Review Article

Biocontrol agents against post harvest decay in fruits and vegetables: A review

Abstract

Post harvest losses are the major threats in the supply chain between harvest and consumption which contribute 44% of the total loss of fruits and vegetables. Among the various causes, post harvest diseases are major post harvest decay of fruits and vegetables accounting for 20-25% losses. The causative fungus belonging to both biotrophic and necrotrophic nature belongs to the genera of *Aspergillus, Penicillium, Botrytis*, etc. Management of post harvest diseases by conventional chemicals is not preferred due to residual effects and toxicity. Considering the global demand and consumer awareness about the health effects of pesticides, biocontrol agents are getting attention in recent times for post harvest disease management. Biocontrol agents like *Trichoderma, Bacillus* and *Pseudomonas* have been explored for successful management of post harvest diseases of citrus, strawberry, tomato, etc. The possible mode actions of the BCAs are competition for nutrients and space, production of antimicrobial compounds, hydrolytic enzymes, and induced resistance. The added advantage of BCAs is that they can be integrated with other physical, natural compounds and additives for coatings due to their synergistic and mutualistic effect. The product development for biocontrol origin must be encouraged to utilize the benefits they provide. Several constraints in process of product development may arise which can be overcome by more research, education, training at the farm level, and multi-omics studies to unravel the potentials of the BCAs.

Key words: Biocontrol agent, biotrophic, necrotrophic, BCA, synergistic

INTRODUCTION

Fruits and vegetables are considered as major source of nutritional security providing vital minerals and nutrients as well as economically viable crops for the growers. Various biotic factors challenge the crops during pre and post harvest, of which, fungal and bacterial pathogens are causes severe losses by causing many diseases resulting in significant economic losses. According to the Food and Agriculture Organization, about 45% loss occurs in harvested fruits, vegetables, roots, and tubers and out of this about 20– 25% loss is caused by pathogens during postharvest handling even in developed countries [1,2,3]. Post harvest loss is the loss of a commodity after it is harvested and such losses cause considerable damage both quantity and quality of fruits. It can be due to various reasons such as environmental factors (temperature, humidity during harvest and transportation); physical injuries during crop harvesting and transportation and, microbial actions [4,5].Post harvest loss is not only the numerical count of lost fruit and the quality or taste. Qualitative food loss can degrade the food's nutrients, texture, shape, or taste. Commercially, these products will fetch a lower price. However, quantitative food losses are the reductions in edible produce mass accessible for personal food across supplier management segments. In simple terms, quantitative food loss is unconsumed food. Pests eating or spoiling the food can cause this quantitative food loss [6]. In developing countries, postharvest losses are often more severe due to pest and pathogen infestation (bacteria, fungi, and insects), unfavourable environmental conditions (rain, humidity, frost, and heat), water loss, saccharification, sprouting, and also inadequate storage and transportation facilities. Traditionally, chemical fungicides and/or food preservatives are used to control postharvest decays [7]. However, exposure to the chemicals is often hazardous to humans, animals, and the environment [8]. Since fresh fruits and many vegetables are consumed raw, fungicide contamination in such commodities pose serious health risk to the consumers. Due to the toxicological risk of residual chemicals in food products, their application in the postharvest period has been limited to a few registered chemicals and is completely prohibited in some European countries [9] restricting export possibilities of many crops. Instead there is growing demand for organic produce or quality food with no chemical residues. The increasing relevance of food and environmental problems, as well as growing demand for energy conservation through natural "green" technologies and organic products, would make it highly desirable to have an approach to the reduction of postharvest food losses that is novel, efficient, environment friendly, and bio-safe [10]. In recent time microbes are being explored as potential; alternative to post harvest fungicides. Biological products with beneficial strains, such as plant growth-promoting bacteria (PGPB), endophytes, and many yeasts are being explored as new strategy against postharvest disease [11,12]. Rise in literature demonstrating role of microbes like Bacillus spp, Pseudomonas, Yeasts amply demonstrates need to introduce these microbes for value chain management including post harvest diseases[7]. The microbe based products establish various physiological changes in host plant metabolism, leading to systemic resistance and prolonged shelf-life without causing adverse effects on plants, humans, or the environment.

POST HARVEST PATHOGENS

Phytopathogenic fungi and bacteria cause postharvest diseases of economically important fruits and vegetables. Different species of bacteria belonging to the major genera viz. *Pseudomonas*, *Xanthomonas*, *Erwinia*, *Xylella*, *Ralstonia*and fungalgenera include*Penicillium*, *Aspergillus*, *Botrytis*, *Fusarium*, *Alternaria*, *Colletotrichum*, etc. The most important pathosystem of postharvest fruits and vegetables includes green mold (*Penicillium digitatum*), blue mold (*Penicillium italicum*), graymold (*Botrytiscinerea*),and white mold (*Sclerotinia* spp.)[13]

Table 1: Postharvest diseases of economically important fruits and vegetables

Nature of disease	Disease name	Crop	References
Fungi	*Botrytis cinerea*	Tomatoes, citrus fruit, grapes, strawberries	[14]
	Penicillium expansum	Apples, citrus fruit	[15]
	Penicillium italicum	Citrus fruit	[15]
	Plasmoparaviticola	Grapes	[16]
	Rhizopus stolonifera	Strawberries	[16]
	Alternaria altrnata	Tomatoes, grapes	[16]
	Fusarium spp.	Melons	[16]
	Trichotheciumroseum	Cucurbits(Melons)	[16]
	Colletotrichum gloeosporioides	Loquats	[16]
	Colletotrichum acutatum	Citrus fruit	[16]
Bacteria	*Clavibactermichiganensis*	Tomatoes	[17]
	Xanthomonas axonopodis	Tomatoes, peppers	[18]
	Salmonella enteric	Tomatoes, melons	[19]
	Escherichia coli	Tomatoes, strawberries	[19]

Virus	Ringspot virus	Papayas	[20]

Post harvest physiological changes like transpiration, respiration, ethylene production and senescence affect the post harvest disease development and deterioration of the fruits.All the changes encourage the biotrophic pathogen to act as necrotrophs and elicit symptoms and also attract opportunistic pathogens which help in disease development and affect the post-harvest quality.

Impacts of post harvest diseases on fruits and vegetables

i) Reduce the quality in harvest fruits and vegetables.

ii) Reduce the shelf life of the products due to rotting.

iii) As a result of decay fruits appear to be ugly in appearance and the market value decreases.

iv) Due to low market value it increases the cost of production and challenges food security globally

v) It also has impacts on human health due to the production of harmful mycotoxins.

Management Strategies

Diseases in harvested fruits are managementthrough different methods like physical treatments by use of heat treatment, irradiation, precooling, modified and controlled atmospheric storage and innovative packaging [21, 22, 23]. Various chemical treatments are used such as sanitizing agents like chlorinated water, sodium hypochloride, etc for surface cleaning of fruits and vegetables [24]. Use of inorganic and organic compounds which belongs to GRAS (Generally regarded as safe) category are and also considered safe for human [25]. Several natural antifungal compounds from plants and animals origin are used to control post harvest diseases which have low phytotoxicity and environmental toxicity [26, 27]. Biocontrol by microbial antagonists (bacteria, yeasts, fungi) is under investigation as an alternative to the application of synthetic fungicides for disease control in the field and postharvest applications [2, 29].

Microbes in Post Harvest Disease Management

Fruits and vegetables produce which contains unauthorized pesticides are being rejected by the international market due to pesticides residues exceeding permissible limits, and with inadequate labelling and packaging. Hence, biological control through bioagents have a great potential, among the important microbes *Trichoderma viride,T. harzianum*, *Pseudomonas flourescens* and *Bacillus subtilis* are most efficient bioagents which act as a producer of biologically active metabolites like antibiotics, bacteriocin and inducers of systemic resistance in plants. Biofilm formed by antagonistic yeast stick to pathogen and parasitize on the hyphae of the pathogen [30, 31].

A potential microbial antagonist should have certain desirable characteristics to make it an ideal bioagent [32, 33]. The antagonist should be: (a) genetically stable; (b) effective at low concentrations; (c) not fastidious in its nutritional requirements; (d) capable of surviving under adverse environmental conditions; (e) effective against a wide range of the pathogens and different harvested commodities; (f) resistant to pesticides; (g) a non-producer of metabolites harmful to human; (h) non-pathogenic to the host; (i) preparable in a form that can be effectively stored and dispensed; and (j) compatible with other chemical and physical treatments. In addition, a microbial antagonist should have an adaptive advantage over a specific pathogen [34].

There are two basic approaches for using microbial antagonists for controlling the postharvest diseases of fruits and vegetables: 1) the Use of microorganisms which already exist on the product itself, which can be promoted and managed or 2) those that can be artificially introduced against postharvest pathogens.

1) Natural microbial antagonist

Natural occurring antagonists are those, which are present naturally on the surface of fruits and vegetables, and after isolation, antagonists are used for the control of postharvest diseases. [35] found that when concentrated washings from the surface of citrus fruit were plated out on an agar medium, only bacteria and yeast appeared while after dilution of these washings, several rot fungi appeared on the agar, suggesting that yeast and bacteria may be suppressing fungal growth. Thus, it indicates that when fruits and vegetables are washed, they are more susceptible to decay than those, which are not washed at all.

2) Artificially introduced microbial antagonist

Although the first reported use of a microbial antagonist was the control of Botrytis rot of strawberry (*Fragaria x ananassa*Duch.) with *Trichoderma*spp. [36], the first classical work was the control of brown rot of stone fruits by *Bacillus subtilis* [37]. The biocontrol potential of several other microbial antagonists has also been demonstrated in several fruits such as banana [38], mango (*Mangifera indica* L.) [39,40], litchi (Litchi chinensis Sonn.) [41}, papaya (*Carica papaya* L.) [42], avocado [43], kiwi fruit (*Actinidia deliciosa* Ber.) [44], jujube [45] and vegetables like tomatoes [46] cabbage (*Brassica oleracea* var. *capitata* L.) [47], chillies (*Capsicum fruitsecence* L.) [48] and potato [49]. Some artificially introduced microbial antagonists are listed below:-

Table 2: List of microbial antagonist

Antagonists	**Disease(Pathogen)**	**Fruits/ vegetables**	**References**
Aureobasidium pullulans	Monilinia rot (*Monilinialaxa*)	Banana	[50]
	Penicillium rots (*Penicillium spp*.)	Citrus	[51]
	Botrytis rot (*Botrytis cinerea*)	Grapes	[52]
	Soft rot (*Monilinialaxa*)	Grapes	[32]
Bacillus subtilis	Brown rot (*Lasiodiplodiatheobromae*)	Apricot	[37]
	Stem end rot (*Botryodiplodiatheobromae* Pat.)	Avocado	[43]
	Green mold (*Penicillium digitatum*)	Citrus	[53]
	Gray mold (*Botrytis cinerea*)	Strawberry	[54]
	Alternaria rot (*Alternaria alternata*)	Muskmelon	[55]
Candida sake (CPA-1)	Penicillium rot (*Penicillium expansum*)	Apple	[56, 57]
	Blue mold (*Penicillium expansum*)	Pear	[5]

Enterobacter cloacae	Rhizopus rot (*Rhizopus stolonifer*)	Peach	[58]
Metschnikowia pulcherrima	Blue mold (*Penicillium expansum*) and Gray mold (*Botrytis cinerea*)	Apple	[59]
*Pseudomonas aeruginosa (*Schroter*)* Migula	Bacterial soft rot *(Erwinia carotovora*sub sp. Carotovora*)*	Cabbbage	[47]
Trichoderma harzianum	Anthracnose (*Colletotrichum musae*)	Banana	[60]
	Gray mold (*Botrytis cinerea*)	Kiwi	[44]
	Anthracnose (*Colletotrichum gloeosporioides*)	Rambutan	[61]
Trichoderma viride	Green mold (*Penicillium digitatum*)	Citrus	[62]
	Gray mold (*Botrytis cinerea*)	Strawberry	[63]
	Stem-end rot (*Botryodiplodiatheobromae*)	Mango	[36]

Mode of Action

1) Competition for space and nutrients

Antagonistic microorganisms trigger inhibition of rapid wound site colonization, which is a crucial step in controlling postharvest decay. The efficiency of the antagonist mainly depends on their ability to outperform pathogens based on their capacity for rapid growth and survival under unfavorable conditions, and is strongly dependent on their initial concentration when applied on the wound site [64].The most effective concentration in controlling postharvest fruit/vegetable diseases is generally considered to be 10^7-10^8 CFU/mL [65].As similar ecological niches exist in both endophytic and phytopathogenic microorganisms, endophytes are considered a prime candidate for the biocontrol of phytopathogens [66]. Due to the stable pH, proper humidity, sufficient nutrient flow, and lack of competition in the endosphere, endophytes have a significant advantage over epiphytic organisms available in the rhizosphere and phyllosphere [67, 68].

Competition for nutrients is another significant contribution to the biocontrol of pathogens.*In-vitro* studies shows that bacterial inoculants take up nutrients faster than pathogenic fungus; this can lead to the inhibition of the germination of pathogen spores at the wound site. A fundamental strategy for nutrient competition is the attachment of microbial antagonists to the hyphae of a pathogen since the antagonists feed on nutrients faster than the target pathogen, thus hampering spore germination and pathogen growth [58]. Nevertheless, in certain cases such as *Aureobasidium pullulans* against *Botrytis cinerea*, *Rhizopus stolonifer*, *Penicillium expansum*, and *Aspergillus niger*, which infect table grapes and *P. expansum* and *B. cinerea* on apple fruit, direct physical interaction is not required for the antagonistic activity [69]. In such circumstances antagonism does not occur via the direct attachment of antagonistic microorganisms to pathogen hyphae. Rather, it is highly likely that other alternative mechanisms, such as the production of a wide range of biologically active molecules, such as antibiotics, biosurfactants, siderophores, hydrogen cyanide, and hydrolases increase their advantage against pathogens as they compete for a suitable niche for colonization [70, 71].

2) Production of Antimicrobial Compounds

Antibiotics are a heterogeneous group of low molecular weight organic compounds produced by bacteria, which suppress or diminish the growth and development of phytopathogenic microorganisms [72]. Antibiotics can cause disruption in a microorganism's cell wall structure or membrane function, disrupt protein synthesis, and inhibit respiratory enzyme function [73]. Thus, most Bacillus antibiotics are active against both gram-positive and gram-negative bacteria, as well as phytopathogenic fungi such as *Aspergillus flavus*, *Alternaria solani*, *Fusarium oxysporum*, *Botryosphaeria ribis*, *Helminthosporium maydis*, *Phomopsis gossypii*, and *Colletotrichum gloeosporioides*. *Bacillus* antibiotic substances break growing hyphae tips of *Sclerotinia sclerotium* (the stimulant of sunflower white rot), *A. alternata*, *Drechleraoryrae* as soil fungi, and *F. roseum*, as well as *Puccinia graminis* (the inducer of cereals rust). It has been found that *B. subtilis* has broad suppressive properties against more than 23 types of plant pathogens in vitro due to its ability to produce a broad range of antibiotics with a wide variety of structures and activities [74]. Some *Bacillus species* may dedicate up to 8% of their genetic potential to the synthesis of a wide range of antimicrobial compounds, among which non-ribosomally synthesized LPs, lytic enzymes, and lantibiotics are suggested to be crucial for pathogen suppression [75].

3) Synthesis of hydrolytic enzymes

Alternative probable mechanisms of the antagonistic function of bioagent can be attributed to the synthesis of extracellular hydrolases such as chitinases and β1,3-glucanases capable of destroying the structural polysaccharides in the cell wall (chitin and glucans) of fungus and lysing the hyphae of fungi [76, 77, 78]. For several bacteria, a correlation between antagonistic activity to various pathogenic fungi and the synthesis of cellulases, mannanases, xylanases, proteases, and lipases has also been established. Research on the complex of mycolytic enzymes of *B. subtilis* showed role of chitinase, chitosanase, β-1,3-glucanases, and proteases showed the most contribution to lysis of the native mycelium of various species of phytopathogenic fungi *Alternaria alternata, Bipolarissorokiniana*, *Fusarium culmorum*, and *Rhizoctonia solani* [79, 72] .

4) Induction of Systemic Resistance in the Host

Antagonists suppress the development of different diseases in harvested fruits/vegetables not only directly through the synthesis of metabolites with fungicidal activity, but also indirectly, through the launch of multiple defence response mechanisms [80]. These indirect mechanisms are linked to the formation of ISR and SAR (in whole host plant organisms) and are regulated by phytohormones such as SA, ABA, JA, ethylene as well as CLPs [81, 82, 83]. To date, induction of auxins, cytokinins, gibberellins, ABA, JA, and SA has been detected in various bacteria. The ability of PGPB to synthesize ABA, especially under stressful conditions, and to influence its level in plants was found in many strains of bacteria including the genera *Bacillus*, *Azospirillum, Pseudomonas, Brevibacterium*, and*Lysinibacillus* [84, 85].

Biocontrol Based Products for post harvest disease management

Although budding research on bioagent based post harvest disease management shows its potential, the application part is still in its infancy. The first product with microorganism as a biocontrol agent effective against brown rot of stone fruits *bacillus subtilis* strain B-3(USA) was patented by [86]. Later on [87] developed another product 'BioSave' with a saprophytic strain of *Pseudomonas syringe* by 'EcoScience' Corp., Orlando, USA, which is highly useful for controlling blue and gray mold on apples and pears (*Pyrus communis* L.). We are summarizing a few products in the following table:

Table 3: Biocontrol Based Formulations

Product	**Microbial agent**	**Fruits/vegetables**	**Target disease(s)**	**Manufacturer/ Distributor**
AQ-10 biofungicide	*Ampelomycesquisqualis*Cesati ex Schlechtendahl	Apples, grapes, strawberries, tomatoes and cucurbits	Powdery mildew	Ecogen, Inc., USA
Aspire	*Candida oleophila* strain 1-182	Apple, pear and citrus	Blue, gray, and green molds	Ecogen, Inc., USA
Rhio-plus	*B. Subtilis* FZB 24	Potatoes and other vegetables	Powdery mildew and root rots	KFZB Biotechnick
Serenade	*B. Subtilis* QST713	Apple, pear, grapes, tomato, potato	Powdery mildew, late blight, brown rot, and fire blight	AgraQuest. Inc., USA
Phytosporin-M Golden Authum, AntiGnilPhytosporin M	*B. Subtilis* 26D	Carrot, tomato, cabbage, sugarbeet, potato	Rots, mold	Bashinkom, Russia
Rhapsody®	*B. Subtilis* QST 713	Tomato	Rots	Bayer, Canada

[88, 89]

Methods of Application

Once an effective and potential antagonist is identified or selected, it is necessary to search for a method which applies it effectively for controlling or suppressing the pathogen. In general, microbial antagonists are applied in two different ways i.e., preharvest application, and postharvest application.

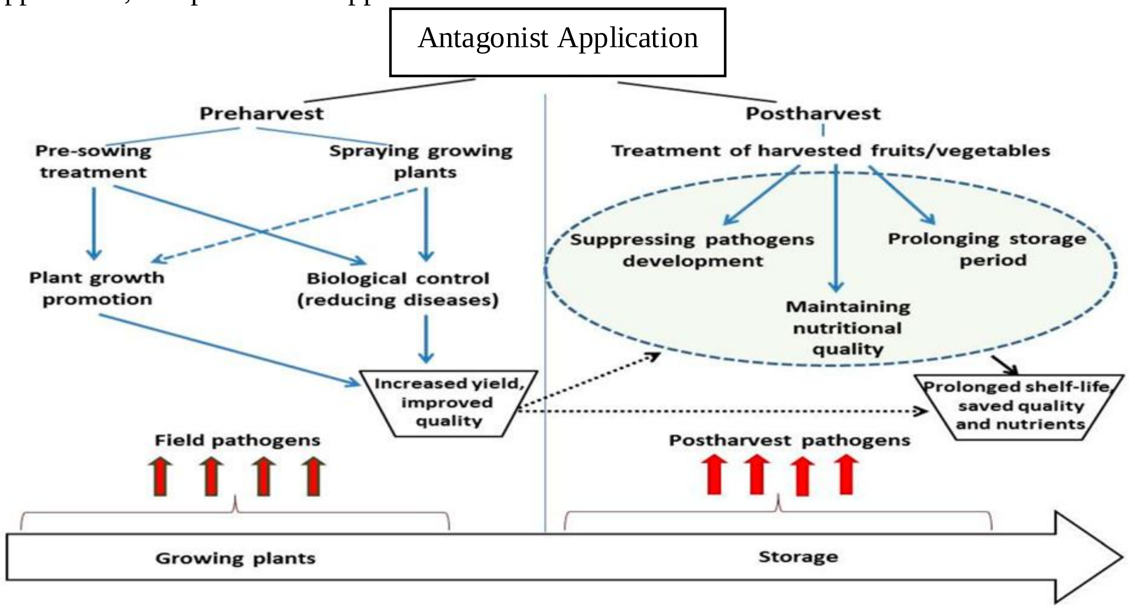

Fig 1:- Antagonists application strategies for diseases management of harvested fruits/vegetables during storage

1) Preharvest Application

In several cases, pathogens infest fruits and vegetables in the field, and these latent infections become a major factor for decay during the transportation or storage of fruits and vegetables. Therefore, preharvest applications of microbial antagonistic culture are often effective to control the postharvest decay of fruits and vegetables. The purpose of the preharvest application is to pre-colonize the fruit surface with an antagonist immediately before harvest so that wounds inflicted during harvesting can be colonized by the antagonist before colonization by a pathogen. Although this approach could not become commercially viable, because of the poor survival of microbial antagonists in the field conditions, however, it has been quite successful in certain cases [90, 91].

2) Postharvest Application

It appears that the postharvest application of microbial antagonists is a better, practical and useful method for controlling postharvest diseases of fruits and vegetables. In this method, microbial cultures are applied either as postharvest sprays or as dips in an antagonist's solution. This approach has been more effective than the preharvest application of microbial antagonists and has had several successes. For example, postharvest application of *Trichoderma harzianum, Trichoderma viride, Gliocladiumroseum* and *Paecilomycesvariotii*Bainier resulted in better control of Botrytis rot in strawberries and Alternaria rot in lemons than preharvest applications [92]. In lemons, postharvest application of *Pseudomonas variotii*was more effective in controlling *Aspergillus* rot than iprodion treatment, and in potatoes (*Solanum tuberosum* L.), postharvest application of *Trichoderma harzianum* controlled Fusarium rot effectively than benomyl dip treatment [35].

Conclusion and Future Prospects

Microbes are considered as next generation alternative for post harvest pathogen management. A significant amount of literatures demonstrate efficacy of a some bacterial and fungal bioagents against post harvest diseases of fruits and vegetables. However, large scale trial of such bioagents/ formulations are required to develop a concrete package. Moreover, effect of such agents on fruit/vegetable quality, nutritional parameters etc also needs investigation. Metagenomic and transcriptomic studies, will provide new insights into biocontrol in postharvest diseases.

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