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## Chapter

# A Review Study on the Postharvest Decay Control of Fruit by *Trichoderma*

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## Abstract

This chapter consists of an overview with the most relevant results about the efficacy of *Trichoderma* on postharvest disease control. The results of investigations demonstrate that this fungus can control several phytopathogens in different fruits. Postharvest losses represent a major problem in several countries. The constant application of fungicides not only at field but also at postharvest stage has led to microbial resistance cases, which make the control of these pathogens difficult. Biological control is a promising alternative to chemical fungicide applications. In this sense, an eco-friendly alternative and effective approach for controlling diseases is the use of microbial antagonists like *Trichoderma*, which have several mechanisms of action to stop disease development. A crucial treat in biological control is related to the maintenance of microbial viability and efficacy, that is why other technologies like their incorporation into edible films and coatings, nanotechnology, microbial mixtures, among others have been applied in combination with *Trichoderma* successfully. An enhancement in biocontrol activity is achieved when alternative systems are combined like GRAS substances, biopolymers, and other antagonists. Thus, *Trichoderma* is an eco-friendly alternative to threat postharvest diseases as an alternative to chemical treatments.

**Keywords:** *Trichoderma*, postharvest, pathogens, fruits, alternative systems

## 1. Introduction

Postharvest diseases represent a major problem through the world causing significant losses at postharvest stage [1]. Postharvest treatments play an important role in the quality preservation of commodities; however, in developed countries, the inadequate storage and transportation systems favor the establishment of diverse pathogens [2]. Traditionally, postharvest disease management is carried out by the application of chemical fungicides; however, environmental and health issues as well as microbial resistance play an important role in the development

of new strategies for controlling diseases [3]. Biological control is an eco-friendly alternative for postharvest disease control. Antagonists can be isolated from diverse sources like soil, fruits, leaves, and from extreme conditions as marine environments [2, 4]. *Trichoderma* is recognized due to their effectiveness in controlling several pathogens in diverse fruits like strawberry (*Botrytis cinerea*), citrus (*Penicillium italicum*), kiwifruit (*Botrytis cinerea*), banana (*Colletotrichum musae*), guava (*Rhizopus* spp.), among others [2]. The efficacy of *Trichoderma* is related to several mechanism of action reported like competition, antibiosis, parasitism (involving lytic enzymes), and the induction of plant defenses [5–7]. Biocontrol activity of *Trichoderma* can be enhanced by the combination of this antagonist with other control systems like the use of GRAS substances, encapsulation in polymeric matrices (chitosan), physical methods, etc. The aim of this chapter was to summarize information about the efficacy and application of *Trichoderma* alone or in combination with other alternative methods in different fruits against the most important postharvest pathogens.

## **2. Fruits: importance at international level**

In recent years, the demand for food has increased dramatically, while the world population has increased by 70%, food consumption per capita has increased more than 20%. According to some reports, it is projected that the production of crops for the year 2030 is 70% higher than the productions that are currently available [8]. Fruits and vegetables will play an important role by providing essential nutrients for the population's diet, both in developed and developing countries, in addition to being associated with the reduction of the risk of suffering from different chronic-degenerative diseases [9]. The United States dominates the international fruit and vegetable trade market and is the first place in terms of imports and exports of food of plant origin, while the European Union, as a whole, is the second importer and exporter of this type of food. On the other hand, some Latin American countries, such as Chile, have become one of the main suppliers in the international fresh fruit market. The increase in the production and commercialization of fruits and vegetables has been increasing, reaching a global production of nearly 1 billion tons of fruits and vegetables [10]. Mexico ranks sixth in the fruit-producing countries, along with China, India, Brazil, the United States, and Italy. China, India, and Brazil concentrate about 30% of the total production of fruits worldwide; however, much of this production is destined for local consumption, so the impact on the world market is minimal [10, 11]. The low international trade attends to the lack of fruit conservation mechanisms, which can suffer damage during the transfer. This implies significant postharvest losses, thus reducing the possibilities of export and international marketing of a large number of vegetable crops.

## **3. Postharvest losses: causes and consequences**

Currently, the losses of food in the world are about 1300 million tons per year; of which, in Latin America, the loss is, on average, 127 million tons of food per year; and with respect to fruits and vegetables, their loss is up to 55% in Latin America [12]. During the postharvest handling of fruits and vegetables, the losses range from 25 to 60% of the total production. This is due to several factors, but one of the most important factors is the poor handling of the products, where mechanical damage and diseases caused by pathogens play an important role [13]. Nowadays, the application of chemical fungicides is widely used as a strategy to control postharvest pathogens; however, even when they are effective,

the environmental impact of these methods is negative since they are toxic. On the other hand, there are several reports about the adaptation of pathogens (resistance) that reduce the antifungal activity and difficult their control; thus, it is necessary to investigate new, secure, and effective alternatives for controlling postharvest diseases [13, 14]. Fungal contamination can occur during the handling of fruits and vegetables at field, postharvest handling, storage, and transport and cause the deterioration of products, and as a consequence, decrease the amount of available products and affect the financial benefits [15].

## **4. Alternative methods for controlling postharvest diseases**

### **4.1 Physical treatments**

Heat treatments are one of the physical methods that have been used most for the conservation of minimally processed postharvest fruits, either alone or combined with other eco-friendly alternatives [15]. Heat treatments have been applied in several fruits against different pathogens with good results, like peach (*Monilinia fructicola*), apple (*Penicillium expansum*), grapefruit (*P. digitatum*), sweet cherry (*P. expansum*), table grapes (*Botrytis cinerea*), among others [16].

### **4.2 Essential oils**

The use of essential oils to control postharvest diseases is gaining popularity due to its safety features, biodegradability, and that are eco-friendly compounds [17]. For a long time, it has been recognized that some essential oils have antimicrobial, antiviral, antifungal, antiparasitic, and insecticidal properties [17]. Besides, in order to protect them and favoring their efficacy for controlling postharvest pathogens, the essential oils can be combined with edible films and coating, as previously reported with good results [17–19].

### **4.3 Edible films and coatings**

The use of edible films and coatings is an alternative to the use of fungicides to preserve the postharvest quality of fruits and vegetables [20]. The application of coatings and edible films in foods is mainly in perishable products, such as horticultural products, due to their properties such as cost, availability, functional attributes, mechanical properties (flexibility, tension), optical properties (brightness and opacity), the barrier effect against the flow of gases, structural resistance to water and microorganisms, as well as sensory acceptability [21]. Besides, edible films and coatings have a high potential to incorporate active ingredients such as antibrowning agents, colorants, flavors, nutrients, spices, antimicrobial compounds, and antagonists; which may favor extending shelf life of the product and reducing the risk of pathogen growth [22]. In this sense, incorporation of different additives into polymeric matrices in several fruits coated has been evaluated successfully in controlling postharvest diseases and maintaining the fruit quality [18, 19, 23–25].

## **5. Biological control at postharvest stage**

There is an international tendency to reduce the use of fungicides in fruit and vegetable products and to develop safer alternatives that reduce postharvest deterioration in fruits [26–28]. One option is the development of alternative control

systems for controlling postharvest pathogens on fruits: as physical treatments (hot water), application of substance of vegetable and animal origin, or the use of antagonistic microorganisms [2, 29, 30]. Biological control is a promising alternative with high application potential for controlling postharvest pathogens. Some of the advantages of biological control compared to the use of fungicides are the absence of toxic waste in the fruits, friendly relationship with the environment, as well as in safety and health for the people who handle the products [14]. For several decades, a large number of microorganisms with potential characteristics of antagonistic organisms have been isolated from different sources (leaves, fruits, marine environment) [4, 31]. Bacteria, yeasts, and fungi are the main antagonistic organisms that have been used at postharvest stage. Countries such as the United States, South Africa, Spain, Italy, and Israel, among others, have developed and, in some cases, commercialized antagonistic microorganisms for their commercial use in postharvest, for the control of different diseases caused by the main pathogenic fungi of fruits of temperate, tropical, and subtropical origin [27, 32, 33]. The most widely used antagonists have an outstanding capacity for rapid growth and colonization of the fruit, nutrient acquisition, ability to withstand extreme temperature conditions, solar radiation, control capacity at low concentrations, as well as genetic stability and development capacity in economic cultivation means, ease of application in the management of postharvest. The main mechanisms of action present in the antagonists are antibiosis, production of lytic enzymes, competence by nutrients and space, or the induction of resistance mechanisms [1, 34].

There is a large number of reports of *in vitro* and *in vivo* tests, using bacteria such as *Pseudomonas cepacia* Van Hall, *Pseudomonas syringae*, *Bacillus subtilis*; as well as yeasts such as *Candida sake*, *Rhodotorula glutinis*, *Debaryomyces hansenii* for the control of the main pathogenic fruit fungi in postharvest, such as *Penicillium digitatum*, *Penicillium italicum*, *Alternaria*, *Aspergillus*, *Botrytis*, *Fusarium*, *Geotrichum*, *Gloeosporium*, *Monilinia*, *Penicillium*, *Mucor*, *Colletotrichum*, and *Rhizopus* [4, 27, 32, 33, 35]. In addition to bacteria and yeasts used in the biological control postharvest pathogens, the application of *Trichoderma*, alone or in combination with other alternative control systems in different fruits for the control of pathogenic fungi of fruits in the fruit, has been evaluated with favorable results [36, 37].

## **6. *Trichoderma*: application on fruits**

*Trichoderma* has been applied as postharvest biocontrol agent in different crops such as papayas, strawberries, tomatoes, apples, pears, and bananas (Table 1). Existing different species of *Trichoderma* with high antagonistic capacity are *T. asperellum*, *T. viride*, and *T. harzianum*. Several authors have investigated different species of *Trichoderma* with the objective to find the most effective biocontrol agent for each crop and pathogen. The efficacy of six species of *Trichoderma* was evaluated on papaya fruit reducing the diameter lesions and disease incidence caused by *Colletotrichum gloeosporioides* by the application of *T. asperellum* and *T. viride* [6]. In a similar study on mango fruit, good efficacy was reported for controlling anthracnose applying *T. harzianum* obtaining a lower disease incidence (41.7% disease incidence) in fruits compared to control [38]. At present, *Trichoderma* is produced at industrial level as active component of biological products “biopesticides”; other ingredients that conform the biopesticides are the edible polymers, which can form coating for easy adhesion to the fruit and give to the product protection and stability during its shelf life. The application of biopesticides is widely used in agriculture and can be applied by immersion or spraying during the industrialization of

Postharvest fruit	Disease	Pathogen	<i>Trichoderma</i> species	Disease inhibition (%)	Reference
Banana	Crown rot	<i>Lasiodiplodia theobromae</i>	<i>T. viride</i>	60.7	Mortuza and Ilag [43]
			<i>T. harzianum</i>	56.2	
Pear	Rotten spots	<i>Rhizopus stolonifer</i>	<i>T. harzianum</i>	62.9	Batta [36]
	Gray mold	<i>Botrytis cinerea</i>		5.3	
	Blue mold	<i>Penicillium expansum</i>		33.3	
Mango	Antracnosis	<i>Colletotrichum gloeosporioides</i>	<i>T. harzianum</i>	60	Prabakar et al. [38]
Apple	Blue mold	<i>Penicillium expansum</i>	<i>T. atroviride</i> cepa P1	70.6	Quaglia et al. [44]
			<i>T. harzianum</i> Rifai cepa T22	72.5	
			<i>T. harzianum</i> Rifai cepa T67	70.6	
			<i>T. reesei</i> cepa T34	71.8	
			<i>Trichoderma</i> spp. cepa 8009	59.3	
Apple	Gray mold	<i>Botrytis cinerea</i>	<i>T. harzianum</i>	50.5	Batta [40]
	Blue mold	<i>Penicillium expansum</i>		40.6	
Pear	Gray mold	<i>Botrytis cinerea</i>		28.7	
	Blue mold	<i>Penicillium expansum</i>		47.6	
Papaya	Anthracnose	<i>Colletotrichum gloeosporioides</i>	<i>T. longibrachiatum</i>	65.0	Valenzuela et al. [6]
			<i>T. viride</i>	77.3	
Tomato	Gray mold	<i>Botrytis cinerea</i>	<i>T. harzianum</i>	96.9	Dal Bello et al. [45]

**Table 1.** Percentage of inhibition of different postharvest diseases by the application of different species of *Trichoderma*.

agricultural products [39]. The incorporation of *T. harzianum* into edible coatings as biopesticide produced higher inhibition of the pathogens *Botrytis cinerea* and *Penicillium expansum* compared to the application as simple conidial suspension of the antagonist on the fruits [40]. The same author previously reported the same effect on other fruits such as pears, grapes, apples, strawberries, kiwis, and peaches [36, 41]. Microbial antagonists not only have antifungal properties, but also can act as inductor agents; some studies report that their application can induce biochemical defense responses by the interaction antagonist-fruit. In this sense, the application of *T. virens* decreases the blue mold incidence of apple fruits caused by *Penicillium expansum* by an increase in the enzymatic activity of peroxidase, catalase,  $\beta$ -1,3-glucanase, and the concentration of phenolic compounds, related as defense mechanisms against pathogens [42]. Studies realized in different plants such as tobacco, broccoli, tomato, lemon, apple, potato, and rice, reported that different *Trichoderma* species promote the expression of

genes dependent on the defense mechanism of plants. Some of the expressed genes were *chit36*, *chit42*, *agn13.1*, and *gluc78*, which correspond to defense enzymes against cellular attack [7].

## **7. *Trichoderma*: mechanisms for controlling pathogens**

The biocontrol mechanisms attributed to *Trichoderma* spp. are: competition for nutrients, parasitism, antibiosis, secretion of enzymes, and the production of inhibitor compounds [46, 47]. This biocontrol agent attacks and penetrates fungal cells, causing an alteration with the consequent degradation of the cell wall, causing retraction of the plasma membrane and disorganization of the cytoplasm [48]. These mechanisms are favored by the ability of *Trichoderma* to colonize the rhizosphere of plants.

### **7.1 Competition**

Competition is defined as the unequal behavior of two or more organisms before the same requirement (substrate, nutrients), if the use of this substrate by one of the organisms reduces the amount or space available to others. This type of antagonism is favored by the characteristics of the biological control agent as ecological plasticity, growth rate primarily as chlamydospores [49], speed of development, and external factors such as soil type, pH, temperature, and humidity [50]. Nutrient competition can occur for nitrogen nonstructural carbohydrates (sugars and polysaccharides such as starch, cellulose, chitin, laminarin, and pectin, among others) and microelements.

### **7.2 Mycoparasitism**

Mycoparasitism is defined as an antagonistic symbiosis between organisms, generally involving extracellular enzymes such as chitinases, cellulases, and which correspond to the composition and structure of the cell walls of parasitized fungi [51]. *Trichoderma* species' mycoparasitism during chemotropical process grows toward the host; hyphae adhere to it, wound on them frequently, and sometimes penetrate. Degradation of the cell walls of the host is observed in the late stages of the parasitic process [52], which leads to almost total phytopathogen weakening. This process is explained in four stages, within which it is recognized [53]:

1. *Chemotrophic growth*: the positive chemotropism directs growth toward a chemical stimulus.
2. *Recognition*: it is based on lectin-carbohydrate interactions. The lectins are proteins linked to sugars or glycoproteins, which agglutinate cells and are involved in the interactions between the components of the cell surface and their extracellular environment.
3. *Adhesion and curl*: occurs when the acknowledgment response is positive, *Trichoderma* hyphae adhere to host-mediated enzymatic processes. Hyphae adhesion occurs through association of a sugar antagonist wall with a lectin present in the wall of the pathogen.
4. *Lytic activity*: this stage is the production of extracellular lytic enzymes, mainly chitinases, glucanases, and proteases, which degrade the cell walls of the host and allow the penetration of antagonist hyphae.

*Trichoderma* can excrete metabolites like cellulases, glucanases, lipases, proteases, and chitinases in order to facilitate the insertion of hyphae for nutrient uptake of the pathogen, ending with the loss of cytoplasmic contents of the host cell [54]. The remaining cytoplasm is mainly surrounding the invading hyphae, showing signs of disintegration.

### 7.3 Antibiosis

Antibiosis is the inhibition of pathogen development by metabolized products and small toxic molecules, volatile and lytic enzymes, which operate structural polymers, such as chitin and  $\beta$ -1-3-glucans of the cell wall in most pathogenic fungi, producing an adverse effect on development and differentiation [55]. Given the above, it is said that the greater the amount of metabolic products, the antagonistic power increases; additionally, some authors mention that this mechanism is not the principle, due to the risk of emergence of the antibiotic-resistant pathogens [55].

### 7.4 Secretion of enzymes

The production of enzymes such as chitinase and/or glucanases produced by the fungus of *Trichoderma* is involved in the control of pathogenic fungi. These hydrolytic enzymes can degrade the cell wall polysaccharides (chitin and  $\beta$  glucans) affecting its stability and integrity [5].

### 7.5 Production of inhibitor compounds

The ability to induce resistance in a wide range of diseases caused by various classes of pathogens (including fungi, bacteria, and viruses) in a wide variety of plants may be an important characteristic of *Trichoderma* [56]. Currently, three classes of compounds are known that are produced by *Trichoderma* strains and that induce resistance in the plant. These are proteins with enzymatic functions, homologs of proteins encoded for avirulence (Avr), and oligosaccharides.

## 8. Advantages and limitations on the use of *Trichoderma*

Fungi possess characteristics that define their potential as biocontrol agents. *Trichoderma* species are cosmopolitan microorganisms, inhabitant natural of soil such as organic matter, decaying wood as well as in crops waste. *Trichoderma* has several advantages as a biological control agent, such as a rapid growth and development as well as good production of a large number of enzymes inducible with the presence of phytopathogenic fungi [57]. In soil, *Trichoderma* has the ability to assimilate nutrients faster than pathogens, favoring its establishment and development, thus controlling pathogen infection and dissemination. Its use as a biocontrol agent can provide excellent advantages from the economic, environmental, and biological point of view, since they do not cause deterioration to the environment, do not affect the development of the plants, their production is cheaper, and its use does not entail the emergence of new pests or secondary pests [58]. However, its production on an industrial scale has some drawbacks that have limited the development of these organisms with wide possibilities as antagonist [59]. Even when, *Trichoderma* already exists in commercial form, its storage life is short, several investigations have been carried out finding that the best methods of fungus conservation [60]. In this sense, the incorporation in the formulations of different additives can improve microbial viability [2]. Other important limitations in the use

of *Trichoderma* involve the lack of precise information to farmers, the little training about how to use the antagonistic fungi, as well as the lack of government economic support [61].

## **9. *Trichoderma* in combination with other control systems at postharvest management**

In the management of postharvest fruit diseases, the use of antagonistic fungi is an alternative with great potential; specifically, the application of *Trichoderma* and its various species has obtained very promising results leading to the fabrication of commercial biocontrol products [62]. In addition, considering their mechanisms of action, such as competition for nutrients, production of lytic enzymes, parasitism, antibiosis, and induction of resistance, ensures the control and destruction of the main postharvest fungi. Even when *Trichoderma* has several mechanisms of action reported [63–65], the sole application of antagonists for controlling postharvest pathogens does not ensure 100% of disease control, that is why the development of new control technologies like combined system with other substances and methods could offer economical treatments and enhance biocontrol activity [66–68]. The combination of strains of *Pseudomonas-Trichoderma* and *Trichoderma viride-Bacillus subtilis* was effective controlling *in vitro* and *in vivo* tests such as *Penicillium digitatum* and *Penicillium expansum* and *Fusarium moniliforme*, respectively, in citrus and grapes. These studies confirm the synergistic effect of the combination of biological control agents [69, 70]. The addition of *Trichoderma harzianum* in polymeric matrices like chitosan has been evaluated against *Fusarium oxysporum* with good results, inducing defense mechanisms and the production of lytic enzymes as well as parasitism. In strawberry fruits, the incorporation of *Trichoderma* in chitosan and the application of a physical treatment (hot air) were effective to reduce microorganisms and maintain the fruit quality [71, 72]. The efficacy of the combination of bacterial (*P. syringae*) and fungal (*Trichoderma*) antagonists has been evaluated against *Botrytis cinerea* and *Fusarium oxysporum* *in vitro* tests, a synergistic effect was observed controlling successfully both fungus by the alteration and, consequently, the degradation of cell wall [73]. It has been reported that the different species of *Trichoderma* present different degrees of pathogen control at postharvest stage applied individually, but if the strains are combining, the potential of biocontrol increases, as previously reported in combinations with *T. viride*, *T. harzianum*, and *T. koningii* in the control of *Colletotrichum musae* reducing the incidence of crown rot [68]. The use of GRAS substances like sodium bicarbonate with *Trichoderma harzianum* was effective to control crown rot in banana fruits (*Colletotrichum musae* and *Fusarium verticillioides*) [74]. Recently, silver nanoparticles with *Trichoderma* have been synthesized and their antifungal capacity against postharvest pathogenic fungi such as *Alternaria*, *Penicillium*, and *Fusarium* has been evaluated; a good control on the mycelial growth and development of the pathogenic fungi was reported at low concentrations of *Trichoderma* spp. [75].

## **10. Conclusions**

*Trichoderma* is a biocontrol agent with several benefits for its application on different commodities; this fungus represents a suitable eco-friendly alternative to fungicides by reducing postharvest losses.

## **Conflict of interest**

Authors declare no conflict of interest.

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