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FUNGI IN BIOLOGICAL MANAGEMENT OF PLANT DISEASES: CURRENT AND FUTURE PERSPECTIVE

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ABSTRACT

The fungal genera have a wide range of natural and commercial applications. Apart from the pertinent role in the ecosystem, the antagonistic characteristics of the fungal species have been utilised as biological control agents (BCA) in plant disease management (PDM). PDM is an integral component of agriculture as millions of agricultural produces are lost due to plant diseases annually. With the current emphasis on sustainable developments in the agriculture sector globally, the green approach offers a safe control against plant pathogens. Disease suppression by fungal biological control agents (FBCAs) is also comparable to the synthetic chemical application due to the current advancement in technology. Alas, despite the huge number of candidates screened and identified as potential FBCAs, the commercialisation of these FBCAs does not succeed as anticipated. Therefore, this review comprehensively highlights and discusses fungal genera as BCA, and the necessary changes required in research and development to enhance PDM. The future research on PDM needs to shift from its current focus on traditional screening and targeting modes of action in FBCA to strengthening the formulation, effective delivery modes, microbial population persistence and influence of environmental parameters to achieve a successful control.

Keywords: biological control agents (BCA), fungi, plant disease management, plant pathogen, mode of applications.

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FUNGI AS BIOLOGICAL CONTROL AGENT

The fungal kingdom has an omnipresent distribution globally with niche roles in the ecosystem. They are typically recognised for their excellent ability in decomposition, nutrient recycling, food source and symbiosis. Fungi emerge as efficient ecosystem engineers, regulators, bioindicators and bio-controllers with no particular reference to the occupied ecosystem (Frąc *et al*., 2018). Soil ecosystem for instance, is most probably one of the ecosystems where fungi elicit a strong influence of three fundamental components: (1) biological controllers, (2) ecosystem regulators, and (3) species participating in organic matter decomposition and compound transformations (Gardi *et al*., 2009; Swift, 2005). Among these niche roles, research on fungi as biological control agents (BCA) has progressed the most in the past 80 years (Tariq *et al*., 2020), with intense acceleration in the last 30 years due to the increased awareness globally for the safe production of food. Evidently, fungi biocontrol agent (FBCA) has been recognised as one of the most potent and powerful alternatives to replace synthetic pesticides and fungicides (Lee *et al*., 2013).

The undesired presence of organism that causes high yield losses in agriculture is termed pests and diseases (P&D). It is reported that approximately 25%-40% of yield losses in agriculture and horticulture systems are due to P&D attacks; this figure constantly changes and sometimes worsens due to biotic and abiotic influence (Lugtenberg,

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2015). Pests and diseases affect the plants' physiological processes in several ways: reduced photosynthesis, disruption in water and nutrient translocation, growth retardment and others. If not attended or intervened at an early stage, these physiological damages will cease the potential growth in plants and eventually affect their yield. The yield losses will immediately affect food security, causing some serious disturbance and balance to the food supply. Other consequences of these P&D damages include financial implications at the local, regional and national levels, with the worst implications leading to famine or loss of life (O'Brien, 2017). To date, the potential threats to plant health have been prevented, mitigated, and controlled using chemical applications, resulting in rapid recovery and reduced losses. However, the continuous and excessive use of these chemicals over the years has brought up environmental and sustainable concerns. Plants developing resistance, environmental pollution, aquatic ecosystem disruption, residual effect on human health, reduced soil fertility and biodiversity loss are among the distresses expressed globally with overusing chemicals (Lee *et al*., 2013; Tariq *et al*., 2020). Therefore, the use of FBCAs in integrated pest management (IPM) is highly recommended since the mode of action of these microbial inoculants is species-specific, resulting in harmless contact with the host and other non-specific residential species within the same ecological niche.

Over the years, advancements in research have enabled plants to achieve exceptional yield with the improvements made through breeding, genetics, good agronomic practices (GAP) and fertiliser applications. However, these improvements can substantially be affected by ineffective P&D management. With considerable interest in securing control measures that are cost effective, green and environmentally friendly, the application of FBCA has been welcomed as an integral part of GAP and plant disease management (PDM), and carefully incorporated into the integrated pest/disease management (IPM/IDM). With an expansive exploration over the years, the approach in FBCA is the deliberate application of indigenous or introduced beneficial microbial inoculants to reduce harmful activities of one or more P&D (Tariq *et al*., 2020). The comprehensive control by FBCA prevents and controls P&D incidences through single or combined mechanisms by limiting the growth factors, such as nutrients and space; parasitising through various physical and chemical modes; enhancing immunity; and finally creating an unfavourable abiotic and biotic microenvironment for pests (Köhl *et al*., 2019; O'Brien, 2017). More recently is the use of endophytes in plant protection consisting of different groups of microorganisms since a broad diversity of fungal and non-fungal endophytes is associated with nearly all plants (Wani *et al*., 2015). Endophytic microorganisms can be represented by bacteria, fungi, actinomycetes or viruses (Bao and Roossinck, 2013; Hassan *et al*., 2017; Sundram, 2013; Sundram *et al*., 2015;) while expressing a variety of symbiotic lifestyles ranging from parasitism to mutualism, and inducing resistance according to the genotype of the host plant and/or environmental conditions. A series of fungal endophytes are already in large-scale productions, such as commercial BCA: *Lecanicillium lecanii*, *Paecilomyces lilicinus*, *Beauveria bassiana*, *Fusarium oxysporum*, *Trichoderma harzianum*, *T. virens, Piriformospora indica* and others (Card *et al*., 2016; Mendoza and Sikora, 2009; Sikora *et al*., 2010; Sundram, 2013).

Fungi are one of the most successful BCA candidates that have been screened, characterised, identified, and formulated into fungal or FBCA products, targeting specific pests or pathogens. Even though other organisms such as bacteria and invertebrates (such as parasitoids) have shown potential as BCA, fungi are the most studied, documented and applied as BCA in PDM (Schrank and Vainstein, 2010). Several species of fungi from various genera, such as *Purpureocillium*, *Metarhizium*, *Beauveria*, *Cordyceps*, *Fusarium*, *Trichoderma*, *Clonostachys*, *Phoma* and others have been reported to have the FBCA, commercialisation and application potential in the field (Baron *et al*., 2019; Sun *et al*., 2020). The list in *Table 1* shows the commercially available products with fungi as the active microbe in the last 10 years. The versatile use of fungi has allowed researchers to utilise these multi-trait microbes by optimising specificity, formulation, delivery, application intervals and dosage to dramatically improve IPM holistically. In this review, we focus on the exclusivity of fungi being utilised as effective BCAs against plant diseases by discussing the process flow in the development of FBCA, followed by underlining its modes of delivery while further emphasising the delivery dynamics, success stories, research priorities and challenges in the strategic direction of future IDM.

PROCESS FLOW – SUCCESSFUL DEVELOPMENT OF BIOCONTROL PRODUCT

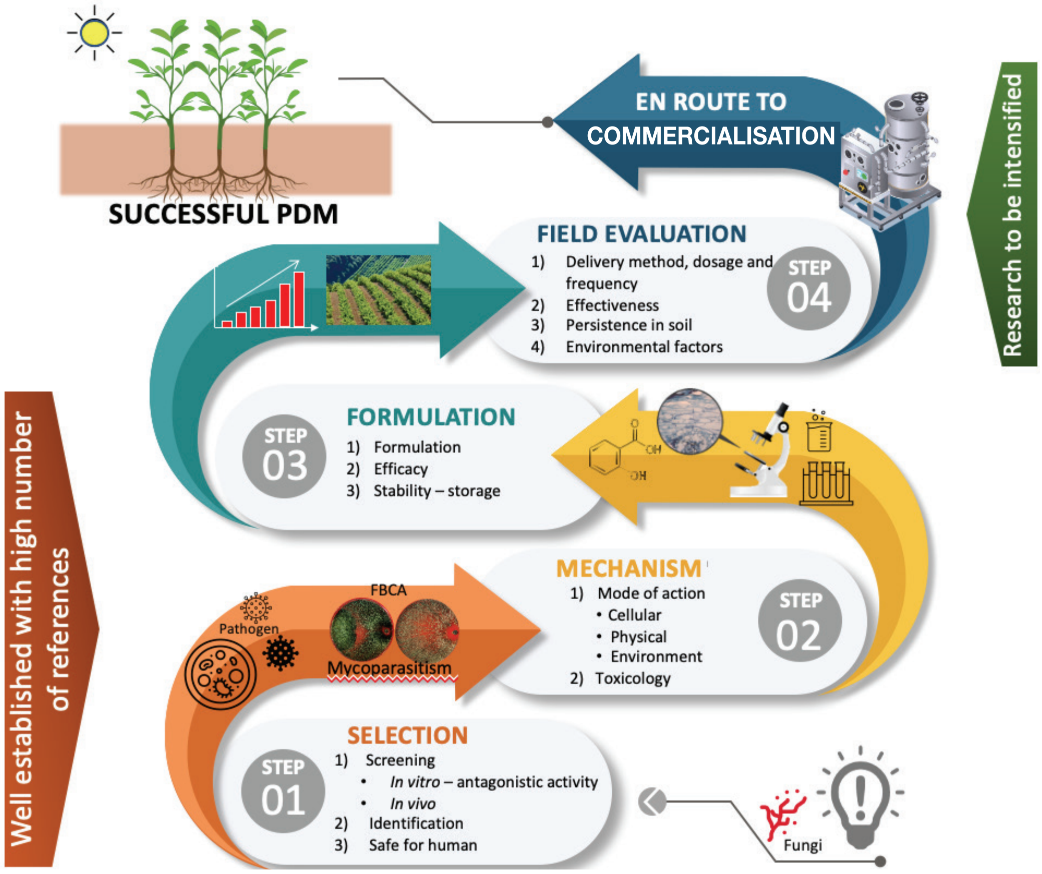
Successful development of a biocontrol product depends on standard process flow involving four important components highlighted in *Figure 1*: (1) selection/screening, (2) mechanism, (3) formulation and (4) field evaluation. Each of the components requires careful planning and execution to eventually determine the success of the developed FBCA. The following section will briefly highlight the four components of the process flow.

Selection

The preliminary selection of potential FBCA is determined based on the inhibition demonstrated on the target pathogen or pest, and this assessment generally takes place in the laboratory via simple screening bioassays. Generally, the selection and screening of FBCA candidates are from the rhizosphere or the endophyte population. The genus *Trichoderma* is prominent among those species of rhizosphere and the endophytic population that are effective as FBCAs (*Table 1*). However, a successful candidate via the *in vitro* screening does not necessarily warrant the successful selection of effective FBCA (O'Brien, 2017). This is because there are other mechanisms other than pathogen inhibition that can be utilised for selection of successful FBCA such as host growth and defence stimulation apart from occlusion of the pathogen but this will require further exploration. The screening assays are followed by the identification, and the preliminary results on the target species will be explored further at the nursery or field. These few initial steps described for the FBCA selection are interchangeable depending on the suitability and funding strength of the research laboratory. Meanwhile, the selected FBCA candidates are also required to be safe to humans, plants and animals. The effectiveness of FBCA in suppressing target organisms is highly dependent on the myriad modes of action inherently present within the FBCA inoculants. This characteristic will determine the success of the application and the consistency of the FBCA in the given environment.

Mechanism

Most microbial inoculants, including fungi, specialise in suppressing the pathogen of interest with either one or more modes of action. Prominent among the fungal genera as FBCA is the genus *Trichoderma* which is the most researched, leading to the successful production of biofungicides (Abbey *et al.*, 2019). The modes of action can be divided according to their nature and the changes taking place: (1) physical, (2) cellular, and (3) environment. For instance, hyperparasitism falls into the physical category because the entire process involves unique physical activity, such as hooking and coiling of the target pathogen as reported by Sundram (2013). Besides that, D'Ambrosio *et al.* (2022) reported the mode of antagonism against soil-borne phytopathogens as deadlock at a distance or with initial contact,



Note: FBCA - Fungal biological control agents.

Figure 1. Flowchart describes four specific processes in developing microbial-based biological control agents and the research needs to be addressed for a successful plant disease management (PDM) in the field.

TABLE 1. EXAMPLES OF COMMERCIALLY AVAILABLE BIOLOGICAL CONTROL PRODUCTS DEVELOPED IN RECENT YEARS USING FUNGI AS ACTIVE INGREDIENT FOR VARIOUS PLANT DISEASES GLOBALLY

Biological control agent (BCA)	Crop/Disease	Causal pathogen	Commercial products/Supplier	References
Coniothyrium minitans	Oilseed rape/ stem rot	*Sclerotinia sclerotiorum*	Contans® WG/ Prophyta Biologischer	Zeng *et al*. (2012)
Fusarium oxysporum non-pathogenic	Wilt	*Fusarium oxysporum*	Fusaclean; Biofox C/ Natural plant protection, France; S.I.A.P.A	Thambugala *et al*. (2020)
Gliocladium catenulatum	Strawberry/ grey mold	*Botrytis cinerea Pers:Fr.*	Prestop-Mix/ Verdera OY, Finland	Karise *et al*. (2016)
Pythium oligandrum	Red clover/ root rot	*Fusarium*	Polyversum/ Bioreparaty spol. s r. o., Czech Republic	Pisarčik *et al*. (2020)
Trichoderma virens G-41	Root disease	*F. oxysporum*	Rootshield® Plus^{+} / BioWorks, Victor, NY, United States	Li *et al*. (2018)
T. harzianum T-22	Root rot	*Sclerotia*	PlantShield® HC/ BioWorks, Victor, NY, United States	Derbyshire and Denton-Giles (2016)
Gliocladium virens GL-21	Root rot	*Pythium, Rhizoctonia,*	SoilGard® / Certis USA LLC	Castañé *et al*. (2020)
T. viride	Citrus/ root rot wilt	*Fusarium solani*	Trieco/ Ecosense Labs Pvt. Ltd., Mumbai, India	Kumar and Ashraf (2017)
T. asperellum T34	Ornamental plants/ root rot	*Fusarium, Rhizoctonia, Pythium, Phytophthora*	Asperello T34/ BIOBEST USA	Preininger *et al*. (2018)
Phlebiopsis gigantea	Root rot diseases	Heterobasidion	Rotstop®/ Kemira Agro Oy, Helsinki, Finland	Bruna *et al*. (2020)
Muscodor albus	Root rot, damping off and wilt	-	Andante™/ AgraQuest, Inc.	Mao *et al*. (2019)
Hendersonia toruloidea	Oil palm/ basal stem rot	*Ganoderma boninense*	GanoEF Biofertilizer/ All Cosmos Industries Sdn. Bhd., Malaysia	Peng *et al*. (2020)
T. virens 159c	Oil palm/ basal stem rot	*G. boninense*	Trichoshield/ True *Trichoderma* Technology Sdn. Bhd., Malaysia	Sundram *et al*. (2016)
Ulocladium oudemansii	Strawberry, grapes/ Fruit rots	*Colletotrichum, Rhizopus, Aspergillus* and *Alternaria*	BOTRY-Zen®/ BOTRYZen Ltd, Dunedin, New Zealand	Thomidis *et al*. (2015)

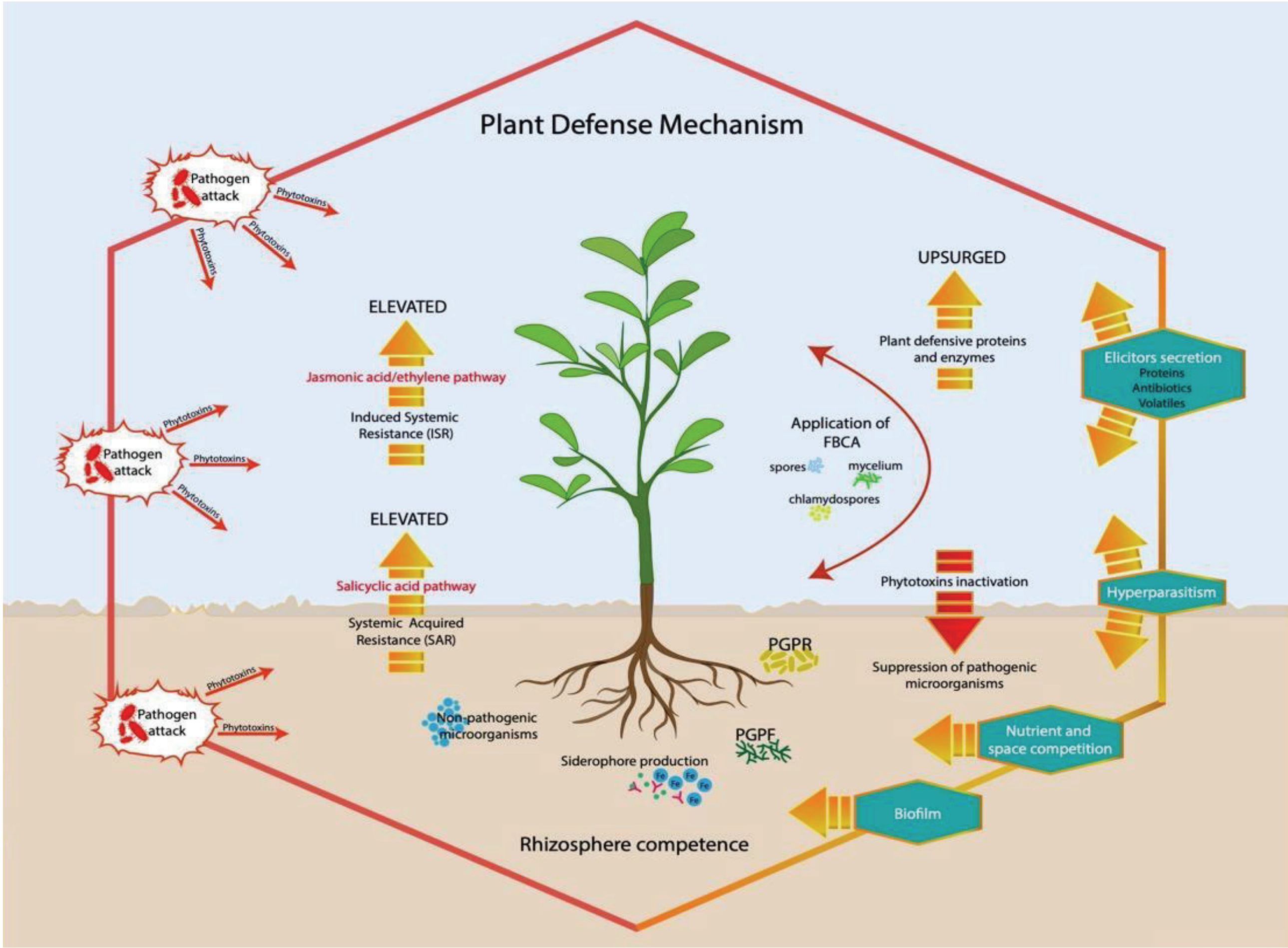
and partial or complete replacement of the tested phytopathogens in laboratory studies.

The cellular production of potent antibiotics, secondary metabolites and induced immunity are categorised under cellular via pathways affecting either the pathogen or the plant directly or indirectly. *Trichoderma virens* strain 7b has been reported as an active producer of siderophore and antifungal compound such as phenylethyl alcohol (PEA) (Angel *et al*., 2016). Besides that, volatile organic compounds of *Muscodor albus*, *Muscodor roseus* and *Fusarium oxysporum* have been reported in reducing the inoculum density of *Verticillium dahliae* in soil, and suppressing *Verticillium* wilt in eggplant and cotton (Stinson *et al*., 2003; Zhang *et al*., 2015).

Finally, the environment category is attributed to the space and nutrient availability to determine the effectiveness of FBCA by competing for the acquisition of nutrients and survival in the field. These modes of action may also intertwine or provide a combined effect on the target pathogen. *Figure 1* illustrates the combination of modes of action through the application of FBCA and their effects on the plant defence system.

Formulation

The selected biocontrol-potential isolates with strong antagonistic activity towards the pathogen will next be tested under a greenhouse condition using the microbial propagule. The main objective of this assessment is the interaction of the host or host tissues, pathogen and antagonist under controlled conditions that represent the targeted epidemiological perspective and environmental conditions of the crop (Köhl *et al*., 2011). The greenhouse conditions would evaluate the mechanisms related to biocontrol activity without significant interference from the external environmental characteristics and parameters, such as soil type, nutrient availability, and microbial diversity under a semi-natural condition. When the application of FBCA leads to positive results in the PDM, a suitable formulation for a large-scale application is developed because the delivery of the free-cell form is usually impractical. A stable formulation that can withstand extreme environmental conditions is required for a high-quality commercial product. These formulations may be simple mixtures of natural ingredients with



Note: FBCAs - Fungal biological control agents; PGPR - Plant growth promoting rhizobacteria; PGPF - Plant growth promoting fungi.

Figure 2. Plant defence mechanism complemented by the various components of microbial community and the different modes of action.

specific activities or complex combinations with multiple effects on the host or the target pest or pathogen (Nega, 2014). Narayanasamy (2013) has listed several desirable attributes of any BCAs that can be selected for the formulation stage. These attributes are the ability to inhibit target pathogens at low doses; production of antimetabolites or toxins against the target pathogen; increased tolerance to fungicides or other plant protection chemicals and plant activators; and improved adaptability to general crop management practices. It is critical to note that a stable formulation improves the shelf life, stability and field performance of the BCAs (Abdallah *et al*., 2018; Ravensberg, 2011). Besides that, the formulation must sustain the FBCA's viability during storage preferably for 18-24 months at room temperature (21°C), and it is still effective when used. The potential FBCA must also be user-friendly and cost-effective. Xue *et al.* (2014) have reported the successful formulation of *Clonostachys rosea* ACM941, with guaranteed efficacy in controlling *Fusarium* head blight in corn, soybean and wheat under field conditions compared to most field trials that used a conidial or spore suspension of the BCA which yielded inconsistent results.

Field Evaluation

Generally, FBCA inoculant often give promising reproducible results under controlled lab and greenhouse conditions. However, not all successful antagonists in the *in vivo* assessments produce similar effectiveness in natural field environments (Abdallah *et al*., 2018). In order to effectively work under the natural environmental conditions, the FBCAs should be able to survive and maintain their population within the habitat that favours the pathogen that causes severe damage to the host plant (Ghorbanpour *et al*., 2018). Various environmental factors, such as the soil characteristics including temperature, UV, low relative humidity, pH, nutrient availability, presence of chemical pesticides or fungicides, metal/heavy metal ions, and microbial community are often reported as the parameters that influence the growth of the FBCAs and eventually affect the biocontrol activities (Abdallah *et al*., 2018; Ghorbanpour *et al*., 2018). Dagno *et al.* (2011) have demonstrated that the percentage of viable conidia and radial growth rate of three fungal biological control agents of water hyacinth (*Eichhornia crassipes*) decreased with the decreasing water activity. Meanwhile, *Cadophora malorum* was found to be more tolerant to low water activity, and *Fusarium sacchari* was more tolerant to high temperature (35°C) (Daryaei *et al*., 2016; Domingues *et al*., 2016). In another study, an optimum *T. atroviridae* LU132 conidia production was observed at pH 6.5, and an optimum conidium fitness with maximum germination and inhibition of *Rhizoctonia solani* colonies occurred at pH 7.5 (Daryaei *et al*., 2016). A better understanding of the effect of abiotic and biotic interactions with FBCA is necessary to determine the optimal dose and timing of FBCA application. The susceptibility of the host, environmental conditions and agricultural practices are some of the factors that affect the FBCAs application timing. The application technology significantly impacts the efficacy of FBCAs. The application technology should involve standard non-costly agricultural equipment, targeted delivery, deposition, and coverage of the infection for significant disease control. Although the field trials are exhausting and time-consuming, the delivery method, application time, effective dose, and best formula are critical to precisely evaluate the performance of the selected FBCA and to ensure effective control of the phytopathogenic microorganisms. The importance of modes of delivery and the different methods of a delivery system in FBCA system is highlighted in the next section.

MODES OF DELIVERY AND APPLICATION OF FBCA

Apart from establishing a suitable formulation for the FBCA application, the concurrent research on delivery methods enables significant FBCA efficiency. The formulation process is imperative to stabilise effective microorganisms (EM), such as fungal propagules during mass production, distribution, and storage (Leggett *et al*., 2011). In addition, effective formulation of FBCAs is essential in handling and applying the final product; protecting the EM from harmful environmental factors (*e.g.*, temperature, moisture, pH); achieving acceptable shelf life; and improving the activity of the FBCAs (Saberi-Riseh *et al*., 2021). Once the formulation is stabilised, it is delivered through several means, relying primarily on the pathogen's survival nature and mode of infection. The introduction of FBCA into the plant ecosystem is the basic principle to enable their multiplication or survival near or within the specific pathogen entry sites in the host plant (Bonaterra *et al*., 2012). The FBCA formulation is applied through inoculative, augmentative, or inundative strategies based on the modes of action of the FBCAs. The inoculative and augmentative strategies involve applying a low initial FBCA population, which then multiplies and reaches an effective population threshold, enabling the control of plant pathogens (Bonaterra *et al*., 2012). On the contrary, the inundative strategy is based on the same principle of microbial pesticides, whereby the FBCAs are applied generally at high concentrations. In order to ensure a wide range of conditions for the application in practice, an approximate dosage of 10^7 CFU/mL is recommended for FBCAs (Bonaterra *et al*., 2012).

A number of liquid or dried formulations are being used as the FBCA application in the market (Bonaterra *et al*., 2012). Therefore, antagonistic FBCAs must be formulated and applied in an appropriate method to allow successful colonisation and persistence in the desired ecological niche, such as soil rhizosphere. These various application modes include seed treatment, soil/foliar application, stump, microencapsulation, organic amendments and a workable combination of different methods. A summary of each effective mode of a delivery system for the application of FBCA is provided in *Table 2*. Apart from identifying an effective delivery method for FBCA application, equally important is the application frequency at the appropriate intervals. Studies have shown that failure in applying FBCA at a suitable interval to suppress a disease will result in insignificant PDM (Bonaterra *et al*., 2012). Moreover, FBCA is effective as a preventive measure and able to be integrated with other disease management options, such as cultural practices, particularly in situations when the disease has established. Generally, the efficiency of any FBCA is strongly dose-dependent because it is affected by the relative aggregates between the pathogen and FBCA association within the plant defence mechanism (Yan and Khan, 2021).

DISCUSSION

Overview of FBCA in IDM

One of the sustainable development goals (SDG) in agriculture is to ensure safe and optimal food production for the booming global population. In line with this, the conventional approach to manage plant diseases using synthetic chemical applications has been flagged due to the implications for human and the environment. Nonetheless, it may be too ambitious to entirely discontinue chemical applications in disease or pest management because they provide an immediate solution to cease a P&D outbreak that may cause even greater damage. Moreover, stringent regulations govern the safe use of synthetic chemicals in the agriculture sector. Leveraging on the sustainable component development in the agriculture sector, the naturally existing microbial communities can be applied in PDM, and this practice has intensified over the past few decades. The traditional approach of using the already naturally occurring microbes as a component in the IPM is well received due to the negligible impact on humans and the environment. It is considered one of the rising alternate solutions due to its conservative, dependable, and eco-friendly approach in PDM. Apart from being a residential or introduced beneficial microbe, the application of FBCA has also demonstrated increased yield, root mass and plant health in general (O'Brien, 2017). Additionally, FBCA fortification in the relevant environmental niche, including rhizosphere, endosphere or phyllosphere, enriches the resident microbiome component as opposed to the chemical application that causes more harm.

The search for a biological agent is an ongoing continuous effort and the existing impactful publications can guide present and future researchers. With the continuous progress in research, the traditional screening of FBCAs will become a thing of the past as biotechnological tools allow for a rapid selection of candidates to leverage specific genes and proteins (Lee *et al*., 2013). Thus, when identifying a potential microbe becomes simpler, we will witness the improvement in the commercialisation of the FBCAs. This is because, despite a large number of effective FBCAs against plant pathogens being identified, only a few have been commercialised for sustainable management of diseases. This applies to not only FBCAs, but also all BCA products. The following section of the paper will underline the challenges and issues leading to the low rate of FBCA commercialisation, and finally, highlight the specific components for a successful field exploration of FBCA. The limitation of the commercial success of FBCA products can be divided into three main components: the technical aspects of the FBCA development, the customer's financial strength, and the manufacturing and regulatory processes to support the adoption and commercialisation of the FBCAs.

Technical

Apart from identifying the potential candidate of FBCA and understanding disease epidemiology and current disease-control strategies, extensive assessments are required to market FBCA products locally or internationally (Velivelli *et al*., 2014). The technical component is by far the most important as it relates to the final product and its efficacy in the field apart from considering all aspects of the formulation and delivery system. Some of the bottlenecks in marketing a good FBCA are impractical dosage recommendations with limited or variation in control efficacy, and an unrealistic delivery system (Singh *et al*., 2016). Additionally, rushing the process from field trials to commercialisation can severely affect the final product efficacy. Besides that, inconsistent effectiveness of FBCA when evaluated in field trials is one of the major constraints that prevent potential control of diseases. This may have been observed due to various reasons, including extrinsic

TABLE 2. MODES OF DELIVERY AND APPLICATION OF FUNGAL BIOLOGICAL CONTROL AGENTS (FBCA) IN PLANT DISEASE MANAGEMENT (PDM)

Plant disease/ host (Pathogen)	Biological control agents (BCA's)	Mode of delivery and application of FBCA	In combination	Observed efficacy	References
Seed treatment					
Wet root rot/ Mungbean (*Rhizoctonia solani*)	*Trichoderma virens*	Seed dressing	+ Soil applications + fungicides carboxin	Field - increasing seed germination, shoot and root lengths and grain yield and reducing wet root rot incidence in mungbean	Dubey *et al.* (2011)
Web blight/ Urd and Mungbean (*R. solani*)	*T. virens* *Gliocladium virens*	Seed treatment	+ Rhizobium + fungicides vitavax	Highest seed germination, plant vigour, number of root nodules and grain yield with minimum seedling mortality and disease intensity	Dubey (2003)
Chickpea wilt/ Chickpea, *Cicer arietinum* (*Fusarium oxysporum* f. sp. *ciceris*)	*T. viride*, *T. harzianum*, *T. virens*	Seed treatment	+ Fungicide carboxin *T. harzianum* (106 spores/mL/10 g seed) and carboxin (2 g kg^{-1} seed) for seed treatment	Green house - enhancing seed germination, root and shoot length, and decreasing wilt incidence	Dubey *et al.* (2007)
Damping-off/ corn (*Phythium* and *Fusarium* spp.)	*G. virens* isolate Gl-3 and *Burkholderia cepacia*	Seed treatment	10 g samples of seeds (infiltrated and non-infiltrated) mixed with 8 mL sticker, Pelgel and 1.2 g biomass powder	Effectively reducing the disease severity of root rot and increasing the plant growth in terms of seedling stands, plant height, and fresh weight	Mao (1998)
Dry root rot (*R. bataticol*a)/ Chickpea, *Cicer arietinum*	*T. harzianum*	Seed treatment (5 g/ kg seed)	+Fungicide mancozeb + carbendazin	Seed treatment with *T. harzianum* @ 5 g/kg seed + *P. fluorescence* @ 5 g/ kg seed followed by soil drenching of mancozeb 50% + carbendazim 25% WS @ 3 g/L water to the infected and surrounding plants showed percent reduction of 71.50% for dry root rot.	Deepa *et al.* (2018)
Cucumber wilt (*F. oxysporum* f. sp. *cucumerinum*)/ Cucumber, *Cucumis sativus* L.	*Penicilium* sp., *Lasiodiplodia theobromae* and *Hypocrea* sp. (endophytic fungi)	Seed treatment (spore suspension 1.6 x 10^6 spores/ mL)	+ Soil drenching	Endophytic fungi suppressed *Fusarium* wilt, and significantly increased the plant height, aerial fresh and dry weight of cucumber plants.	Abro *et al.* (2019)
Cucumber wilt (*Phythium* spp.)/ cucumber	*T. harzianum* strain 1295-22	Seed treatment	+ Binder, Pelgel or Polyox N-10	The coating provided a physical barrier that delayed the pathogen attack and resulted in a conducive environment for *T. harzianum* growth (sown in a *Pythium*-infested soil in a laboratory bioassay).	Taylor *et al.* (1991)
Wet root rot (*R. solani*)/ Mungbean	*T. virens*	Seed dressing	+ Soil applications + fungicides carboxin	Field - increasing seed germination, shoot and root lengths and grain yield and reducing wet root rot incidence in Mungbean	Dubey *et al.* (2011)

TABLE 2. MODES OF DELIVERY AND APPLICATION OF FUNGAL BIOLOGICAL CONTROL AGENTS (FBCA) IN PLANT DISEASE MANAGEMENT (PDM) (continued)

Plant disease/ host (Pathogen)	Biological control agents (BCA’s)	Mode of delivery and application of FBCA	In combination	Observed efficacy	References
Foliar treatment					
Powdery mildew (*Uncinula necator*)/ grapes	*T. harzianum* and *T. atroviride*	Foliar suspensions (applied at 10^8 spore/L)	none	Improving crop yield and increasing the total amount of polyphenols and antioxidant activity in grapes	Pascale *et al.* (2017)
Stem rot (*Botrytis cinerea*)/ tomato	*T. harzianum*	Spore suspension in water 0.1 g/100 mL, 10^6 cfu/mL	none	Simultaneous inoculation and pre-inoculation with *T. harzianum* gave good control of *B. cinerea* (50% and 90% disease reduction, 10 days after inoculation)	O’Neill *et al.* (1996)
Powdery mildew and Gray mould (*Sphaerotheca fusca* and *B. cinerea)*/ cucumber	*T. harzianum* T39 (TRICHODEX) and Ampelolyces quisqualis (AQ10)	Foliar spray	none	Reducing powdery mildew severity by up to 97%-98% but its efficacy declined as the epidemic progressed	O’Neill *et al.* (1996)
Grey mould (*B. cinerea*)/ tomato	*Fusarium semitectum* 252 and *T. harzianum* 118	Foliar suspension (applied at 10^7/ mL spore/mL)	none	Foliar spray with both strains significantly reduced disease incidence (65%-95%) and severity (50%-77%) on tomato crop	Dal Bello *et al.* (2011)
Root treatment/Root dipping					
Root-rot nematode (Meloidogyne javanica) and *Fusarium* wilt (*F. oxysporum* f. sp. *lycopersici*/ tomato (*Solanum lycopersicum L.*)	*Purpureocillium lilacinum* (PL) and *Trichoderma harzianum* (TH)	Soil drenching (fungal suspension 1 x 10^7 spores/ g soil)	+ FBCA’s + neem + resistant tomato cultivars.	*P. lilacinum* - *T. harzianum*, *P. lilacinum* and *T. harzianum* reduced *Fusarium* propagules and *M. javanica* juveniles in the roots and performed even better when combined with neem in two resistant tomato cultivars	Mwangi *et al.* (2019)
Root treatment/ Root dipping					
Fusarium wilt (*F. oxysporum* . f. sp. *lycopersici*)/ tomato (*S. lycopersicum* L.)	*Funneliformis mosseae* and *Acaulospora laevis* (mycorrhizal fungi, AMF)	Soil inoculation (AMF)	+ *Trichoderma viride* was applied via soil drenching (*T. viride*)	Remarkable increase in the plant phosphorus and nitrogen content. Maximum reduction in disease incidence and severity was recorded in combined inoculation of *F. mosseae*, *A. laevis* and *T. viride*	Tanwar *et al.* (2013)
Fusarium wilt, tomato (*F. oxysporum* f. sp. *Lycopersic*/ tomato (*S. lycopersicum* L.	*T. asperellum* MSST	Talc-based bioformulation	none	Reducing disease incidence by up to 85%, increasing the vegetative parameters and inducing PR proteins	Patel and Saraf (2017)
Fusarium wilt, (*F. oxysporum* f. sp. *lactucae)*/ lettuce	*T harzia*num T22 and *F. oxysporum* *I*F 23	Talc based formulation	none	Disease control as well as increased growth response were shown by *T. harzianum* T 22	Gilardi *et al.* (2007)
Powdery mildew (*Sphaerotheca fusc*a)/ cucumber	*T. harzianum* T39 (TRICHODEX)	Soil application	none	Application of *T. harzianum* T39 to soil instead of spraying, 75%-90% lower coverage of powdery mildew on the leaves, and induced resistance as the mode of suppression	Elad *et al.* (1998)

environmental factors, reflecting the biological nature of the FBCA (Nega *et al*., 2014). A better understanding of the ecological and epidemiological relationships between the active ingredient (AI) and surrounding residential microorganisms, and the appropriate delivery systems at the correct intervals will allow the fungal strains significantly suppress diseases while reducing the gap between the experimental and commercial results of FBCA use. Therefore, the technicality involving the formulation and the delivery system are the two essential researches to successfully develop FBCA.

Formulation. During the early years of FBCA's research, emphasis was largely placed on the screening of potential candidates, with very little attention to the field implementation success. Subsequently, the research that follows the successful selection of FBCA is critical, requiring careful validation. Evidently, chemical products have a very long shelf life with an easy application methodology. On the contrary, FBCA formulation requires comprehensive studies for large-scale production, including selecting suitable and inexpensive carrier medium either solid or liquid for the proliferation of FBCA, and various additive materials. The addition of carrier and additive materials, such as wetting and dispersal agents, nutrients and UV- and osmotic-protection agents would improve field performance, shelf life and stability (Parnell *et al*., 2016; Ravensberg, 2011). The liquid formulation is typically aqueous, oil-based, or polymer-based, containing FBCA's biomass suspensions. FBCA products with an extended shelf life, good solvability in storage, capability of proliferating under extreme soil conditions, efficient disease control in the field, cost-effective, easy and safe handling have been achieved (Parnell *et al*., 2016; Ravensberg, 2011). For example, Prasad *et al*. (2020) have demonstrated the effectiveness of a chitosan biopolymer-based *Trichoderm*a (Cts-PEG-Th) liquid blend in inhibiting *Aspergillus* collar rot and *Macrophomina* root rot in groundnut and safflower under a greenhouse condition. The viability of *Trichoderma* spores was sustained over a period of six months in two different temperatures of the developed formulation. There is also a high preference for the solid formulation of FBCA, generally in the form of direct application dusts (DP), seed dressing formulations-powders for seed dressing (DS), granules (GR), microgranules (MG), dry formulations for dilution in water-water dispersal granules (WG), and wettable powders (WP). Similarly, liquid formulations have been developed for dilution in water emulsions, suspension concentrates (SC), oil dispersion (OD), suspoemulsion (SE), capsule suspension (CS), and ultra-low volume formulations (Singh *et al*., 2016). The FBCAs can either be applied directly onto the soil, suspended in water or used as seed coatings. Carrier materials such as peat, talc, lignite, kaolinite, zeolite, montmorillonite, alginate, press mud, sawdust, vermiculite and rice husk are among the prominent carriers selected for the preparation of solid formulation. The usage of these carriers increases the survival rate and shelf life of FBCA by protecting them from desiccation and providing a conducive microenvironment for rapid growth after release.

Delivery. The delivery system, bioecology of FBCA, pathogen, and host play an important role in choosing the type of FBCA's formulation. The list of delivery systems available for FBCA has been highlighted in the earlier part of this paper (*Table 2*). For instance, a granular material would be the most suitable for distributing in-furrow or incorporation as a potting mix for a horticultural crop while a wettable powder suspended in water is the most appropriate to be applied as soil drenching and root dips (Lecomte *et al*., 2016). Developing a precise and adequate delivery methodology to introduce the FBCA is one of the crucial steps to ensure the success of biocontrol activity under field conditions. Several factors, such as the type of pathogen, the stage of the crop to be protected, the severity of disease, and the climatic conditions of the region have been reported to influence the selection of an effective delivery method (Desai *et al*., 2002). FBCA products have generally been introduced into the plant ecosystem through direct soil application, seed coating, foliar spray or root dipping (Mahmood *et al*., 2016) to enable multiplication and survival near or at the specific entry sites used by the pathogen to enter the host plant (Bonaterra *et al*., 2012). In addition to an adequate formulation and delivery method, determining the application intervals is critical for effective disease management. For instance, a single application of FBCA may not allow the persistence of the microbe in its environment for effective disease control. In addition to the application intervals, identifying the effective microbial concentration is vital so that the product persists and competes with other microbial species within the niche environment. Meanwhile, various types of propagules persist and interact variably within the environment, contributing to the population dynamics of FBCA in the environment. Failing to deliver the FBCA correctly using the appropriate delivery method and at suitable intervals will result in unsuccessful management of the disease.

Financial Strength

A cost-effective mass production of a stable FBCA formulation would require the use of relatively cheap raw materials. Many chemical-based fungicides, pesticides and herbicides available in the market are comparatively affordable and more effective than FBCAs. Thus, the potential FBCAs need to be formulated with hassle-free delivery systems, or the products will never reach the market or be used by growers despite the demonstrated effectiveness (Nega *et al*., 2014). An affordable pricing for the end-users of FBCA is crucial to compete with the conventional and effective chemical application (Moosavi and Zare, 2015). Misunderstanding or lack of knowledge on the handling of FBCA products often leads to ineffective disease control, affecting the product sales and customer feedback. Additionally, it is critical to disseminate the updated knowledge and advances in the technology and effectiveness of FBCA in order to educate growers and users on the misconception of FBCA; and the know-how to achieve effective implementation for significant disease management.

It is also essential for a timely analysis of the market size, the return on investment and the potential market for FBCA investors. Surveys on the availability of the potential market and the willingness of growers to invest in biological control will also provide an overview of the potential uptake of FBCA in a particular industry. It must also be taken into consideration that the real market size may be smaller than the potential market size (Ravensberg, 2011), which, in most cases, does not meet the expectation for commercialisation standards by companies. Thus, to be commercially successful, the strategic plan and directions should be aimed to develop products that are consistent in their performance, with a broad spectrum of activities to target different groups of diseases and deliver high investment returns. Therefore, a product that yields reproducible results in several sites with a wide spectrum of activities and target pathogen guarantees a larger market size and greater product sales, making the product development more attractive and economically feasible (Nega *et al*., 2014). There should also be strong support by the local government in providing the necessary funding or subsidies to entice companies to invest in green technologies, such as biofungicides. For instance, in Malaysia, large funding is provided for start-up companies that are interested in biological and sustainable technologies. The funding is managed by the Ministry of Science, Technology and Innovation (MOSTI) with a pre-assessment conducted by the Malaysian Global Innovation and Creative Centre (MAGIC). Pitching sessions for funding allocations are offered for potential companies with good preliminary results even though the companies are not necessarily prepared for a commercial market. Therefore, the strength in technical efficacy while gearing towards developing an end product is an added advantage in acquiring the available funding.

Regulatory

Regulatory frameworks and product registrations are used worldwide to guide the commercial development of microbial fungicides such as the FBCA. The pre-regulatory framework of a new microbial fungicide or pesticide varies depending on the country, the characteristics of the products and their intended usage. These national and international regulations must be taken into consideration at every stage of the product development cycle, starting from its initial stages of the FBCA establishment. The regulatory cycles for developing new bioinoculants and biocontrol products are generally streamlined and well-articulated according to the country's agricultural policies. As a result, microbial products are an appealing and cost-effective option to implement an integrated and system-level approach toward crop productivity and agricultural pests' management. Nevertheless, public health, public safety and protection of the environment must remain important, serving as a basis for judgment on the product's suitability. However, more flexible regulatory requirements could be achieved without compromising this if the regulatory bodies are provided with valid product information, especially on the environmental impact and safety of the microbial agents used in FBCA products.

Trichoderma sp. is one of the successful examples of FBCA genus that has been widely used in agriculture. The genus has long been recognised as a promising FBCA to control diseases and increase plant growth and development. Its biology and uses have been well established and documented. Waghunde *et al.* (2016) described more than 50 formulations of *Trichoderma*-based FBCA products that have been patented and registered worldwide. However, despite the relatively abundant number of patents filed for microbial pesticides, the number of commercial applications is underwhelming. Limitations in registration include multiple levels of following up, adding more cost and slowing down the process to commercialise new technologies. Furthermore, the registration procedure for approving any biopesticide formulation in the market has not been revised to consider the biological aspects of the product, which are far different from those for chemical-based product testing. The regulations involved in registering a microbial product are a very important, complex and dynamic process. Therefore, from developing to registering a biological control system, the cost may be higher compared to other products. For instance, prior to field application on a large scale, the formulation of an FBCA should first be assessed by analysing the risk assessment. According to Chandler *et al.* (2011), these regulations are to protect humans and the safety of the environment and secondly, to characterise the products to ensure constant and consistent biopesticides quality. According to the Organization for Economic Cooperation and Development (OECD), the guideline for microbial pesticides before commercialisation is as follows: the microorganism and its metabolites do not pose pathogenicity or toxicity to mammals other than the non-target organisms that are likely to be exposed to the microbial product. Besides that, all additives present in microbial formulations are non-toxic, suggesting little risk to human health or environmental hazard. The product includes information on the mode of action, toxicological and ecotoxicological assessments, host range testing and others. These are complex processes that require a considerable amount of time, resources and expertise from the registration authority.

Future Perspective

Therefore, a set of multiple factors should be deciphered so that biofungicides effectively manage the disease. In general, the ecological parameters are the most neglected elements in managing plant diseases. The success of a FBCA may depend entirely on the ecological changes in the environment that may influence the product efficacy. Any changes in the environment may favour the plant, pathogen or microbial product. The ten principles of agricultural practices, include (1) soil, (2) nutrition, (3) water, (4) seed, (5) population density, (6) plant protection, (7) field management, (8) farming machinery technology, (9) light and (10) air, are known to influence the effectiveness of FBCA either independently or in combination (He *et al.*, 2016). In order to be economically feasible as an alternative to chemical control, FBCAs must at least comply with the following requirements:

- An effective microbial strain that shows reliable and repeatable effectiveness.
- Understanding of the mechanism underlying biological control activity, ecological requirement, and ecotoxicological safety.
- Development of a cost-effective process for mass production and suitable formulation for long-term storage.
- Ease of application with an effective delivery system in the field.
- Financial support to business investors, *e.g.*, start-up companies.
- Regulatory support for registration, quality assurance and bridging communication barriers.

In the Malaysian palm oil industry, for instance, the government has been advocating the use of green technologies in its supply chain, including the PDM. The industry has been largely scrutinised for its non-sustainable approaches in its early days, but significant changes have been made over the past decades to provide a comprehensive sustainable supply chain. Leveraging on this development, green technologies have been actively explored for a number of P&D problems, namely *Ganoderma* basal stem rot (BSR) disease and bagworm. The BSR disease causes devastating productivity loss since the disease mainly manifests during the prime age of the crop. Two FBCA products, namely GanoEF™ and Trichoshield® have been developed to manage BSR disease and have been well received by the industry. Both formulations went through the registration process and improvements in several ways to cater to the local market. This included switching to cheaper substrates and conducting ongoing awareness programmes on green approaches to the growers. Although growers have been sceptical in the early years, this mindset has changed due to the industry's commitment to sustainable approaches and the long-term effect. Therefore, it is essential to develop a cost-effective formulation of FBCA by exploring the use of low-cost growing media, such as agricultural wastes (rice bran, empty fruit bunch, sawdust, and palm kernel cake or paddy husk) to provide affordable green technologies. Research on FBCA should not only be considered as a scientific and academic achievement but also pursued as a potential precursor study for product development. Thus, to completely realise the benefit and to tap the advantages of FBCA in the management of plant diseases, the research must shift its current focus on conventional screening and identification towards perfecting formulation, delivery, storage stability and suitable application intervals; and understanding population dynamics, persistence and ecological parameters, all of which play a bigger role than anticipated.

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