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Biocontrol potential of trichoderma and yeast against post harvest fruit fungal diseases: A review

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ABSTRACT

Crop protection is vital to maintain high productivity and high quality of crops. Over the past years, people used different fungicides, herbicides and good agronomical practices to control fungal diseases and pests to increase productivity. However, extensive use of chemicals in controlling pests and diseases resulted in negative impacts on the environment, producing inferior quality and harming consumer health. In recent times, diverse approaches are being used to manage a variety of pathogens for control of plant diseases. Biological control is the alternative approach for disease management that is eco-friendly and reduces the amount of human contact with harmful chemicals and their residues. A variety of biocontrol agents including fungi and bacteria have been identified; In this regard, yeast and trichoderma species are the most researched microbes in biocontrol research area. But, despite the presence of many reports on biocontrol, practicability of the biocontrols requires effective adoption and a better understanding of the intricate interactions among the pathogen, plants and environment towards sustainable agriculture. To this end, this review attempts to find and compile previous works done on the role of trichoderma and yeast as a biocontrol agent against postharvest fungal pathogens. Moreover, this review analyzes the mechanisms of biocontrol activity, their means of application and future prospects on the use biogents and the challenges that encounter during the commercialization process.

Keywords: Biocontrol, Trichoderma, Postharvest, Preharvest, Yeast

1. INTRODUCTION

To date, Postharvest loss of fruits and vegetables is a major challenge throughout the world. Plant pathogens are among the most important biotic agents causing serious losses and damages to agricultural products. Hence, plant pathogens need to be controlled to ensure food, feed and fiber production quantitatively and qualitatively (Hasan *et al.*, 2013). Decays of fruits and vegetables caused by fungal pathogens account for significant levels of postharvest losses. It is estimated that about 20–25% of the harvested fruits and vegetables are decayed by pathogens during postharvest handling even in developed countries ((FAO, 2011)). Postharvest diseases of fruits mainly spread during sale, transport and storage (Janisiewicz and Korsten, 2002) and result in reduced food supplies, products of poorer quality, economic hardships for growers and higher prices (Monte, 2001). In addition to quality deterioration and economic losses, fruits infected with fungal pathogens can cause health risk since several fungal genera produce mycotoxins. According to the report of Andersen *et al.* (2006), *Penicillium expansum*, an etiological agent of blue mold in a variety of harvested fruits, produces potential carcinogenic metabolites including citrinin, patulin and chaetoglobosins. Moreover, mycotoxins such as aflatoxins, ochratoxins, alternaria and fumonisin were also found to be produced in fruits and vegetables contaminated with fungal genera such as *Aspergillus*, *Alternaria* and *Fusarium* (Sanzani *et al.*, 2016). Traditionally, postharvest spoilage caused by fungal pathogens is mainly controlled through the use of chemical fungicides either in the field or during storage (Vitoratos *et al.*, 2013). However, the use of many synthetic fungicides in postharvest disease control has become unacceptable due to various reasons including emergence of pathogen resistance to many key fungicides, development of new pathogen biotypes, lack of effective alternative fungicides, increasing levels of fungicide residues in agricultural produce, toxicological problems related to human health and negative environmental impacts (Droby, 2006). As a result of, the global trend is shifting towards the search for safer and eco friendly alternative approaches to control postharvest diseases and decay in fruits and vegetables (Mari *et al.*, 2012). Among the different biological approaches, use of the microbial antagonists like yeasts, fungi, and bacteria is quite promising and gaining popularity (Droby, 2006).

Biological control is defined as the reduction of inoculum density or disease producing activities of a pathogen or parasite in its active or dormant state, by one or more organisms accomplished naturally or through manipulation of the environment or host or antagonist or by mass introduction of one or more antagonists (Baker and Cook, 1974). Biological control is found to be both environmentally and economically sound. Various biocontrol agents such as fungi and bacteria have been identified for the control of postharvest diseases of many fruits and play an important role in sustainable agriculture and management of plant pathogens (Montesinos, 2003, Sobowale *et al.*, 2008, Montesinos and Bonaterra 2009, Junaid *et al.*, 2013). It has been also been investigated that the potential of antagonistic microorganisms to control decay of fruits and vegetables depends on their ability to colonise fruit surfaces and adapt to various environmental conditions (Sharma *et al.*, 2014). Biological control using antagonistic microbes to control postharvest disease and decay caused by pathogens is nowadays an emerging and attractive option (Dukare *et al.*, 2011; Liu *et al.*, 2013). The use of antagonist microbes in postharvest disease is advantageous over synthetic fungicides in that antagonist microbes do not produce toxic residues, environmentally friendly, safer application method, easy to deliver and economical to produce (Bonaterra *et al.*, 2012).

In the last one decade so many authors reviewed biocontrol of post harvest fruit pathogens using microbial antagonists in general. However, it is very difficult to address all reports of microbes having antagonistic property at a time as many reports are coming from different corners of the scientific world. Therefore, this review is intended to provide a comprehensive understanding of fungal antagonist-mediated postharvest biocontrol systems, including mechanisms of their biocontrol actions, application and the current perspectives of yeast and trichoderma biocontrol agents in controlling fungal pathogens.

2. MAJOR POSTHARVEST PATHOGENS AND THEIR PATHOGENECITY

Several fungal infections are responsible for postharvest disease and loss of fruits and vegetables. Fungal genera such as *Alternaria*, *Aspergillus*, *Botrytis*, *Fusarium*, *Geotrichum*, *Gloeosporium*, *Monilinia*, *Colletotrichum*, *Penicillium*, *Mucor* and *Rhizopus* are the major postharvest pathogens responsible for fruits and vegetables diseases (Barkai-Golan, 2001). The symptoms of the disease appear in fungal infected crops during the transportation and storage. Postharvest disease development is affected by several factors and processes, including ripening, harvesting, and mechanical injuries. The diseases development is initiated when fungal pathogens germinate and penetrate the host tissue cuticle through wounds and injuries (Alkan and Fortes, 2015). Fungal pathogens use three main routes to penetrate the host tissue: through wounds, natural openings, such as lenticels, stem ends, and pedicels and by direct breaching of the host cuticle (Emery, et al., 2000). Pathogens also enter through the lenticels; pedicel-fruit interphase and sometimes reside endophytically in the stem ends. Moreover, the pathogens can also directly penetrate in the host cuticle throughout the fruit growth period. Several plant fungal pathogens genera like *Alternaria*, *Botrytis*, *Botryosphaeria*, *Colletotrichum*, *Lasiodiplodia*, *Monilinia* and *Phomopsis* reside quiescently at the initial introduction site of unripe fruits (Prusky *et al.*, 2013). As a result, these pathogens remain inactive and invisible during the storage until the fruits ripen. But when the fruit begin to ripe, fungal pathogens grow aggressively and kill the host tissues necrotrophically and take nutrients from the host, leading to decomposition of the tissues and decay initiates. The pathogenic fungi may live dormant either endophytically or hemibiotrophically on fruit tissue till ripening. However, during ripening, the intrinsic disease resistance mechanism protecting the fruits from fungal attack becomes weak or inefficient and then fruits become vulnerable to fungal attacks (Prusky *et al.*, 2013). Therefore, postharvest disease biocontrol becomes vital to prevent post harvest crop loss both in quantity and quality.

3. POTENTIAL BIOCONTROL AGENTS AS A POTENTIAL SUBSTITUTE FOR CHEMICAL FUNGICIDES: TRICHODERMA AND YEAST AS PROMISING BIOAGENTS

Biological control using microbial agents has been reported among several alternatives to be an effective approach, to the use of synthetic chemical fungicides for managing postharvest fruit decay (Droby et al., 2009; Spadaro and Gullino, 2004). Indiscriminate use of chemicals to control the pathogens has caused enhancement of overhead costs, accumulation of toxic chemical residues in food chain and soil pollution leading to loss of soil health.

Table 1. Some reports on the use of trichoderma and yeast as biocontrol agent against plant pathogens.

Antagonist (yeast & trichoderma)	Pathogen	Host Plant	Citation
<i>Trichoderma harzianum</i>	Anthracnose (<i>Colletotrichum musae</i>)	Banana	Devi and Arumugam (2005)
	Anthracnose (<i>Colletotrichum gloeosporioides</i>)	Mango	Alvindhia (2018):
	Gray mold (<i>Botrytis cinerea</i>)	Grape	Batta (2007)
	Anthracnose (<i>Colletotrichum gloeosporioides</i>)	Mango	Sivakumar <i>et al.</i> (2000)
<i>Trichoderma viride</i>	Stem-end rot	lentil	Sharfuddin <i>et al.</i> (2012)
<i>Trichoderma spp</i>	Root rot	Pine	Dar <i>et al.</i> (2011)
	<i>Sclerotium rolfsii</i>	Mango	Bastakoti <i>et al.</i> (2017)
	Chocolate spot disease (<i>Botrytis fabae</i> Sard)	Faba Bean	Sahile <i>et al.</i> (2011)
	Anthracnose	Papaya Fruit	Valenzuela <i>et al.</i> (2015); Admasu <i>et al.</i> (2014)
<i>Trichoderma asperellum</i>	-		Santos-Villalobos (2013)
<i>Trichosporon pullulans</i>	Fruit rots	Mango	Pathak (1997)
	Alternaria rot	Cherry	Qin <i>et al.</i> (2004)
	Gray mold (<i>Botrytis cinerea</i>)	Cherry	Qin <i>et al.</i> (2004)
Yeast spp (as general)	Anthracnose	Mango	Montiel <i>et al.</i> (2017)
Yeast	<i>Penicillium italicum</i>	Citrus	Da Cunha <i>et al.</i> (2018)

<i>Saccharomyces cerevisiae</i>	<i>Colletotrichum acutatum</i> (fruit drop)	Citrus	Lopes <i>et al.</i> (2015)
<i>Rhodotorula minuta</i>	Sour rot	Citrus	Ferraz <i>et al.</i> (2016)
<i>Candida azyma</i>			
<i>Aureobasidium pullulans</i>			
<i>Rhodotorula glutinis</i>	Gray mold (<i>Botrytis cinerea</i>)	Tomato	Liu <i>et al.</i> (2013)
<i>Leucosporidium scottii</i>	<i>P. expansum</i> and <i>B. cinerea</i>	Apple	
<i>Candida oleophila</i> (I-182)	<i>Penicillium digitatum</i>	Grapefruit	Droby <i>et al.</i> (2002)
<i>Candida saitoana</i>	<i>Botrytis cinerea</i>	Apple	El-Ghaouth <i>et al.</i> (2003)
<i>Pichia membranaefaciens</i>	<i>Penicillium expansum</i>	Peach	Yang <i>et al.</i> (2011)
<i>Debaryomyces hansenii</i> , <i>Rhodotorula minuta</i> , <i>Cryptococcus laurentii</i> and <i>Cryptococcus diffluens</i>	Anthraco-nose	Mango	Luis <i>et al.</i> (2017)

Apart from this, the chemicals tend to become less efficient due to the development of resistance among the pathogens over time scale. Under these circumstances, the use of various ecofriendly biocontrol agents is increasingly being emphasized as an important component of plant disease management. Moreover chemical measures may establish imbalance in the microbiological community i.e. unfavorable situation for activity of beneficial organisms (Suryawanshi, 2018). So, direct application of antagonist would be safer method for introducing microorganisms into the soil for biological control of soil borne plant pathogen. Many authors reported that trichoderma and yeasts are promising antagonists against many phytopathogenic diseases of crops (Bastakoti *et al.*, 2017; Liu *et al.*, 2013; Antos-Villalobos *et al.*, 2013; Alvindia, 2018; Marques, 2018).

4. ORIGIN OF MICROBIAL ANTAGONISTS

Most of the microbial antagonists naturally reside on fruit and vegetable surfaces. Many effective antagonists have been isolated and characterized as suitable biocontrol agents for the

control of postharvest pathogens from fruits and vegetables (Janisiewicz *et al.*, 2013). In addition to the fruit surface, phyllosphere is a best source of microbes with antagonistic role (Kalogiannis *et al.*, 2006). Many antagonistic microbes were also isolated and characterized from roots and soil. For instance, the yeast *Rhodotorula glutinis*, obtained from the tomato phyllosphere impedes the growth of *Botrytis cinerea*, a causative agent of grey mold on tomato leaves and fruits (Kalogiannis *et al.*, 2006). The yeast *Kloeckera apiculata* isolated from citrus roots, effectively control the postharvest pathogens *Penicillium italicum* on citrus and grapes (Long *et al.*, 2007). Some effective microbial antagonists have also been investigated in unique natural habitats. For instance, the cold-tolerant yeast *Leucosporidium scottii*, an isolate from the Antarctic soil, was identified as an efficient microbial antagonist to *P. expansum* and *B. cinerea* responsible for blue and grey mold of apples, respectively (Vero *et al.*, 2013). Similarly, the marine yeast *Rhodospiridium paludigenum*, an osmotolerant yeast isolate, inhibits *P. expansum* growth on pear fruits, while *Alternaria alternata* inhibits *P. expansum* growth on Chinese winter jujube (Wang *et al.*, 2011). Marine yeasts typically have greater osmotolerance ability compared to yeasts isolated from the fruit surface and therefore be more suitable candidates for use under conditions with high abiotic stress (Hernandez-Montiel *et al.*, 2010). *Trichoderma* is an asexually reproducing fungal genus usually found in soil. They are known to be strong opportunistic invaders, fast growing, prolific producers of spores and also powerful antibiotic producers even under highly competitive environment for space, nutrients, and light (Montero-Barrientos *et al.*, 2011).

These properties collectively make *Trichoderma* ecologically very dominant and ubiquitous strains able to grow in native prairie, agricultural, marsh, forest, salt and desert soils of all climatic zones. Recently, marine *Trichoderma* isolates were characterized to evaluate their potential use as halotolerant biocontrol agents and found effective against *Rhizoctonia solani* inducing systemic defense responses in plants (Gal-Hemed *et al.*, 2011).

5. MECHANISMS OF ACTIONS OF MICROBIAL ANTAGONISTS

Several studies have shown the antifungal potential of many microbial antagonists against postharvest fungal pathogens (Nunes, 2012; Gbadeyan *et al.*, 2016; Wisniewski *et al.*, 2016). Biological control can result from interactions between organisms of different types. In the process of interaction, pathogens are antagonized by the presence and activities of other organisms that they encounter. Competition for nutrients and space, antibiosis through antibiotic production, mycoparasitism, production of cell wall lytic enzymes, and induction of host resistance are major biocontrol mechanisms displayed by antagonists (El-Ghaouth *et al.*, 2004; Sharma *et al.*, 2009; Di Francesco *et al.*, 2016). Recent studies have showed the roles of biofilm formation, quorum sensing, alleviation of host oxidative damage and antifungal volatile compound production in suppressing the activity of postharvest fungal pathogens on fruits (Liu *et al.*, 2013). Often, there is more than one mechanism in successful postharvest biological control and some of the different mechanisms of antagonism are mentioned below:

5. 1. Competition

Biological agents compete with plant pathogens for space, organic nutrients and minerals. An effective competition for nutrients such as carbohydrates, amino acids, vitamins and minerals as well as for oxygen and/ or space is vital to suppress postharvest pathogens of fruits

(Spadaro *et al.*, 2016). As the major postharvest diseases are caused by fungi, the majority of antagonists are highly efficient biocontrol agents by successfully competing with fungi for nutritional resources (Zhang *et al.*, 2010). Under nutrient starvation, the antagonists diminish the available nutrients in the wound site and make nutrients inaccessible for the pathogens to germinate, grow and infect. The capability of antagonistic yeasts to attach with their pathogen hyphae increases nutrient competition and thus obstructs the initiation process of the pathogenic infection (Talibi *et al.*, 2014). Yeasts can use most of the carbohydrate and nitrogen sources for cell growth by competing for space and forming an extracellular polysaccharide matrix at the wound site and as a result of this, the growth rate of yeast antagonists is generally high (Spadaro *et al.*, 2010). The analysis of the radio-labelled glucose distribution pattern among the antagonistic yeast *Sporobolomyces roseus* and an etiological agent of grey mold disease (*B. cinerea*) revealed strong sugar use by the antagonistic yeast, which ultimately blocked the conidial germination of the pathogen due to sugar deficiency (Spadaro and Gullino, 2004). Similarly, the important role of the competition for sugars and nitrates was observed in the interactions of *Pichia guilliermondii* with *B. cinerea* on apples (Spadaro and Droby, 2016) and *Colletotrichum* spp. on peppers (Chanchaichaovivat *et al.*, 2008). In fruit wounds, competition is extended to other essential nutrients such as oxygen, amino acids or vitamins when present at low concentrations. A significant decrease in the efficacy of a yeast strain of *A. pullulans*, an antagonist against *P. expansum* was observed when high concentrations of amino acids were applied exogenously to apple wounds (Bencheqroun *et al.*, 2007).

This shows the important role of competition for nutrients, which represents major mechanism in biocontrol activity. Surface residing non-pathogenic natural microbiota of fruits can also intervene in nutrient and space competition by effective colonisation and toxic metabolite production (Galvez *et al.*, 2010; Di Francesco *et al.*, 2016). Further, the rapid colonisation of wound site also depends on the antagonist concentration and the host fruit species, as certain antagonists prefer certain nutrient types. Most aerobic and facultative anaerobic micro-organisms respond to low iron stress by producing extracellular, low molecular weight (500- 1000 daltons) iron transport agents, designated as Siderophores, which selectively make complex with iron (Fe⁺) with very high affinity. Iron starvation prevents the germination of spores of fungal pathogens in rhizosphere as well as rhizoplane. Eg. Siderophores produced by *Pseudomonas fluorescens* (known as pseudobactin or pyoverdins) helps in the control of soft rot bacterium, *Erwinia caratovora*. suppression of *Pythium ultimum* by *Enterobacter cloacae*, *P. putida* colonize the root system in the rhizosphere and a corresponding reduction in Fusarium wilt suppression in cucumber (Maindad et al, 2014).

5. 2. Induction of host resistance

Plants actively respond to a variety of environmental stimuli, including gravity, light, temperature, physical stress, water and nutrient availability. Several studies have investigated that the application of microbial biocontrol agents to fruit surfaces induced systemic resistance against invading fungal pathogens (Janisiewicz *et al.*, 2008; Romanazzi *et al.*, 2016; Droby *et al.*, 2016). Induction of resistance to biotic or abiotic stresses involves accumulation of structural barriers and elicitation of many biochemical and molecular defense responses in the host, including mitogen activated protein kinase signaling (MAPK), reactive oxygen species generation (ROS), biosynthesis of terpenoid and phytoalexin, production of phytoalexins and PR-proteins, enhanced accumulation of phenolic compounds, lignification at the infection site

and strengthening of host cell wall by formation of glycoproteins, lignin, callose, and other phenolic polymers (Shoresh *et al.*, 2010).

Trichoderma spp. are well-known for their ability to promote plant growth and defense. The previous studies showed that *Trichoderma* can produce gluconic and citric acids that decrease the soil pH, enhance the solubilization of phosphates, micronutrients, and mineral components such as iron, magnesium, and manganese (Vinale *et al.*, 2016). It was shown that the bean plants treated with *T. harzianum* T019 always had an increased size respect to control. In addition, this strain induced the expression of plant defense-related genes and produced a higher level of ergosterol, indicating its positive effects on plant growth and defense in the presence of the pathogen (Mayo *et al.*, 2015).

Moreover, the roots of maize plants treated with *T. harzianum* strain T-22 were about twice as long compared to untreated plants after several months from treatment (Harman, 2004). Saravanakumar *et al.* (2016) showed that *Trichoderma cellulase* complexes trigger the induced systematic resistance (ISR) against *Curvularia* leaf spot in maize by increasing the expression of genes related to the jasmonate/ethylene signaling pathways. Furthermore, Rao *et al.* (2015) suggested that treatment of legume seeds (*Cajanus cajan*, *Vigna radiate* and *Vigna mungo*) with *T. viride* induces systemic resistance by reprogramming defense mechanisms in these legumes.

Treatment of peaches with yeast *C. laurentii* and methyl jasmonic acid (MeJA) stimulated the activities of enzymes chitinase, b-1, 3-glucanase, phenylalanine ammonia-lyase (PAL) and peroxidase (POD) in comparison to the application of yeast or MeJA alone (Yao and Tian, 2005). The treatment reduced the diameter of disease lesions on fruit caused by *M. fructicola* and *P. expansum*. Higher levels of the enzymes, such as catalase (CAT), glutathione peroxidase, methionine sulfoxide reductase peroxiredoxin, and polyphenol oxidase (PPO), protect the host tissues against oxidative damage by *P. expansum* pathogen. Application of antagonistic yeast *R. paludigenum* on mandarins at pre-harvest stage also induced defense response by increasing production of defense-related enzymes, including b-1, 3-glucanase (Spadaro *et al.*, 2016).

5. 3. Antibiosis

Antagonism mediated by specific or nonspecific metabolites of microbial origin, by lytic agents, enzymes, volatile compounds or other toxic substances is known as antibiosis. Most microbes produce and secrete one or more compounds with antibiotic activity. In some instances, antibiotics produced by microorganisms have been shown to be particularly effective at suppressing plant pathogens and the diseases they cause. *Trichoderma* can produce low molecular weight diffusible compounds or antibiotics that inhibit the growth of other microorganisms. There are several metabolites or antibiotics secreted from *Trichoderma* against their pathogens such as: harzianic acid, tricholin, peptaibols, 6-pentyl- α -pyrone, viridin, gliovirin, glisoprenins, and heptelidic acid (Gajera *et al.*, 2013).

Sadykova *et al.* (2015) tested the antibiotic activity in 42 strains of 8 species of the *Trichoderma* genus (*Trichoderma asperellum*, *T. viride*, *T. hamatum*, *T. koningii*, *T. atroviride*, *T. harzianum*, *T. Citrinoviride*, and *T. longibrachiatum*) isolated from Siberian. Vinale *et al.* (2014) also showed that the pyrone 6-pentyl-2H-pyran-2-one is a metabolite purified from the culture filtrate of different *Trichoderma* spp. (*T. viride*, *T. atroviride*, *T. harzianum* and *T. koningii*) and has shown both *in vivo* and *in vitro* antifungal activities towards several plant pathogenic fungi. Furthermore, Howell (1999) reported that strains of *Trichoderma virens* (P group) produce the antibiotic gliovirin which is very active against *P. ultimum*, while the Q group of these strains can produce gliotoxin, which is very active against *R. solani*.

5. 4. Mycoparasitism

Direct parasitism, mycoparasitism or hyperparasitism, is the ability of antagonistic microorganism to attach with the hyphae of fungal pathogens to produce extracellular cell wall lytic enzymes. Mycoparasitism of antagonist depends upon the sequential occurrence of the following events: come into close contact of fungal pathogens, mutual recognition by antagonist and pathogen, lytic enzymes secretion and, active growth of antagonist into the host (Spadaro and Gullino, 2004; Talibi *et al.*, 2014). Parasitism causes either complete killing of fungal propagules or destruction and lysis of their structure. Wisniewski *et al.* (1991) reported mycoparasitism initially in the studies on biocontrol of *Botrytis cinera* by yeast antagonist *P. guilliermondii*. Disintegration of pathogenic fungal cell wall by the action of extracellular hydrolytic enzymes of antagonists, such as chitinases, chitosanases, glucanases, cellulase and/or protease, individually or in combination, contributes to biocontrol activity (Spadaro and Droby, 2016). The lytic enzymes also impede pathogen spores germination, elongate of germ-tube and destroy oospores (El-Tarabily, 2006). Banani *et al.* (2015) also reported chitinase activity of antagonistic yeast *Metschnikowia fructicola* and demonstrated that chitinase gene MfChi was over induced in the presence of yeast *Monilinia fructicola* cell wall. An overexpressed MfChi chitinase in *Pichia pastoris* controlled the growth of *M. fructicola* and *Monilinia laxa* under in vitro and in vivo studies on peach fruits. Similarly, antifungal activity of alkaline serine protease, secreted by yeast-like fungus *A. pullulans*, is documented as mycoparasitism (Zhang *et al.*, 2012). Enzymatic breakdown of fungal pathogens hyphae results in cellular deformities, including cytological damages, lysis and distortion in mycelia, altered cell membrane permeability and leakage of cytoplasmic content (Di Francesco *et al.*, 2016). It may, therefore, be inferred that enzymatic dissolution of cell walls leads to the loss of fungal protoplasm and accountable for antagonistic activity (Kim and Chung, 2004).

5. 5. Production of antifungal volatile compounds (VOCs)

Microbial antagonists produce several antifungal metabolites of high VOCs also play important role in inhibition of fungal pathogen growth (Mari *et al.*, 2016). VOCs are low molecular weight lipophilic compounds mixture. Role of VOCs produced by fungi (Morath *et al.*, 2012); yeast (Di Francesco *et al.*, 2015); and bacteria (Zhang *et al.*, 2013) have been reported to control postharvest disease of fruits. Fungal species, such as *Trichoderma harzianum*, *Fusarium oxysporum* and *A. pullulans*, produce volatile antifungal substances in low concentrations (Mari *et al.*, 2012). Yeast antagonist *A. pullulans* produces VOCs, including 2-methyl-1-butanol, 3-methyl-1-butanol, phenethyl alcohol and 2-methyl-1-propanol, which showed fungistatic activity against *C. acutatum*, *B. cinerea* and *Penicillium* species (Di Francesco *et al.*, 2014). Fungi having ability to produce VOCs may be used as bio-fumigant. Endophytic fungus *Muscodora albus*, isolated from *Cinnamomum zeylanicum* in a botanical garden of Honduras, is a typical example of volatile producing bio-fumigant fungi for control of postharvest decay (Strobel, 2011).

6. APPLICATION METHODS OF BIOCONTROL AGENTS

Microbial antagonists are used either those that exist on the produce itself which can be promoted and managed or those that can be artificially introduced against post harvest

pathogens (Sharma *et al.*, 2009). Many investigators have provided strong evidence that several pathogens infest fruits and vegetables in the field, and these infestations become critical factors for decays during transportation or storage of the commodities and hence argued that preharvest application (s) of microbial antagonistic culture are often effective to controlling postharvest decays in fruits and vegetables (Ippolito *et al.*, 2004; Irtwange, 2006).

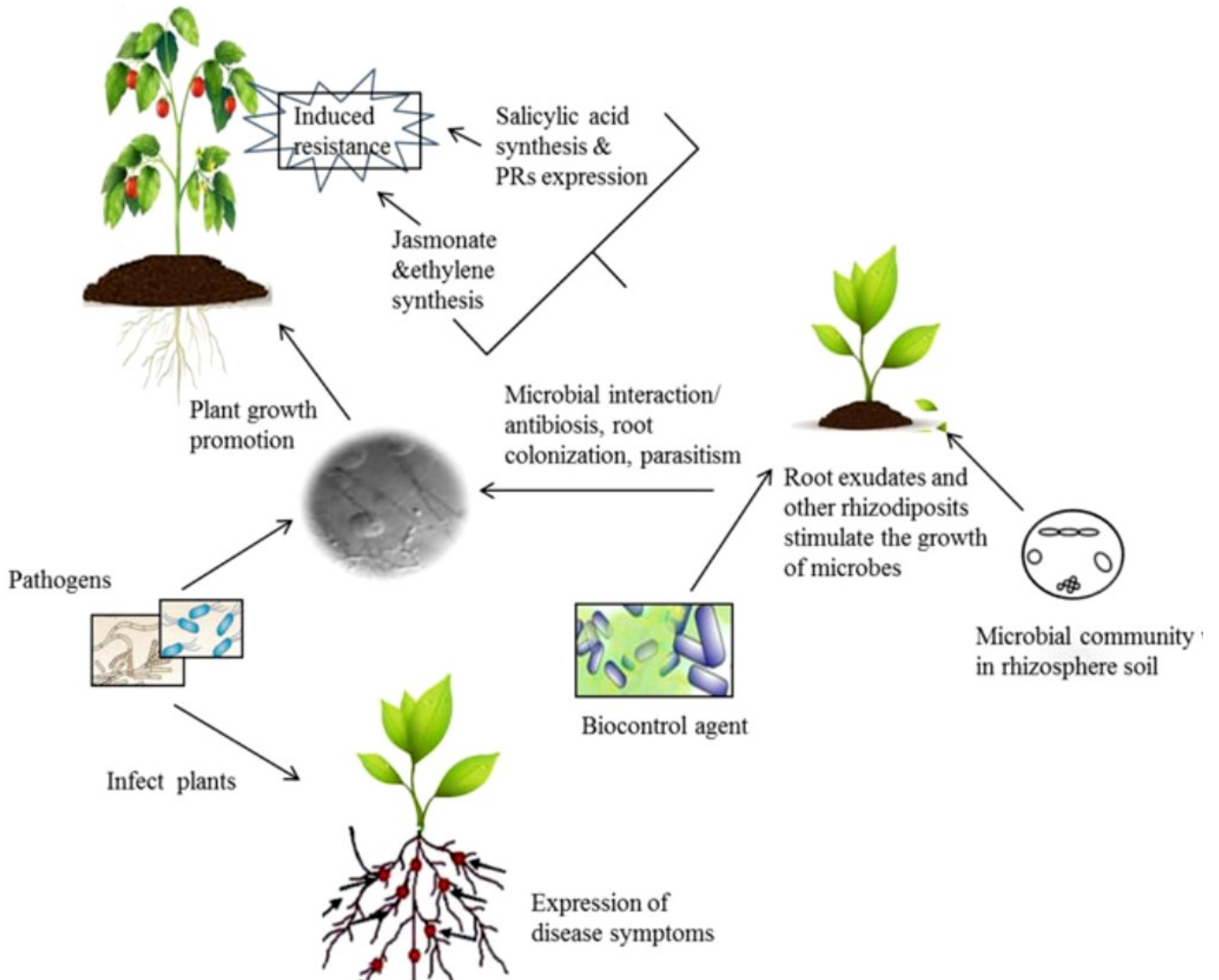


Fig. 1. Interaction within the plants, pathogenic microorganisms and biocontrol

Typically, pre harvest application is done to pre-colonize the fruit surface with the antagonistic microbes so that fruits can be colonized by the antagonists before colonization by the pathogens (Ippolito and Nigro, 2000).

Preharvest applications with yeast antagonists *Tricho sporonpullulans* (Lindner.), *Cryptococcus laurentii* (Kuffer.) *Rhodotorula glutinis* (Fresenius) (Tian *et al.*, 2004),

Trichoderma harzianum (Zhang *et al.*, 2007) and *Epicoccum nigrum* (Larena *et al.*, 2005) have proved postharvest control of strawberries where synthetic fungicides proved ineffective. In a different study, the preharvest application of *Aureobasidium pullulans* reduced significantly the storage rots in strawberry (Lima *et al.*, 2003), grapes and cherries (Scheda *et al.*, 2003), and apples (Leibinger *et al.*, 1997). There was a related reducing trend of incidence of green mold (*Penicillium digitatum*) on grape fruit by preharvest spray of *Pichia guilliermondii* (Droby *et al.*, 1992). In pear, field application(s) of *Cryptococcus laurentii* and *Candida oleophila* was reported to reduce storage rots (Benbow and Sugar, 1999) whereas preharvest applications of *Pantoea agglomerans* CPA-2 and *Epicoccum nigrum* were reported to be effective against citrus rots and peach brown rot under laboratory and field conditions respectively (Sharma *et al.*, 2009). Similarly, Canamas *et al.* (2008) reported that preharvest application of different concentrations of *Pantoea agglomerans* was effective against *Penicillium digitatum* during storage of oranges.

Postharvest application of antagonistic microorganisms is common and appears to be better for controlling post harvest diseases of fruits and vegetables. In the application process, the antagonists are sprayed directly onto the surfaces of the fruits and vegetables or are applied by dipping (Sharma *et al.*, 2009). Investigations by many authors show that the postharvest application of microbial antagonists for controlling diseases in fruits and vegetables are more effective than the preharvest approach for citrus (Long *et al.*, 2007), apples (Morales *et al.*, 2008; Zhang *et al.*, 2009; Mikani *et al.*, 2008), peach (Mandal *et al.*, 2007), banana (Lassois *et al.*, 2008), mango (Kefialew and Ayalew, 2008), tomato (Zhao *et al.*, 2008), and cabbage (Adeline and Sijam, 1999). In strawberries and lemons for example, Pratella and Mari (1993) found that postharvest application of *Trichoderma harzianum*, *Trichoderma viride*, *Gliocladium roseum* and *Paecilomyces variotii* Bainier resulted in a better control of *Botrytis* and *Alternaria* rots respectively than preharvest application(s). In a related development, postharvest applications of *Pseudomonas variotii* and *Trichoderma harzianum* were more effective in controlling *Aspergillus* and *Fusarium* rots in lemons and potatoes than their respective dips in iprodion and benomyl. Identification and selection of promising antagonists are generally followed by the selection of the appropriate time and application method for the effective suppression of postharvest pathogens. In general, both pre-harvest and postharvest application approaches are practiced.

7. CHALLENGES AND FUTURE PROSPECTS OF COMMERCIAL BIOCONTROL AGENTS

In the present crop production scenario, the biocontrol is of utmost importance, but its potential is yet to be exploited fully mainly because the research in this area is still confined to the laboratory and very little attention has been paid to produce the commercial formulations of bio agents. Moreover, whatever has been commercially produced has not been used efficiently by the farmers owing to the lack of information regarding its use. In order to be successful, a biocontrol product needs to provide an acceptable and consistent level of control of target diseases in the target commodity under commercial processing and storage conditions. These conditions may vary significantly for different commodities and in different packinghouses (Droby *et al.*, 2009). Pilot studies, semicommercial, and large-scale commercial tests requiring large amounts of formulated product are needed to obtain the data necessary to

evaluate the efficacy of a biocontrol agent (Abadias *et al.*, 2003). It is important for these tests to be conducted in packinghouses at different locations under conditions of natural infection (Long *et al.*, 2007). Regulatory approval of a formulation by government agencies is also required to produce a commercial product. The application package for approval must contain a third-party evaluation of the safety of the formulated product to human health, as well as efficacy data.

The registration of biocontrol products for postharvest use in the USA is the responsibility of the Environmental Protection Agency (EPA) and on average has required about two years. In contrast, the registration process in Europe takes almost seven years (Nunes, 2012). Although many different yeasts, isolated from a variety of sources, have been reported as good postharvest biocontrol agents, only a few yeast-based biocontrol products are available in the market: Shemer™ (*M. fructicola*, Bayer, Germany), Candifruit™ (*C. sake*, Sipcam-Inagra, Spain), and Boni-Protect™ (the yeast-like fungus, *A. pullulans*, Bioprotect, Germany). These products are registered for use on several different commodities and for several different pathogens. The ability to control different rots on different commodities is essential for the economic viability of a postharvest biocontrol product. For example, Shemer, based on a heat-, oxidative stress- and osmo-tolerant strain of *M. fructicola* NRRL Y-27328 (Droby *et al.*, 2009; Kurtzman and Droby, 2001; Liu *et al.*, 2011), has been shown to be effective against rots caused by *Botrytis*, *Penicillium*, *Rhizopus* and *Aspergillus* on strawberry (Karabulut *et al.*, 2004), grape, sweet potato, carrot and citrus (Blachinsky *et al.*, 2007).

Most of the bio agents perform well in the laboratory conditions but fail to perform to their fullest once applied to the soil. This is probably attributed to the physiological and ecological constraints that limit the efficacy of bio agents. To overcome this problem, genetic engineering and other molecular tools offer a new possibility for improving the selection and characterisation of bio control agents. Various methods that can contribute to increase the efficacy of bio agent include mutation or protoplasm fusion utilising poly ethylene glycol. There is also an urgent need to mass produce the bio agents, understand their mechanism of action and to evaluate the environmental factors that favour the rapid growth of bio control agents

The first fungus *Trichoderma harzianum* ATCC 20476 was registered with the EPA for the control of plant diseases. Currently a total of 14 bacteria and 12 fungi have been registered with the EPA for the control of plant diseases (Fravel, 2005). Most of these are sold commercially as one or more products. The technology of commercialization is still in its initial phase. 65% of the EPA registered organisms have been registered within the past 10 years while the remaining 36% registered over the past 5 years. Although the number of bio control products in plant disease management is increasing, these products still represent only 1% of the agricultural control measures while fungicides account for 15% of total chemicals used in agriculture (Fravel *et al.*, 2005). I

n the past five decades, an increasing number of chemical fertilizer and biocidal molecules were the main cause for a substantial increase in crop production and quality. Because of environmental issues and health concerns, continuous and extensive use of those molecules has raised serious debate, and often various biological control methods based on natural pest and pathogen-suppressing organisms are being recommended as a substitute. Globally the registrations of microbial biocontrol agents are increasing significantly. The changes in legislation in the country level, development of new policies and management structures to address the reduction of chemical uses are the expanding scope of biocontrol agents.

On the other hand, the researchers worldwide have been supported to discover new biocontrol agents to reinforce for entering in the industry. Being practical, at present biocontrol agents are not comparable to chemical pesticides in meeting efficacy which is needed for market expectations, but they still have a promising future if knowledge and methods of various fields of biotechnology are utilized. The availability of recent molecular technologies has significantly facilitated for surveying and identification of candidate agents, and helped to interpret the modes of action after field applications. These new technologies like proteomics and functional genomics will give new possibilities for insights in ecological constraints and will help to see unseen possibilities to determine the physiological status and expression of crucial genes present within the biocontrol agents during mass production, formulation, storage and application.

8. CONCLUSIONS

Pesticide residues in fresh fruits and vegetables have been and will continue to be one of the main concerns of regulatory agencies and consumers. Therefore, reducing or eliminating the pre- and postharvest use of synthetic chemical fungicides by developing alternative management strategies remains a high research priority. This review article has provided a brief overview on the use of yeast and trichoderma antagonists as a viable alternative to synthetic, chemical fungicides. It is anticipated that the continuing withdrawal of key postharvest fungicides from the market, due to exclusion by regulatory agencies or the high-cost of re-registration, will lead to an absence of effective tools for reducing postharvest losses. Hence, the use of biocontrol products is expected to gain momentum in the coming years and become more widely accepted as a component of an integrated approach to managing postharvest diseases. Many challenges need to be addressed in order to develop a commercially successful postharvest biocontrol product. These include the improvement and enhancement of biocontrol efficacy under commercial condition, the development of high quality, economical methods of fermentation and formulation, the maintenance of cell viability and biocontrol efficacy in the formulated product, the identification of yeast and trichoderma antagonists that exhibit a wide spectrum of activity against several different pathogens on different commodities; e) the establishment of an effective marketing outlet, preferably by a multinational based company; and f) developing a fundamental understanding of how biocontrol systems operate and how the environment affects the interactions between the host, pathogen, and biocontrol agent.

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