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BIOCONTROL OF POSTHARVEST FUNGAL DISEASES BY MICROBIAL ANTAGONISTS - minireview

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Abstract: This paper summarizes the data on use of antagonistic microorganisms as biocontrol agents against fungal phytopathogens that affects postharvest fruits and vegetables. The use of synthetic fungicides has been the dominant control strategy for diseases caused by fungi. However, their excessive and inappropriate use in intensive agriculture has created problems that have led to environmental contamination, considerable residues in agricultural products, and phytopathogen resistance. Therefore, there is a need to generate alternatives that are safe, ecological, and economically viable to face this problem. Inhibition of phytopathogen in fruit/vegetable utilizing antagonistic microorganisms as biological control agents (BCA) could represent a viable and environmentally safe alternative to synthetic fungicides.

Key words: *postharvest, biocontrol, microbial antagonists, biological control agents*

INTRODUCTION

Fresh fruits and vegetables are exposed to different disease between postharvest and consumption period which leads to significant economically and food losses. The biggest losses are during storage due to pest and pathogen infections (bacteria, fungi, insects), unfavorable environment conditions (rain, humidity, frozen, heat), water lost, saccharification and germination (Hodges et. al., 2010; Buccholz et al., 2018). Classic treatments with fungicides and/or food preservatives are used for postharvest decline control, although the exposure to these chemicals are in many cases dangerously for human, animals and environment (Droby et al., 2016). Cause to toxicological risk of residual pesticides in food, the postharvest application of chemicals has been limited at few substances and completely banned in some European countries (Wisniewski et al., 2016). Currently, it's want a new approach that is efficient, friendly and environmentally safe in order to reduce post-harvest food losses, as a result of the increasing demands for energy conservation through “green” technologies and organic products. The bioproducts based on useful strains such as plant growth promoting bacteria (PGPB) could be considered an alternative to chemical fungicides and/or food preservatives in the reduce postharvest diseases. PGPB are a group of beneficial non-pathogenic bacteria that can directly and/or indirectly stimulate the plant growth promoting, disease resistance and tolerance to abiotic stress. These microorganisms can live autonomously in the soil or colonize the rhizoshere, phylosphere (epiphytic bacteria) and the inner plant tissues (endophytic bacteria) (Baez-Rogelio et al., 2016; Lastochkina et al., 2017, 2019). A wide range of antagonistic bacteria, fungi and actinomycetes are used for the management of diseases of horticultural crops. Antagonists like *Bacillus subtilis, B. cereus, Bradyrhizobium japonicum, Enterobacter cloacae, Pseudomonas aeruginosa, P. fluorescens, P. corrugata, P. putida, Rhizobium* spp., *Streptomyces* spp., *S. griseoviridis, Penicillium* spp., *Trichoderma harzianum, Chaetomium* *globosum, Cladorrhinum foecundissimum, Coniothyrium minitans,* non-pathogenic strains of *Fusarium* e.g., F047 strain of *Fusarium oxysporum and F. oxysporum* f. sp. *niveum, Gliocladium virens, Glomus* spp., *Glomus fasciculatum, G. intraradices, G. mosseae, Penicillium oxalicum, Sporidesmium sclerotivorum, Talaromyces flavus, Vertilicillium biguttatum* and *Trichoderma* spp., are used as potential biocontrol agents against the pathogens causing diseases in horticultural crops (Whipps, 1997).

A particularly interesting PGPB belongs to the genus *Bacillus* spp., which is one of the most attractive biocontrol agents for the development of plant protection bioproducts. *Bacillus* species are generally recognized as safe microorganisms for the application in food industry, and having the ability to produce a wide range of compounds with antibiotic activity. Moreover, *Bacillus subtilis* produces endospores resistant to physical and chemical treatments such as heat, desiccation, organic solvents and UV irradiation, this makes it possible to easily formulate and store *Bacillus*-based bioproducts as a potential bioactive component against phytopathogens. The use of *Bacillus* species for postharvest diseases biocontrol of various fresh fruits /vegetables has been frequently reported (Arroyave-Toroa et al., 2017). These effects may consist of increase the resistance of fresh products to broad spectrum of postharvest diseases and unfavorable storage conditions correlated to prolonging shelf life and maintaining of nutritional qualities (Jiang et al., 2001).

For example, the ability of this antagonist to suppress postharvest pathogens development that cause gray rot (*Botrytis cinerea* and *B. mali*) to strawberries, pears, apples and tomatoes has been demonstrated (Kim et al., 2016; Singh & Deverall, 1984; Mari et al., 1996; Touré et al., 2004; Zhao et al., 2007; Jamalizadeh et al., 2010; Kilani-Feki et al., 2016; Fan et al., 2017).

Potential action modes of microbial antagonists

The elucidation of action mechanisms of microbial antagonists is essential in determining the most effective formula of application that inhibits the maximum growth and functioning of the pathogen on a harvested host plant (Droby et al., 1992). Although the suppress mode of postharvest pathogens by antagonistic microorganisms is not fully understood, more mechanisms of action have been suggested, including: i) competition with pathogenic microflora for nutrients and niches supply for colonization (Beneduzi et al., 2015; Jiang et al., 2001; Janisiewicz et al., 2000); ii) producing of antibiosis metabolites e.g. antibiotics, biosurfactants, siderophores, hydrogen cyanide (Fan et al., 2017; Jijakli et al., 2001; El-Ghaouth et al., 2004; Cawoy et al., 2015; Baindara & Korpole, 2016); iii) synthesis of hydrolytic enzymes such as chitinases, glucanases, proteases and lipases, that can destroy the pathogenic fungal cells and a number of compounds (Van der Ent et al., 2009; Maksimov et al., 2011) and, iv) the elicitors activity and systemic induced resistance (ISR and SAR) (Van der Ent et al., 2009; De Vleesschauwer & Höfte, 2009) (Figure 1).

The metabolism of these compounds is mainly regulated by host plant hormones e.g. salicylic acid, abscisic acid, jasmonic acid, ethylene and they appear due to bacterial determinants like flagelines, lipopolysaccharides, siderophores, antibiotics, biosurfactants and volatile compounds (Maksimov & Khairullin, 2016; Cawoy et al., 2015; De Vleesschauwer & Höfte, 2009).

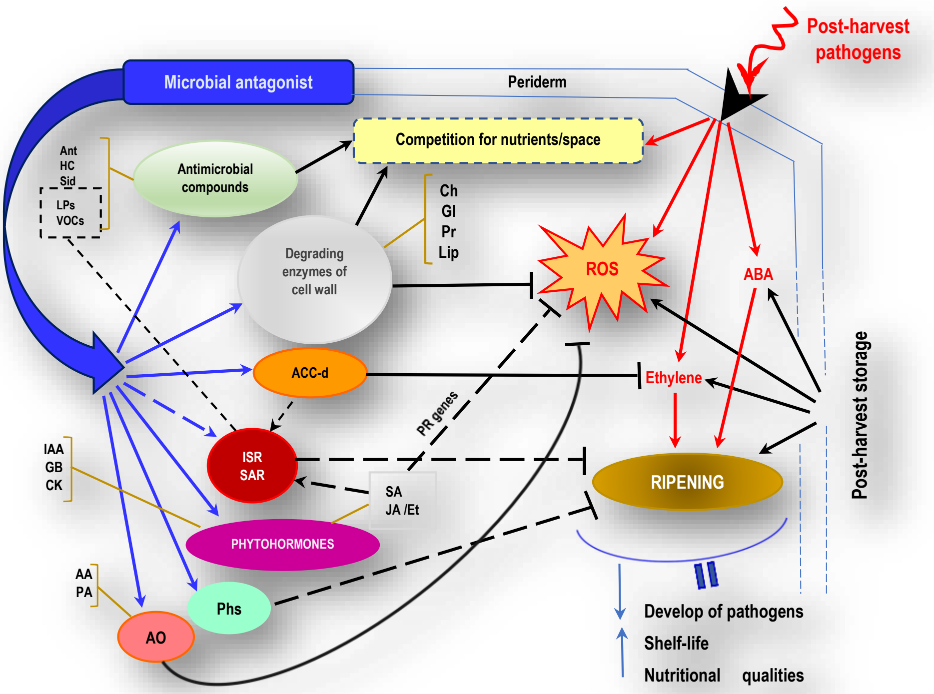

Figure 1. The diagram of microbial antagonist mechanisms against pathogenic infections and their interaction in harvested fruits/vegetables during storage. AA-ascorbic acid; ABA-abscisic acid; ACC-d-1-aminocyclopropane-1-carboxylate deaminase; Ant-antibiotics; AO-antioxidants; Ch-chitinases; CK-cytokinins; Et-ethylene; GB-giberellins; Gl-glucanases; HC-hydrogen cyanide; IAA-indole -3-acetic acid; ISR- induced systemic resistance; JA-jasmonic acid; Lip-lipases; PA-peroxidase; Pr-proteases; Phs-phytoalexins; ROS- reactive oxygen species; SA-salicylic acid; SAR-systemic acquired resistance; Sid-siderophores; LPs-lipopolysaccharide; VOCs-volatile compounds (according to Lastochkina et al., 2019, modified)

Methods of application of antagonists

The efficacy of potential antagonistic microorganisms to suppress the pathogens of harvested fruits/vegetables depend not only the strain features but method of application too. Generally, biocontrol agents can be applied using pre-or post-harvest strategies (Figure 2).

Pre-harvest application Pathogens often infect fruits and vegetables in the field and living asymptomatically in plant tissues during the growing season; however, 'hidden' infections can develop during storage of products and can become a major degradation factor, leading to significant food losses (Coates et al., 1997). The research regarding use of antagonistic microorganisms as microbial inoculants suggests that their application reduces stress-induced effects and has a positive effect on crop yield during storage (Gao et al., 2016; Maksimov et al., 2011). According to Ippolito and Nigro (2000), the pre-harvest soon application of antagonists help to colonize of fruit surfaces and protect them against storage phytopathogens. Although this approach has successfully in several cases, commercially it has not become viable due to poor survival rate of microorganisms in field conditions. However, there is proof regarding the efficacy of *Bacillus* species in biocontrol of postharvest diseases to avocado, by using of bacteria during vegetation and pre-vegetation period, before fruits storage (Korsten et al., 1991).

The *post-harvest application* of microbial antagonists on fruits and vegetables has been introduced as an appropriate and practical method for disease control. According to this method, bioproducts based on antagonistic microorganisms can be applied as sprays by post-harvest spraying or as solutions in harvested fruits/vegetables (Figure 2) (Irtwange, 2006; Barkai-Golan, 2001).

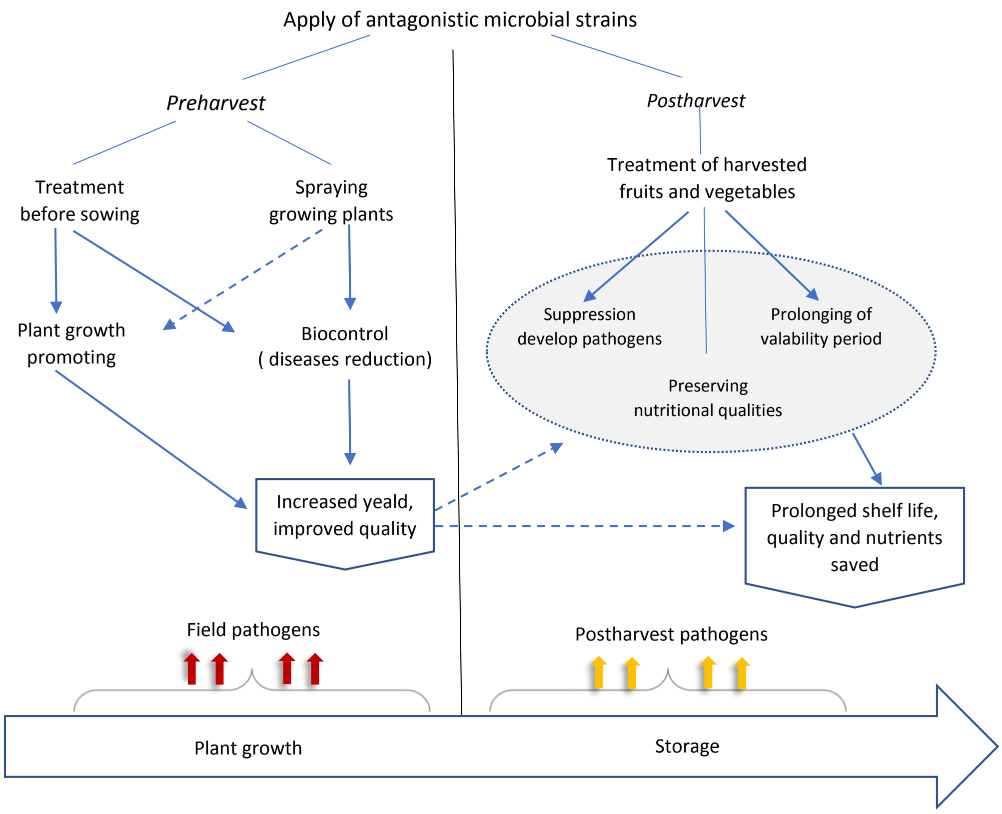

Figure 2. The diagram for methods of antagonistic microorganisms application in the control of fruits/vegetables diseases during storage (according to Lastochkina et al., 2019, modified)

However, the application of a single antagonist cannot completely prevent degradation of fruits/vegetables during storage, as it is difficult to select only microbial strain with broad spectrum of action against various phytopathogens (El-Ghaouth et al., 2004; Singh & Sharma, 2009; Barkai-Golan, 2001; Droby, 2006). Thus, some producers use mixed of microbial strains to increase the effectiveness of bioproducts e.g. “Companion” (Growth Products Ltd., USA) formulated based on GB03 strain of *Bacillus subtilis*, *B. lichenoformis* and *B. megaterium*, or the bioproduct “Bactril” (Biopharmatec, Russia) containing a mixture of 132 *B. nigrum* strain, MBI600 strain of *B. subtilis* and *Bradyrhizobium japonicum*. It has been shown that mixtures of different broad spectrum antagonists and combination of different biocontrol qualities control two or more postharvest diseases when applied under different environmental conditions (Sharma et al., 2009). The efficacy of microbial anatgonists to control of postharvest fruit/ vegetable degradation can be achieved by adding intensifying elicitors such as calcium chloride, calcium propionate, sodium bicarbonate, ammonium molibdate, sodium carbonate, potassium methabisulphite, salicylic acid, or by combining pre- and postharvest with wax based agents (Droby, 2006). Also, microbial antagonists integration with physical methods like preservation or termic treatments, can improve the bio-effectiveness of microbial agents (El-Ghaouth et al., 2004; Sharma et al., 2009).

Management of diseases

Pre-harvest factors affecting post-harvest degradation For good management of postharvest diseases it is important to make right choice, the first steps been applied to field cultivation. The cultivars used in organic production systems must well functional under fertilization condition and pest management strategies, which are limited and significantly different from conventional methods. It is essential to be chosen both plant cultivars suitable for the growing area and growing areas which does not favor the development of pathogens, such as *B. cinerea* or *R. stolonifer*. Also, the management of weeds is important, as excessive weeds reduce fruit yields and increase moisture in the field. Soil solarisation or mulching are used to manage weeds. Besides to weed control, mulching has multiple functions such as heating or cooling the soil, protecting the fruit and foliage from soil, and / or improving moisture retention. Sanitation of crop plant is another key point. To prevent infection with gray rot, dead tissues should be removed from the plants and soil surface, both in the field and greenhouse. Also, all ripe fruit should be removed during harvesting, as well as any fruit with damage signs or rain damage, and plant debris should not be left under the workbench or on access roads. Strawberry plants should be grown using proper fertilization as high levels of nitrogen favor pathogen development.

Post-harvest factors affecting post-harvest degradation The most effective way to extend shelf life of fruits/vegetables is low temperature. Fast and constant cold storage is the key to ensuring a good quality of harvested fruits and vegetables. There is a direct relation between the time of harvesting and cool keeping and percentage of damaged fruits during storage. Thus, the faster cooling of harvested products it is, the lower post-harvest decomposition rate (Nunes et al., 1995). Furthermore, cool storage must be constant, as any interruption of cold chain may allow an infection development of a latent pathogen. A study by Boer et al., (2009) consisted to daily monitoring of some *Rhizopus* isolates on potato-dextrose-agar medium at different temperatures, from to 3°C at 20°C for 2 hours, then again at 3°C. They found a small difference regarding the global growth of fungal isolates depending on the number of interruptions, which indicates that cold storage requires only one interruption for the fungus to be activated (Boer et al., 2009).

The second important parameter during cold storage of fresh products is humidity, as low relative humidity dehydrated fruits while high relative humidity favor fungal growth. Usually, the optimum relative humidity to preserving fresh fruits, e.g., strawberries is between 90% and 95%. The atmosphere of during storage may be change so that, as the fruit breaths decrease the oxygen level and increase carbon dioxide level (Kader, 1995). Under these atmospheric conditions, the respiration rate of fruits is low and metabolic processes including ripening and senescence are slowed down. However, in some cases, there may be adverse effects on colour and flavor after exposure to very low levels of oxygen and very high levels of carbon dioxide (Ke et al., 1991; Shamaila et al., 1992). To improve the quality of post-harvest strawberries, very high levels of oxygen and enriched atmosphere in gases such as ozone (Ayala-Zavala et al., 2007; Nadas et al., 2003; Perez et al., 1999), nitric oxide (Soegiarto & Wills, 2006; Wills et al., 2000), nitrous oxid (Qadir & Hashinaga, 2001) or hydrogen sulphide (Hu et al., 2012) have been successfully tested as alternative atmosphere for preserving of fruits. A modified atmosphere is created when fruits are sealed in polyethylene bags with relative low gas permeability. Therefore, the quality of fresh fruits could improve although the gas concentrations in the bags during storage cannot be accurately controlled.

In recent years, demands for new packaging and technologies that are both safe and environmentally sustainable has increased considerably e.g., the use of biodegradable or edible packaging. Examples of edible layers for strawberries are those based on chitosan (Romanazzi et al., 2014) and methylcellulose (Nadim et al., 2014). These edible films act as a barrier against mechanical shocks but also, may contain antimicrobial compounds that inhibit the growth of surface microorganisms or nutraceutical compounds with nutritional value for the fresh products.

Application of conventional fungicides The infections caused by *Botrytis cinerea* that induce postharvest degradation usually occur before field harvest and may remain dormant until storage fruits. Due to the lack of infection symptomatology and some effective methods to anticipate the risk of disease, preventive applications of synthetic fungicides during the crop growing cycle are recommended. The treatments with fungicides are applied around of plant in flowering period and repeated until harvest, depending on the environmental conditions and pre-harvest interval (Feliziani & Romanazzi, 2013). The active compounds against to *B. cinerea* belong to class of aniline-pyrimidines and include cyprodinyl, mepanipyrim, pyrimethanil that block fungal aminoacids synthesis. *B. cinerea* is a classic “high risk” phytopathogen because develop resistance to several class of fungicides, frequently reported worldwide. The mutant strains of *B. cinerea* fungicide-resistanced have been isolated by laboratory and field studies, for many of active fungal agents (Myresiotis et al., 2007). The fungicides with broad spectrum of action or them mixtures can be used in controlling of fruit soft rot during ripening period. The main synthetic fungicides use for rot control caused by *Rhizopus* are boscalid, fludioxonil and fenhexamid.

Alternatives to conventional fungicides At present, use of synthetic fungicides is the most efficacy and common method of rottenness fruits and vegetables control. However, increasing of the consumer demands for fruits and vegetables without residual fungicides caused the interest of researchers and food industries to develop the control alternatives of degradation postharvest fresh products. These alternative compounds should be integrated with synthetic fungicides or, if it possible completely replaced.

Subsequently, some of studied alternatives for postharvest degradation control of strawberry fruits are summarised. These include: i) biocontrol agents (BCA); ii) natural compounds or decontamination agents; iii) physical methods; iv) combination of all three groups mentioned above (Poling, 2008; Mari et al., 2009).

BCAs are, mainly antagonistic bacteria and yeasts against to pathogens that cause the degradation of post-harvest fruits. These useful microorganisms have different mechanisms of action such as competition for space/nutrients, producing of antibiotics and volatile compounds, which are harmful to pathogens or induce resistance in host plant to defence against them (Romanazzi et al., 2012).

In recent years, many studies have been conducted on the isolation and testing of antagonistic microorganisms against to strawberry pathogens. Table 1 presents the most significant BCAs and their mode of action.

Table 1. Use of biocontrol agents on strawberry tissues, in the pre-harvest / post-harvest stages, against *Botrytis cinerea* and / or *Rhizopus stolonifer*

BCA	Target pathogen	Time of application	Mode of action	References
Trichoderma atroviride	*B. cinerea*	spraying on detached or senescent strawberry leaves and in field conditions	a combination for sugars competition, non-volatile compounds production and possibly mycoparasitism	[Card et al., 2009]
halophilic bacteria	*B. cinerea*	immersion of strawberry fruits in bacterial suspension	enzymes and volatiles production	[Essghaier et al, 2009]
Trichoderma harzianum	*B. cinereal* *R. stolonifer*	inoculation of healthy and diseased fruits	direct parasitism	[Batta, 2007]
Candida intermedia	*B. cinerea*	immersion of fruit in yeast cell suspension or exposure to yeast volatiles	volatiles production	[Huang et al., 2011]

Aureobasidium pullulans	*B. cinerea*	fruit softening	antibiotics production	[Liu et al., 2007]
Lactobacillus plantarum combined with cumin oil	*B. cinerea*	spraying on diseased fruits	competition for space/ nutrients	[Zamani-Zadeha et al, 2014]
Metschnikowia fructicola	*B. cinerea*	spraying in greenhouses, low-level plastic tunnels and in open field culture	–	[Karabulut et al., 2004]
Cryptococcus laurentii combined with immersion in hot water	*R. stolonifer*	fruits inoculation	competition for nutrients	[Zhang et al., 2007]

At present, there are already several marketed biofungicides based on antagonistic microorganisms against to *B. cinerea,* such as *Pseudomonas syringae* (BioSave; JET Harvest Solutions, Longwood, FL, SUA), *Bacillus subtilis* (Serenade; Bayer, Leverkusen, Germany), *Candida sake* (Candifruit; IRTA, Lleida, Spain) and *Metschnikowia fructicola* (Shemer; Bayer, Leverkusen, Germany). Compared to post-harvest application, the field treatments have a preventive effect against infection and disease development, as they allow the antagonist to have longer interactions with pathogen and colonize plant tissues before the infection. However, the biological treatments applied before harvest to be successful, the potential CBAs must tolerate low nutrient availability, UV radiation, high temperatures and drought conditions (Jamalizadeh et al., 2011). Also, before marketing a bioproduct, it must be shown that it has no phytotoxic effects or produces secondary metabolites that can be harmful to human health, but is also effective against post-harvest pathogens (Ippolito & Nigro, 2000; Wisniewski & Wilson, 1992; Nunes, 2012).

Natural substances or decontamination agents The compounds “generally recognized as safe” (GRAS) do not harm the environment and human health and are used for their antimicrobial features or inducing resistance in plant defence. Among the natural compounds, plant extracts and essential oils have been reported in the control of post-harvest diseases confirmed by *in vitro* and *in vivo* tests, as well as for prolonging the quality and shelf life of fresh goods (Oro et al., 2014; Antunes & Cavaco, 2010). Some examples of essential oils and vegetable extracts tested for post-harvest strawberry diseases are shown in table 2.

Table 2. Essential oils and plant extracts tested against postharvest diseases of strawberries

Scientific name	**Common name**	**Target pathogen**	**Mode of application**	**Reference**
Ocimum basilicum	basil	*B. cinerea*	–	[Giampieri et al., 2013; Alvarez-Suarez et al., 2014]
Lavandula angustifolia	lavander	*R. stolonifera,* *A. niger*	–	[Marjanlo et al., 2010]
Rosmarinus officinalis	rosemary	degradare post-recoltare	microencapsulation	[Asghari et al., 2010]
Foeniculum vulgare	fenician	*B. cinerea, A. niger, R. stolonifer*	–	[Marjanlo et al., 2010]
Thymus vulgaris	thyme	post-harvest decay	microencapsulation	[Asghari et al., 2010; Hadian et al., 2008]
Eucalyptus globulus	eucalyptus	post-harvest decay	exposure to vapors	[Alikhani, Daraei Garmakhany, 2012]

Cuminum cyminum	cumin	*B. cinerea, A. niger, R. stolonifer*	–	[Alvarez-Suarez et al., 2014; Marjanlo et al., 2010]
Mentha piperita	mint	*B. cinerea, A. niger, R. stolonifer*	–	[Marjanlo et al., 2010]
Cinnamonum zeylanicum	cinnamon	post-harvest decay	exposure to vapors	[Alikhani, Daraei Garmakhany, 2012]
Urtica dioica	nettle	*B. cinerea, P. expansum, R. stolonifer*	immersion	[Romanazzi et al., 2013]
Abies sibirica	fir	*P. expansum, R. stolonifer*	field treatments	[Romanazzi et al., 2013, Landi et al., 2014]

In some cases, even though essential oils are effective in controlling of postharvest degradation, sensory fruits testing should also be performed before and after marketing because the strawberries taste may be affected by their application. Inorganic salts have been shown to be active antimicrobial agents against a number of phytopathogenic fungi. Among these, bicarbonate has been proposed as a safe and effective alternative to control postharvest rot of fruits and vegetables, including strawberries (Feliziani et al., 2013). Treatments with calcium-rich formulations can strengthen the middle lamellae of strawberry cells to increase their resistance to mechanical and biological damage (Romanazzi et al., 2013; Sanzani et al., 2009). Also, successful results had been obtained by using of ethanol and chlorine dioxide for prolonging storage period of postharvest strawberries (Ayala-Zavala et al., 2007; Mari et al., 2009; Rahman et al., 2013) and maintaining of fruit qualities, respectively (Karabulut et al., 2004; Shin et al., 2012; Vandekinderen et al., 2009). One of the most widely used postharvest treatments to control fungal growth and reduce the respiration rate of strawberries is a modified storage atmosphere, enriched with carbon dioxide, to reduce the incidence and severity of decomposition and thus to prolong fruit life. However, in some cases have been reported adverse effects on fruit colour and flavor after exposure to low oxygen levels and very high levels of carbon dioxide (Ke et al., 1991; Shamaila et al., 1992). Thus, very high conditions of oxygen had been tested as alternative to the conventional packaging atmosphere of fruits/vegetables inclusive strawberries, because inhibit the growth of bacteria, yeasts and molds and can prevent undesirable anoxic fermentation (Ayala-Zavala et al., 2007; Aday & Caner, 2014; Zheng et al., 2008). In addition, the installation of ozone generators in strawberry cooling chambers has increased in recent years due to the fact that ozone is considered a safe element, does not deposit residues on fruits, is allowed in organic farming and is effective in controlling post-harvest degradation (Allende et al., 2007). When strawberry fruits were cold stored into experimental enriched atmosphere in ozone, them quality was maintained more long time (Ayala-Zavala et al., 2007; Nadas et al., 2003; Allende et al., 2007).

Physical methods to protecting of fruits/vegetables include heat treatment, UV-C exposure and hypo-/hyper-baric treatments (Mari et al., 2009). The storage of strawberry fruits under environment conditions with hypo-baric pressure to reduce the post-harvest decay (Mohammadi et al., 2015; Romanazzi et al., 2001) and induce resistance in the fruit tissues has been successfully demonstrated (Hashmi et al., 2013).

Heat treatments are carried out by fruit immersion in hot water or exposing them to hot steam or air. The duration of process is variable, depending on cultivar and fruits. Strawberries are very sensitive therefore the time and temperature of heat treatment must be accurately monitored. Some studies have investigated optimal combinations of time and temperature for heat treatments and concluded that the treatment between 44°C and 46°C, for 15 minutes is the best treatment combination to maintain firmness and initial qualities of strawberries, no colour or flavor changes.

Ultraviolet (UV) treatment is a technique that uses different energy levels and is applied to many fruits and vegetables (Jing et al., 2010; Pombo et al., 2011; Kim et al., 2010). The treatment causes stress that determine intensify of defence enzymes expression and implicit, fungal rot decrease. A recent study, which conducted field UV-C treatments on strawberry plants, showed that these treatments did not affect the phytochemical profiles of fruits (Nigro et al., 2000).

Ultrasound is one of the newest non-thermal methods that has been experimentally tested to extend the shelf life of fresh fruit (Xie et al., 2015). The effectiveness of ultrasound depends on the wave frequency and strength and the treatment duration. Thus, 30W and 60W ultrasound, applied of strawberries for 5-10 min have been shown to be effective in reducing the growth of rot and extending the fruit shelf life (Xie et al., 2015).

CONCLUSIONS

The use of biological control agents (BCA) as an alternative to synthetic products has been a focus of research in the last 30 years by many researchers and several commercial enterprises worldwide. The application of these alternative products has already been integrated into common cultivation practices, many of which are available to farmers. Although are generally less effective in controlling of fruits/vegetables decay than fungicides, studies are needed on their applicability as well as the number / time / and concentration of treatments.

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