follow similar patterns of infection that involve attachment, host penetration, and subsequent necrosis and decay of plant tissues. Some examples of fungal necrotrophs are *Cochliobolus* that causes corn leaf blight, *Alternaria* that causes early blight of potato and *Botrytis* that causes grey mold (Agrios 2005; Ellis et al. 2006; Latijnhouwers 2003; Ab Rahman et al. 2018). Mechanisms of plant immunity against these pathogens are in the form of phytohormones, pathogenesis proteins and secondary metabolites (Laluk and Mengiste 2010). Some of the important cash crops that are infected by necrotrophic fungi like *Fusarium* and *Rhizoctonia* include wheat, maize, and rice (Savary et al. 2012; Van Bruggen et al. 2016; Singh et al. 2016). Even if a percentage of the crop genotype does not respond to the toxins produced by the necrotrophic fungi and evades necrosis, these pathogens are still capable of inflicting a much greater loss of productivity and overall destruction in comparison to the biotrophs (Laluk and Mengiste 2010).

**Hemibiotrophic pathogens**

Hemibiotrophic pathogens are an interesting group of pathogens as they display characters of both biotrophs and necrotrophs and are capable of switching between the two modes. The transition from the asymptomatic biotrophic phase to the destructive necrotrophic phase is accompanied by suppression of the host’s immune response at the required time resulting in extensive damage to the host leading to its decay and death (Ab Rahman et al. 2018). Hemibiotrophic characteristics are shown by fungi like *Magnaporthe grisea*, *Phytophthora*, *Pythium*, *Fusarium*, *Colletotrichum* and *Venturia*, and the bacterium *Pseudomonas syringae* all of which are capable of a prior biotrophic existence with the host but ultimately shift to a necrotrophic mode of nourishment by killing the host cells (Münch et al. 2008; Latijnhouwers 2003; Walters and McRoberts 2006; Mang et al. 2009; Wiermer et al. 2005; Trigiano 2007; Münch et al. 2008; Bhadaura et al. 2009).

**BIOCONTROL MANAGEMENT**

**Microbial Biocontrol**

The rhizosphere is the soil area that surrounds the roots and is composed of microbes capable of repressing plant pathogens. It, therefore, aids in providing natural protection to the plants against a variety of organisms either directly by synthesizing metabolites antagonistic towards the pathogens or indirectly by suppressing pathogen growth and improving the host’s defense mechanisms. Antibiosis caused by the release of antibiotics, organic compounds, toxins, and various hydrolytic enzymes like beta-xylosidase, chitinase, pectin methylesterase, β-1,3-glucanase, etc is one of the mechanisms employed by the rhizosphere microbial population to carry out the destruction of the pathogen including disintegration of the glycosidic linkages in its cell wall (Ab Rahman et al. 2018). Plant growth-promoting rhizobacteria (PGPR) residing in the rhizosphere also perform biocontrol by reducing the incidence of plant disease thereby assisting
in plant growth. The PGPR also promote antibiosis, competition, production of metabolites that induce systemic acquired resistance (SAR) and induction of systemic resistance (ISR), parasitism, production of hydrolytic enzymes such as cellulase, glucanase, chitinase, and protease that break down the cell wall along with a number of antibiotics like oomycin A, 2,4-diacetyl phloroglucinol (DAPG), pyoluteorin etc against the pathogens (Jadhav et al. 2017). For example genus, *Serratia* belonging to Enterobacteriaceae is a PGPR that produces secondary metabolites having attractive biocontrol properties (Soenens and Imperial 2019).

Rhizobia are symbiotic microbes found on the roots of leguminous plants that not only play an important role in nitrogen fixation but also in biocontrol. They promote plant growth by secreting antibiotics, mycolytic enzymes, siderophores, and hydrocyanic acid (HCN) that prevent the growth of pathogenic fungi belonging to genera like *Fusarium*, *Rhizoctonia*, *Sclerotium*, and *Macrophomina*. They enhance plant immunity by increasing the expression of defense-related genes and instigating systemic resistance.

Seed quality can be improved by bacterization with the correct rhizobial strain to cause activation of various enzymes involved in isoflavonoid and phenylpropanoid pathways, accumulation of phenolic compounds and isoflavonoid phytoalexins that enhance the biocontrol capability of the cultivars thereby improving plant growth and productivity (Das et al. 2017). Examples of protection by rhizobia can be seen in the use of a colloquium of *Pseudomonas* strains that were isolated from potato phyllosphere and rhizosphere and used to fight the late blight of potato caused by *Phytophthora infestans*. The colloquium of different strains proved to be far more effective compared to the use of individual strains (De Vriez et al. 2018). Plant disease management also engages endophytes as biocontrol agents. These microbes can reside asymptotically in different parts of a plant like a shoot, leaves, or roots (Schulz and Boyle 2005; Sun et al. 2014, Grünig et al. 2008; Rodriguez-Cabal et al. 2013; Sokolski et al. 2007; Verma et al. 2007). Potential antagonistic strains of endophytes can be screened for biocontrol capability as all strains do not exhibit similar activity. This was exhibited by Gonthier et al. (2019) on the use of *Suillus luteus* against the fungal pathogens *Heterobasidion irregular* and *Heterobasidion annosum* that infect Scots pine (*Pinus sylvestris*) that resulted in diminished susceptibility to only *H. annosum*, and not to *H. irregular*. They can also be engaged as control methods against threats such as the spotted lanternfly that causes severe economic loss in North America (Eric et al. 2019; Rabiey 2019). Endophytes use varied mechanisms like lytic enzymes, activation of host defenses, synthesis of antibiotics, and mycoparasitism against pathogens. In-depth research on their biocontrol activity is much required in order to exploit their full potential as future disease and pest management agents (Dutta et al. 2014; Gao et al. 2010).

**Fungal Biocontrol**

Apart from their ability to improve nutrient uptake and nitrogen use in plants, fungi also have biocontrol capabilities. They can aid in the fight against pests like nematodes and microbial pathogens that infect various parts of the plant such as roots, foliage, and fruits. They offer protection against diseases with the help of processes like mycoparasitism, competition for resources with pathogens, antibiosis, conferring ISR to the host plant, and mycovirus mediated cross-protection or MMCP (Singh and Giri 2017). Some of the well-known fungal biocontrol agents include the *Trichoderma* species, ectomycorrhizas, arbuscular mycorrhizas (AMF), yeasts, and endophytes. Even the nonvirulent strains of certain pathogens can utilize hypovirulence-associated mycoviruses in order to function as biocontrol fungi (Ghorbanpour et al. 2018). With improved biotechnological and genetic advances it is not only possible to
introduce beneficial fungal genes into the genome of the host plant but also to interrupt or overexpress these genes in order to improve biocontrol ability (Ghorbanpour et al. 2018).

**Arbuscular Mycorrhizal Fungi (AMF) Biocontrol**

A number of studies lay emphasis on the biocontrol abilities of Arbuscular Mycorrhizal Fungi (AMF) as they have been shown to reduce the incidence of fungal diseases and nematode attacks on host plants by 30 to 42% and 44–57% respectively (Veresoglou and Rillig 2012; Bagyaraj 2014; Mishra et al., 2018; Ellouze et al., 2019). The biocontrol properties of AMF are broad-spectrum and more pronounced against fungal root pathogens in comparison to the shoot ones (Whipps 2004; Ronsheim 2016). AMF offers defense against a number of fungal pathogens belonging to the genera *Colletotrichum*, *Alternaria*, *Erysiphe*, *Gaeumannomyces*, *Macrophomina*, *Botrytis*, *Rhizoctonia*, *Fusarium*, *Cylindrocladium*, *Sclerotium*, and *Verticillium*. On the other hand, they do not offer much protection against a large number of bacterial and viral pathogens but some bacteria like *Pseudomonas syringae pv. glycina* that causes bacterial blight on soybean can be checked by AMF. In the case of viral pathogens, the presence of mycorrhizal fungi seems to increase the damage caused by viral infections (Singh and Giri 2017) as seen with Tomato spotted wilt virus (TSWV) (Miozzi et al. 2011), Potato virus Y (Sipahioglu et al. 2009), Citrus tristeza virus and Citrus leaf rugose virus (Nemec and Myhre 1984) and Tobacco mosaic virus (Shaull et al. 1999). Therefore the role of AMF against viral pathogens is largely unclear and mostly points towards a supportive influence resulting in intensified disease rigor (Miozzi et al. 2011; Maffei et al. 2014). Moreover, reduced colonization and spore formation is shown by the AMF when the host plant is infected with a viral pathogen like the yellow mosaic virus (Jayaram and Kumar 1995).

**Biocontrol Yeast**

Yeasts such as *Aureobasidium pullulans*, *Cryptococcus albidus*, *Candida oleophila*, *Saccharomyces cerevisiae*, and *Metschnikowia fructicola* are currently being employed as biocontrol agents as they are effective adversaries of various plant pathogens. Yeasts are a category of unicellular fungi that grow in most environments, have simple culture needs and few if any biosafety concerns. They apply competition, volatiles, enzymes and toxins, mycoparasitism, and initiation of immune response mechanisms for plant protection. Due to these properties, they can be exploited as biocontrol effectors but a paucity of studies on their role limits their full utilization (Freimoser et al. 2019).

**Phage based Biocontrol**

Phages have been in use as biocontrol agents against bacterial pathogens for a long time. The earliest study demonstrating their biocontrol ability was done by Mallmann and Hemstreet in 1924 in which they isolated *Xanthomonas campestris pv. campestris* from plant tissues suffering from the cabbage-rot disease. Future studies showed that phages could inhibit soft-rot caused by *Pectobacterium carotovorum subsp. carotovorum* in carrots (Coons and Kotila 1925), a bacterial spot of tomato by *X. campestris pv. vesicatoria* (Flaherty et al. 2000) and *Pectobacterium atrosepticum* in potato slices (Kotila and Coons 1925). More recent explorations into phage biocontrol usage have focussed on improving their durability under field conditions (Balogh et al. 2003). Exploring the use of phage cocktails and systemic acquired resistance activator in disease management against *X. citri subsp. citri* and *Xanthomonas axonopodis pv. citrulme* that causes citrus bacterial canker and citrus bacterial spot respectively showed positive results in field trials (Ibrahim et al. 2017). On the other hand, some studies showed a better disease management response in laboratory-based bioassays rather than in field trials, like in the case of phage treatment against *Pseudomonas syringae pv. porri* that causes bacterial blight of leek.
(Rombouts et al. 2016). However various economically significant bacterial pathogens like *Xanthomonas* spp. and *Pseudomonas syringae* can be effectively controlled by phages. Peptidoglycan hydrolases, lysins from phages Atu_ph02 and Atu_ph03 are capable of blocking cell division in *Agrobacterium tumefaciens* (causes crown gall disease) resulting in its lysis (Attai et al. 2017). Other lysins from CMP1 and CN77 phages have also shown lytic capacity against *Clavibacter michiganensis* subsp. michiganensis, that causes bacterial wilt and canker of tomato (Wittmann et al. 2010). The incorporation of phage lysins into transgenic crops can aid in their easy application and overcome production issues (Wittmann et al. 2016). Mostly, the application of phages and phage lysins in plant disease management is a progressive step and has shown positive outcomes in a number of instances. Focus now needs to be on developing better delivery methods and guaranteeing a longer shelf life for the phage and its enzymes on the host plant (Dy et al. 2018).

### Natural Compounds against Plant Diseases

Bioactive natural compounds can be of plant or animal origin and are capable of controlling plant diseases thereby promoting plant growth. A number of bioactive molecules belonging to phenolic, terpenoid, or alkaloid categories (Freeman and Battie 2008) such as chitin, laminarin, allicin, terpenes, chitosan, naringin, and carrageenans have been identified for use as biopesticides in organic cultivation. Allicin, acquired from garlic exhibits antibacterial and antifungal properties under field conditions (Göellner and Conrath 2008; Pohl et al. 2019; Koziara et al. 2006), garlic juice inhibits the growth of a number of bacteria belonging to the genus *Pseudomonas*, *Agrobacterium*, *Xanthomonas*, and *Erwinia* and fungi *Cercospora arachidicola*, *Botrytis cinerea*, *Rhizoctonia solani*, *Alternaria alternata*, *Fusarium moniliforme*, *Colletotrichum coccodes* (Abdulrahman and Alkhail 2005; Jamiołkowska and Wagner 2011). Naringin (40,5,7-trihydroxyflavanone-7-β-d-α-l-rhamnosyl(1→2)-β-d-glucoside) is another potent bioactive molecule found in seeds and pulp of grapefruit (Céliz et al. 2011) that displays effectiveness against fusariosis, alternariosis, and gray mold infections in soybean, ornamental plants, and vegetables such as potato (Pastucha 2008; Saniewska 2006; Jamiołkowska 2011). Tea tree oil (*Melaleuca alternifolia* L.) contains terpenes like terpinen-4-ol, gamma-terpinene, 1,8-cineole and exhibits strong antimicrobial properties against a variety of bacteria and fungi. It is particularly effective against *Bremia lactucae* and downy mildew that attack lettuce (Angelini et al. 2006; Terzi et al. 2007; Yu et al. 2015). At times the use of bioactive compounds like garlic pulp is more beneficial than synthetic compounds like azoxystrobin as seen in the case of sweet pepper plants (Jamiołkowska and Wagner 2011). Chitin which is the second most abundant polysaccharide in nature and a component of the fungal cell wall and exoskeleton of crustaceans and insects shows bioactivity against a number of bacterial, viral, and fungal pathogens (El Hadrami et al. 2010). It is known to have a strong antifungal influence against soil-borne pathogenic fungi that infect soybean (Pastucha 2008) and is a fungal microbe-associated molecular pattern (MAMP) molecule that is able to activate immune responses in the host plant (Jamiołkowska 2020). It can be isolated using enzymatic reactions and chitosan distillation (Kurita 2006). Bioactive compounds, therefore, show a variety of modes of action not only to limit pathogen growth and multiplication but also inactivation of the host defense response (Babosha 2004). They usually act via binding to the membrane receptors on plants and produce a signal that is capable of initiating an immune response.

### Algal and Cyanobacterial Biocontrol

Apart from being an abundant source of vitamins, saccharides, enzymes, amino acids, phytohormones and elements like molybdenum, boron, manganese, iron, iodine, and zinc, algae...
and cyanobacteria extracts are a rich source of bioactive elicitors (Jimenez 2011; Sultana et al. 2011) with antifungal, antiviral and antibacterial properties (Arunkumar et al. 2010). These extracts are usually applied in agriculture to improve productivity and plant vitality. Use of extracts from the algae *Sargassum filipendula*, *Ulva lactuca*, *Caulerpa sertularioides*, *Padina gymnospora* and *Sargassum liebmannii* ease symptoms of fungal infection on tomato produced by *Alternaria solani* and *Xanthomonas campestris pv. vesicatoria* (Ramkissoon et al. 2017; Hernández-Herrera et al. 2014). Studies on tomato seedlings infected by *Macrophomina phaseolina* showed improvement after the application of *Kappaphycus alvarezii*. The algal action was propagated through improved levels of phytohormones (salicylic acid, indole-3-acetic acid and abscisic acid), transcription of PR-1b1, PR-3, and PR-4 genes, and the cytokinin zeatin (Agarwal et al. 2016). The activity of polyphenol oxidase and peroxidase enzymes important in plant defense in tomatoes was also shown to improve when extracts from *Cystoseira myriophylloides*, *Laminaria digitata* and *Fucus spiralis* were utilized against *Verticillium dahliae* wilt (Esserti et al. 2017). Cyanobacteria against plant pathogens have been applied both at the levels of soil and leaves. Employing *Nostoc entophytum* and *Nostoc muscorum* in the soil against *Rhizoctonia solani* greatly enhanced seedling endurance along with improving root and shoot dry weight and plant length (Osman et al. 2011). In tomato, application of *Nostoc lincizia* in soil against *Fusarium oxysporum* f. sp. *lycopersici* decreased wilt while an improved state of similarly infected seedlings of tomato was observed with *Nostoc commune* (Alwathnani and Perveen 2012; Kim and Kim 2008). Usage of cyanobacteria, *Anabaena* sp. on zucchini cotyledons infected with powdery mildew (*Podosphaera xanthii*) resulted in enhanced enzymatic activity of peroxidases, endochitinase, chitin 1,4-β-chitotriosidase, β-N-acetylhexosaminidase, and β-1,3-glucanase (Roberti et al. 2015). Similar enzymatic activation was observed by Prasanna et al. (2015) upon employing a biofilm composed by *Anabaena* sp. on maize roots and shoots. Cyanobacteria, like algae are also capable of high polysaccharide production in response to various categories of plant pathogens but there is a lack of data which limits their use as biocontrol agents (Singh 2014; Righini et al. 2019; Righini and Roberti 2019).

**EMERGING BIOCONTROL STRATEGIES**

**Microbial Volatilome and its role in the biological control of Plant Pathogens**

One of the most resilient and encouraging solutions in biocontrol approaches is the employment of microorganisms as biological control agents (BCAs). Among the several microbiological strategies used by BCAs, the production of volatile organic compounds (VOCs) is a method that is helpful in situations where the straightforward association between the pathogen and its competitor is not possible. All living forms synthesize VOCs and these can be exploited for usage in biocontrol of plant pathogens like bacteria, oomycetes, and fungi. VOCs are a sustainable preference for synthetic fungicides due to their ease of application, low residue deposition in the environment and on crops, and their biocontrol efficacy (Tilocca et al. 2020). According to Tahir and colleagues (2017), VOCs produced by *Bacillus* species are known to function at a number of levels against the tobacco wilt agent *Ralstonia solanacearum*. In vitro studies indicate that *Bacillus* volatile compounds reduced *Ralstonia* growth and viability and caused significant problems in cell integrity and motility in addition to considerable alterations in *Ralstonia* genes expression that controls disease progression (Tahir et al. 2017). Furthermore, tobacco plants treated with *Bacillus* emissions and purified detected VOCs elevated transcription levels in critical defense-related genes such NPR1 and EDS1, leading to inhibition of systemic
resistance (Tahir et al. 2017). It's possible that bacterial volatiles has a role in *Bacillus* reported biocontrol properties both directly and indirectly, and that bacterial VOC bouquets function as multifactorial, sequential, or simultaneous signals on pathogens and hosts. (Bailly and Weisskopf 2017).

**Microbiome-based solutions for plant protection**

New findings demonstrate a remarkable microbial diversity among all plants, as well as unique phytopathogen antagonistic bacteria. Mosses, which are the world's oldest land plants, exhibit a unique microbial diversity, and their ecology allows them to contain a large number of enemies (Opelt et al. 2007; Bragina et al. 2015). Apart from mosses, medicinal and endemic plants are also likely sources of rare biodiversity and enemies. A characteristic acquired by them due to their unique metabolism, that alters the architecture of the plant microbiome (Köberl et al. 2013b; Zachow et al. 2014). We expect endophytes, particularly seed endophytes, to serve as sources for novel biocontrol agents as a result of new discoveries. Until now, bacteria and fungi have been used mostly for biocontrol. Archaea have just recently been recognized as part of the plant microbiome (Müller et al. 2015); their effects on plants and potential for biocontrol are unknown. Microbial invasion can affect the network of microorganisms that are linked with plants. These network models of soil and plant microbiomes can be interpreted for biocontrol and present new prospects for disease management. While single organisms were commonly utilized in the past, their effects were often uneven, microbiome-based biocontrol techniques are now possible (Berg et al. 2013).

In the future, microbial consortia and biocontrol agents can be employed to improve biodiversity associated with crops via microbiome engineering so as to achieve definitive microbiome outcomes as desired (Erlacher et al. 2014). Crop-specific biological consortia can be assembled from a pool of selected biocontrol agents in this setting. Taking a holistic approach and incorporating microbiome-based solutions allows for targeted and predictive biocontrol measures. Furthermore, integrated breeding and biocontrol measures are essential to sustain ecosystem variety and health. These systemic techniques are necessary to prevent further biodiversity losses and promote sustainable agriculture operations (Berg et al. 2017).

**Phage Cocktail**

Phage cocktails are a feasible option for controlling a variety of plant diseases; however, further study and solutions to technical obstacles are needed to achieve successful biocontrol. Thorough knowledge of interactions between plant, phage, and pathogen is required since the habitat of each plant system is unique and complex. This can only be achieved by conducting more extensive field experiments, as *in vitro* and *in vivo* tests under laboratory conditions do not accurately reflect the real circumstances in the field. More advanced protective formulations are needed to ensure the survival of phage mixtures during long-term storage under ambient conditions. The use of already existing phages from the phyllosphere can provide better protection against the phytopathogens in that environment. To improve phage persistence in the phyllosphere, light-absorbing compounds and/or protective formulations could be added to phages that have evolved to resist UV-induced damage. Synthetic phage cocktails with customized host ranges can also be created using genetically engineered phages. More research is needed in order to obtain well-characterized phages with defined and configurable host ranges. Finally, due to the great diversity of phytobacteria, a single universal phage cocktail for all diseases is not viable. Designing tests that can identify the disease-causing bacteria and its antagonistic phage can greatly aid in its control. To date, no such simple and economical option is either available or implemented (Kering et al. 2019).
Genetically Modified Biocontrol Agents

To improve the efficacy of BCAs, techniques for genetic engineering of all organisms can be used. *Rhizoctonia solani* infection in beans can be brought under control by transferring a gene coding for the enzyme chitinase from *Serratia* to a *Pseudomonas* endophyte (Downing and Thomson 2000). While transferring a gene for glucanase to *Trichoderma* produced resistance to pathogens such as *Rhizoctonia, Rhizopus,* and *Pythium* (Djonovic et al. 2007). Cloning of 2,4-diacetylphloroglucinol (2,4-DAPG) biosynthetic locus phlACBDE from strain CPF-10 into a mini-Tn5 transposon by Zhou et al. (2005) into a mini-Tn5 transposon and its insertion into the chromosome of *Pseudomonas fluorescens* P32 improved resistance of wheat to *Gaeumannomyces graminis var. tritici* and tomato to *Ralstonia solanacearum* bacterial wilt.

Regardless of the findings of this research, these newly produced BCAs are subject to the same restrictions that apply to organisms that have been genetically changed using recombinant DNA technology. Clermont et al. (2011) employed genome shuffling to create superior *Streptomyces melanoporfaciens* EF76 biocontrol strains. Four strains with improved antagonistic activity against the potato diseases *Streptomyces scabies* and *Phytophthora infestans* were isolated after two rounds of genome shuffling. Biological control ability can also be improved by employing chemical mutagenesis. Examples include the use of nitrosoguanidine mutagenesis in *Pseudomonas aurantiaca* B-162 to produce a strain with better phenazine synthesis leading to improved biocontrol activity (Feklistova and Maksimova 2008) and *Trichoderma harzianum* strains that exhibited enhanced biocontrol ability after UV mutagenesis (Marzano et al. 2013). The addition of the required mutation can at times produce altered gene expression in non-targeted genes resulting in undesired effects. These constraints can be overcome using more recently established genome editing approaches. We can insert mutations into specific regions in the genome with high precision and efficiency using techniques like Crispr/Cas (Barrango and van Pijkeren 2016). Another benefit is that mutations can be induced in numerous genes at the same time, which will aid in determining the role of different genes in biocontrol (O'Brien 2017). The gene editing approach could also help in commercialization of BCA’s through ease of regulatory clearances.

Microbiome engineering

Many researchers suggest the microbiome to be representative of a “second genome” (Clavel et al. 2016; Zmora et al. 2016) but some prefer the term “holobiont” to describe the variety of microbes linked with plant and animal hosts (Zilber-Rosenberg and Rosenberg 2008). Microbiome engineering has the potential to have a big impact on agriculture (Mitter et al. 2017). As a result, the creation of an altered microbiome with the desired properties is required. Many recent investigations have revealed that certain endophytic strains can alter the structure and species richness of plant tissues (Patel and Archana 2017; Timm et al. 2016). Very few studies have investigated the internal microbiome of plants for subsequent generations post introduction of a specific strain(s) (Mitter et al. 2017). Moreover, little research has been carried out on the importance of manipulated microbiomes from disease-suppressive soils on control of phytopathogens (Xue et al. 2015). From a practical standpoint, it would be immensely beneficial to establish microbiomes that are durable and stress-tolerant thereby capable of increasing agricultural output (Mueller et al. 2016). Finally, plant microbiome bioengineering is an intriguing option for improving a plant's biological capabilities, an approach that, while still in its infancy, has the potential to be of immense agricultural value. (Del Carmen Orozco-Mosqueda et al. 2018).
CONCLUSIONS
Finally, the ever-growing demand for food has led to dependence on chemicals in agriculture that are hazardous to human health. These chemical-based formulations not only create ecological imbalance but also result in ecotoxicity. Organic methods of farming are preferred for sustainable agriculture but their use incurs high costs that make them inaccessible for most farmers in poor countries. The adoption of diverse biocontrol methodologies, such as those used in organic agriculture, to control plant diseases is environmentally benign, relatively inexpensive, harmless, and have enough potential to significantly boost plant production. As a result, these biocontrol techniques offer enormous benefits for successful rhizosphere management for a sustainable agriculture. The ultimate goal for biocontrol agents is to integrate microbial biofertilizers, biocontrol microorganisms, phages and phage-based technologies and cocktails, biocontrol yeasts, algae, and cyanobacteria, optimized microbiomes, genetically modified biocontrol techniques, and microbiome engineering. An intelligent experimental trial using a combinatorial approach utilizing all the resources from the strategies discussed would invariably provide enormous leads that could be harnessed by the field plant growers to combat plant diseases. At present, this is an under-researched subject that has the potential to increase crop yields while also addressing food security in an environmentally safe and sustainable manner.

Table 1: Plant pathogen and their biocontrol strategies

| PATHOGEN | HOST | BIOCONTROL STRATEGIES | REFERENCES |
|----------|------|-----------------------|------------|
| Phytophthora sojae, Pythium heterothallic, Pythium irregulare, Pythium sylvaticum, and Pythium ultimum, | Glycine max | Pseudomonas water derived strain, 06C 126, effectively inhibited oomycetes | Wagner et al. (2018). |
| Soilborne fungal pathogens, | Pulses, grapes, cotton, onion, carrot, peas, plums, maize, apple, etc. | The fungal genus Trichoderma has biocontrol activity against fungi and nematodes | Kumar and Ashraf (2017). |
| Phytopathogenic microorganisms in agriculture or even in other areas | Endophytic *Bacillus toyonensis* BAC3151 | Lopes et al. (2017) |
| Phytopathogenic fungi | *Trichoderma* spp potential biocontrol agents | Silva et al. (2019). |
| Phytophthora spp. and Pythium spp | Aquaponics | Antagonistic microorganisms |
| Soil-borne pathogens | Pathogen-suppressing microorganisms | Meisner and De Boer (2018). |
| Category                        | Description                                                                 | Cause                                                                                           | Reference                  |
|--------------------------------|------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|----------------------------|
| Broad range of plant pathogens | Antibiotics, lipopeptides, and enzymes with antagonistic properties against a range of plant pathogens are produced by *Bacillus* species. These bacteria also influence resistance development in plants and stimulate plant growth. | *Ralstonia solanacearum, R. pseudo solanacearum, and R. syzygii subsp. indonesiensis* causative agents of bacterial wilt | Shafi et al. (2017).       |
| Hosts                          | Ralstonia solanacearum, R. pseudo solanacearum, and R. syzygii subsp. indonesiensis | Losses range from 100% in banana, 90% in potato and tomato and around 20-30% in peanuts and tobacco. | Álvarez and Biosca (2017). |
| Fungal and bacterial phytopathogens | Many crops                                                                  | Streptomyces spp as Endophytes mediated biocontrol of phytopathogens                             | Vurukonda et al. (2018).   |
| Pathogens in the crop residues | Cereal crops                                                                 | Microbiome-based biocontrol strategies                                                             | Kerdraon et al. (2019).    |
| Fungal pathogens | Cereal crops | Streptomyces species produce a range of secondary metabolites that can inhibit the growth of phytopathogens | Newitt et al. (2019). |
|------------------|-------------|-------------------------------------------------------------------------------------------------|---------------------|
| Plant fungal pathogen | Improved control obtained with by combinations of fungicides and BCAs (Trichoderma spp. or Bacillus spp.,) | Ons et al. (2020) |
| Diseases caused by fungi, bacteria, viruses, viroids, nematodes, and oomycetes | Citrus sp | Employment of antagonists produced by Bacillus sp. offers superior capacity to restrict diseases in citrus plants | Chen et al. (2020) |
| Rhizoctonia solani that induces stem canker, Fusarium solani causes tubers dry rot, and black scurf and Alternaria solani that induces early blight. | Potato | Endophytic bacteria from Romanian potato tubers isolate 6T4 identified as B. atrophaeus/subtilis revealed promising perspectives for biocontrol strategies. | Boiu-sicuia et al. (2017) |
| Phytopathogen                          | Host                  | Microorganism                                      | Reference                        |
|---------------------------------------|-----------------------|----------------------------------------------------|----------------------------------|
| *Fusarium oxysporum* and other phytopathogens | Wheat                | *Bacillus amyloliquefaciens subsp. plantarum* XH-9 is a rhizobacterium with antagonistic potential against a variety of phytopathogens. It discharges antibiotics and enzymes that are capable of bringing about hydrolysis in the pathogen. | Wang et al. (2018) |
| *Verticillium dahliae* soil borne pathogen | Cotton              | Endophytic *Fusarium solani CEF559* against *Verticillium dahliae* in Cotton Plant | Wei et al. (2019) |
| Fungal Pathogens                      |                      | *Trichoderma* is a fungal genera having antagonistic activity against disease causing fungal pathogens. | Adnan et al. (2019) |
| **Fusarium** head blight (FHB) | **Wheat** | Endophytic **Anthracocystis floculossa** P1P1, **Penicillium olsonii** ML37, **Sarocladium strictum** C113L, and **A. floculossa** F63P exhibit the ability to act as biocontrol agents against FHB. | Rojas et al. (2020) |
| --- | --- | --- | --- |
| Fungi **Ustilaginoidea virens**, **Alternaria alternata**, **Fusarium oxysporum**, **Botrytis cinerea**, **Fulvia fulva**, and **Fusarium graminearum** | **Tomato** | Antifungal metabolites of **Bacillus velezensis** NKG-2, | Myo et al. (2019) |
| Bacterial phytopathogen **Pseudomonas syringae** pv. **tomato** | **Tomato** | **Pseudomonas segetis** strain P6 isolated from the rhizosphere has the ability to induce plant growth and inhibit quorum sensing abilities of bacterial pathogens. | Rodríguez et al. (2020) |
| Pepper gray mold caused by **Botrytis cinerea** | **Pepper** | Can be controlled efficiently by the biocontrol mediator **Bacillus velezensis**. | Jiang et al. (2018) |
| Seed and soil borne pathogens. | Chaetomium globosum functions as an effective potential biocontrol agent | Aswini (2019) |
|-------------------------------|-------------------------------------------------------------------|----------------|
| Fungal Pathogen | Endophyte and epiphyte microbiome of Grapevine leaf as biocontrol agents against phytopathogen | Bruisson et al. (2019) |
| Fungal pathogen | Vitis vinifera | Bacillus licheniformis GL174 culturable endophytic strain isolated from Vitis vinifera cultivar Glera, | Elsayed et al. (2020) |

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