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Bio-Control Potential of Microbial Antagonists Against Post-Harvest Diseases of Fruit Crops: A Review

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ABSTRACT

Postharvest diseases cause severe economic losses to horticultural fresh produce during the transportation and storage. The incidence of postharvest diseases can affect the quality and restrict the shelf life of the horticultural fresh produce. Synthetic fungicides, when applied are the primary factor to control post-harvest diseases. At present legal regulations are enforced by the perishable produce importing nations regarding the minimum pesticide residue levels in the edible portion of the perishable produce. Most of fungal pathogens were reported to develop indigenous resistance against synthetic fungicides. Open disposal of metallic and synthetic fungicides has a hazardous impact on environmental footprint. All these valid reasons have demanded the lookup for a natural novel biological fungicide to replace the synthetic fungicide application in the supply chain system as postharvest application. Microbial antagonists were emerged as a promising eco-friendly alternative to chemicals. During the past twenty years, several microbial antagonists have been widely evaluated against various post-harvest fungal and bacterial pathogens. Therefore, this current review summarizes the use and novelty of antagonists in the control of postharvest diseases of fruit crops, their mode of actions, effects on the defence mechanism and quality of fresh fruit.

INTRODUCTION

Fruits are an important part of balanced diet being a source of essential vitamins and minerals and rightly referred to as healthy food. Apart from direct consumption, fruits are highly utilized for medicinal values viz., antioxidants and anticancer, aesthetic and religious purposes. Among the potential threats in fruit production, the losses due to postharvest decay caused by fungal pathogens constitute an important part. Postharvest losses of fruits have been estimated to be in the range of 20% to 25% or more of the harvested produce [1]. Even in the countries with most advanced technologies available, the postharvest losses are substantial [2]. Harvesting, handling and postharvest chemical treatments can increase the cost of fruits and vegetables several folds from field to consumer. Considering economics and the problems of world hunger, it is imperative to understand the importance of postharvest diseases and to continually strive to reduce postharvest losses from pathogens [3]. Postharvest losses representing up to 25% of total production in industrialized countries and more than 50% in developing countries have been reported [4]. The primary means of controlling postharvest diseases is by application of synthetic fungicides [2]. However, public opinion over food safety has placed the use of synthetic fungicides under study, thus creating a need to develop alternative methods to control these diseases. This move is further found to be accelerated by possible deregistration of some of the more effective fungicides and the development of resistance to fungicides by some of the storage pathogens [1,5-8]. The review deals with the use of microbial antagonists for minimizing post-harvest diseases of tropical fruits.

Considerations for Biological Control of Postharvest Diseases

Postharvest application of biocontrol agents has acquired much importance in the recent years. The confined environment for the storage of harvested perishable commodities provides a great opportunity for applying biocontrol agents since temperature and humidity are stable and controllable [9]. In addition, biocontrol agents are easy to apply, they act on more concentrated plant parts, and the value of the harvested crops is great enough to justify expensive control procedures. But relatively few studies have been carried out on the control of postharvest diseases by pre-harvest application of microbial antagonists. The major aspects

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taken care of by pre-harvest application of biocontrol agents are, management of latent or quiescent infections, controlling wound infections during harvest and postharvest handling and suppression of pathogen inoculum.

Microbial Antagonists for the Management of Post-Harvest Fruit Diseases

Biocontrol agents are harmless; cheapen than pesticides, highly effective throughout the crop growth period with high rhizosphere competency and competitive saprophytic ability. The other avenues include, ease to deliver improve plant growth, activate resistance mechanism in the host, increase biomass production and yield. A wide range of antagonistic bacteria, fungi and actinomycetes are used for the management of diseases of horticultural crops. Antagonist like *Bacillus subtilis, B. cereus, Bradyrhizobium japanicum, Enterobacter cloacae, Pseudomonas aeruginosa, P. cepacia, P. fluorescens, P. corrugata, P. putida, Rhizobium* spp.*, Streptomyces* spp.*, S. griseoviridis, Laetisaria arvalis, Penicillium* spp.*, Pythium oligandrum, P. nunn, Rhizoctonia* spp.*, Trichoderma harzianum, Chaetomium globosum, Cladorrhinum foecundissimum, Coniothyrium minitans,* non-pathogenic *Fusarium oxysporum* F047, *F. oxysporum* f. sp. *niveum* (non-pathogenic), *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 [10].

Managing Quiescent Infections

The phenomenon of latent or quiescent infection in *Colletotrichum* was defined as 'the inhibition of the pathogen through physiological conditions imposed by the host until some stage of maturation has been accomplished' [11]. Latency is defined as a quiescent or dormant parasitic relationship, which after a time changes to an active one [12]. Molecular and physiological basis of quiescent infection during transition from immature to ripening stage was clearly described [13]. Latent infections have been reported in stone fruits, apples, avocados, mangoes, papayas, citrus fruits, grapes and strawberries [14-17]. In strawberries, *Botrytis* rots start on infected, senescent floral parts that become latent infections which develop into active rots on ripe fruits [18]. The yeast-like fungus *Aureobasidium pullulans* and the yeast *Candida oleophila* was effective against *B. cinerea* storage rots on strawberries when applied in the field at bloom compared to application immediately after harvesting [19]. Also, the application of *A. pullulans* and *Epicoccum purpurascens* to sweet cherry blossoms reduced the number of latent infections by *Monilinia laxa* in green strawberry fruits [14]. The importance of establishment of a biocontrol agent before pathogen arrival especially, when short-lived colonizable substrates (e.g. petals, stamens) act as infection foci [20]. Repeated applications of antagonists was required to control postharvest decays originating from latent infections in the orchard as demonstrated by three sprays of *Bacillus subtilis* to control avocado anthracnose [21]. In addition, continuous application of *B. subtilis* over a four-year period resulted in a gradual build-up of the antagonist population, reduction in the level of pathogen inoculum and more effective disease control. The population dynamics of *A. pullullans*, *Rhodotorula glutinis* and *B. subtilis* on apple fruits, for which the antagonists were applied to trees in the field late in the growing season [22]. After transfer from field to cold storage, the population of the introduced antagonists remained higher than in control treatments, except for *B. subtilis* which declined. The efficacy of preharvest application of *A. pullulans* against *Botrytis* rot of sweet cherry and table grapes and *R. glutinis* against *Botrytis* rot and blue mold of pear were also reported. Field sprays of *C. guilliermondii* A42 and *Acremonium cephalosporium* B11 two to five times at 7-10 days interval on table and wine grapes could reduce decay caused by *B. cinerea* and *A. niger* [23-26].

Controlling Wound Infection during Harvest and Post-Harvest Handling

Mechanical injuries created during harvest and postharvest handling operations may result in infection by wound pathogens [17]. In such cases, a near harvest treatment with an effective antagonist seems an appropriate strategy to prevent the colonization of wounds by weak resident antagonists and by pathogens [27]. The efficacy of preharvest application of *B. subtilis* against anthracnose, stem-end rot and *Colletotrichum* fruit rot of avocado [21]. On strawberries treated with *A. pullulans* immediately before harvest, *Rhizopus* rot was reduced by 72% while only slight activity was observed against *Botrytis* rot [19]. This difference in efficacy signifies the need of knowledge on the epidemiology of the target disease for choosing the right time of application of biocontrol agents. *B. cinerea* being present inside the fruits as latent infection could not be effectively checked, but *Rhizopus stolonifer*, being an external contaminating pathogen could easily be inhibited by *A. pullulans*. Post-harvest apples treated two days prior to harvest with *C. sake* had less blue mold after four months of storage [28]. Pre-harvest application of antagonists permits a pre-emptive colonization of the wounds inflicted during harvest or handling, and thus allows more time to the antagonist to colonize and saturate the fresh wound before the arrival of the pathogen [29,30]. Such type of competitive or pre-emptive exclusion is considered as one of the mechanisms of protection by the antagonists against the pathogen infection.

Cases of Unadvisable Post-Harvest Handling

In certain cases, postharvest applications are not feasible. For example, fruits that is easily damaged or cannot be exposed to water-based treatment such as strawberries and fruits with a waxy bloom on the surface such as table grapes. Post-Harvest treatment with *Pichia guilliermondii* or *Hanseniaspora uvarum* markedly suppressed the postharvest decay of table grapes, but it tended to remove the surface bloom; this problem was avoided by applying *P. guilliermondii* on plants three days before harvest [31]. To avoid handling and related deleterious effects, it was preferred to harvest strawberries and pack them directly into plastic baskets where the fruit remained until it reached the consumer. In such conditions, spraying suspensions of antagonist propagules, even one day before harvest seemed to be the chief method of applying the biocontrol agents.

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Essential Traits of Antagonists for Pre-Harvest Application

1. A successful antagonist must be able to tolerate low nutrient availability, ultraviolet radiation, desiccation, rapid climatic changes [22] and presence of agrochemicals [32,33].

2. Attachment to the fruit surface is probably an important trait for the antagonists since persistent attachment would contribute to a better colonization and avoids dislodging due to wind, rain or water level fluctuations [34].

3. A crucial trait of a good biocontrol agent is its ability to colonize and to survive on the target host tissue. Colonization of the host surface is an important and multifaceted process needed for the competitive and pre-emptive exclusion of the pathogen. The antagonist should be well established in the carposphere before the pathogen arrives, or that when the antagonist population declines, products triggering the infection have been removed from the host surface [35].

Mechanisms Involved in Biocontrol of Post-Harvest Diseases by Microbial Antagonists

Relatively few attempts have been made on studying the mechanisms involved in biocontrol of postharvest diseases of fruits. Various mechanisms *viz*., antibiosis, competition for limiting nutrients and space, production of lytic enzymes, parasitism and induced resistance have been reported.

1. Antibiotic production: The antibiotic iturin produced by *B. subtilis* B-3 and pyrrolnitrin produced by *Pseudomonas cepacia* LT-4-12W reduced *in vitro* growth and conidial germination of the stone fruit pathogen *M. fructicola* and pome fruit pathogens *P. expansum* and *B. cinerea* respectively [36,37]. Both these strains could control fruit decays caused by the respective pathogens [37,13]. The fruit decays were also controlled by applications of the respective antibiotics alone [38]. *A. pullulans* may produce the antibiotic aureobasidin [39]. *P. syringae* strains ESC-10 and ESC-11 produced the antibiotic Syringomycin E on some media and that the purified compound could control green mold of lemons [40].

2. Competition for nutrients and space: Competition for nutrients has been elucidated between *Enterobacter cloacae* and *R. stolonifer* on peach and *P. guilliermondii* and *P. italicum* on citrus [41,42]. The antagonists *Cryptococcus laurentii* and *Sporobolomyces roseus* used the apple volatile butyl acetate as food source and reduced the stimulatory effects of this chemical compound on germination of *B. cinerea* conidia *in vitro* [43]. Also, it was noticed that these two antagonists exhibited greater uptake and utilization of ^{14}C fructose suggesting that competition for nutrients might have played a major role. The same phenomenon was also revealed in the interaction of *E. herbicola* B66 and B 90 with *B. cinerea* and *P. expansum* in diluted apple juice [44]. The very high density (10^{10} cfu/ml) of *E. cloacae* needed to inhibit conidial germination of *R. stolonifer* and decay of peach suggested exclusion as a mechanism of biocontrol [41].

3. Production of lytic enzymes: The lysis of *B. cinerea* hyphae by β-1,3-glucanase produced by *P. guilliermiondii* on apple [42]. *P. anomala* strain K, effective in control of grey mold of apple, increased production of exo-β-1,3-glucanase threefold in the presence of cell wall preparation of *B. cinerea* in apple wounds, reducing lesion size by more than half compared to the antagonist alone [45,46]. Higher β-1,3-glucanase and chitinase activity was also detected in apple wounds treated with strains of *A. pullulans*, effective in controlling various decays of apple, and table grapes [3,47].

4. Induced systemic resistance: The antagonistic yeast *P. guilliermondii* US-7 was shown to induce PAL and the phytoalexin scoparone in citrus fruit peel [48]. Also, treatment with *C. saitoana* induced chitinase and caused deposition of papillae along host cell walls in apple and citrus [16,49]. *A. pullulans* L47 upon wound application on apple increased β-1,3-glucanase, chitinase and PO activities 24h after treatment which reached maximum levels at 48 and 96h after treatment [35]. *C. saitoana* when applied on apple fruit 48h to 72h pre-inoculation with *B. cinerea*, reduced lesion diameter by more than 50% and 70% respectively [50]. In addition, treatment with the yeast antagonist increased chitinase and β-1,3-glucanase activities in fresh apples. *C. famata* reduced green mold (*P. digitatum*) decay on oranges and increased phytoalexins scoparone and scopoletin 12-fold in fruit wounds after four days when inoculated alone [51]. The application of *Pseudomonas fluorescens* FP7 water in oil emulsion formulation increase in the activity of defense related enzymes viz., phenyl alanine ammonia lyase, peroxidase, polyphenol oxidase, catalase and b-1,3-glucanase was observed [52]. Postharvest application of *Pseudomonas fluorescens* Pf1 and *Bacillus subtilis* EPCO16 induced defence related compounds *viz*., peroxidase, polyphenol oxidase, phenylalanine ammonia lyase, superoxide dismutase and catalase was observed on treated mango fruits against stem end rot disease [53].

Bioformulations for Management of Post-Harvest Diseases

Some of the antagonistic microorganisms have been patented and commercial products such as Aspire, recently named Decco I-182 (*C. oleophila* I-182, Ecogen Corp., Langhorne, PA) as wettable powder, Bio-Save 100, 110 and 1000 (*P. syringae* ESC-10 and 11, EcoScience Corp., Orlando, FL) as frozen cell concentrated pellets and Yield *Plus* (*Cryptococcus albidus*, Anchor Biotechnologies, Cape Town, South Africa) have been registered for use against postharvest decay of citrus and pome fruits. Viable cells of *C. sake* CPA-1 grown in sorbitol modified liquid molasses-based medium and preserved with isotonic solution of trehalose (0.96 M) after storage for seven months maintained their viability and efficacy against *P. expansum* infection of apples [54]. Coating forming dispersions with *C. sake* improves the inhibition of *B. cinerea* on grapes [55]. Additional benefits, such as enhancement of fruits firmness and extended shelf-life, have also been correlated with microbial antagonists [56].

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Biological Control of Mycotoxigenic Fungi

The use of microbial antagonists for the control of postharvest pathogens, including mycotoxigenic fungi is most important. Recently, experiments showed some *A. pullulans* strains are highly suppressive to rot caused by *Aspergillus carbonarius* on detached and wounded wine grapes [57]. Interestingly, these strains are pertaining potential to degrade ochratoxin A to the less toxic compound ochratoxin *in vitro*. Furthermore, in the few grape berries that were contaminated by the fungus and were pre-treated with the biocontrol agents, ochratoxin A contamination was lower than in infected nontreated berries [6]. In apples pre-treated with the microbial antagonist, the overall accumulation of patulin in the low percentage of *P. expansum* infected fruits was significantly lower than in infected, non-pretreated apples [58]. Interestingly, the major patulin degradation product formed by *Rhodotorula glutinis* LS11 characterized structurally by NMR analyses was identified as desoxypatulinic acid which was reported to be nontoxic to multiple microorganisms that are inhibited by patulin.

Challenges for Transformation of Biocontrol Agents into Practical Usage

The first and rational step to prevent pathogen contamination is a good agricultural practice in the field and at the storage. The inherent ability of microbial bio-agents to prevent postharvest diseases in perishable commodities relies on their potential to outcompete the hazardous pathogen for required nutrients in the wound site and/or to form a biofilm layer that prevents pathogens physically from receiving nutrients and make starvation. Many postharvest pathogens need an infection site to settle them and initiate an infection [59]. Despite their ability to use as an alternative approach, the use of postharvest biocontrol agents as commercial products remains narrowed. This lack of widespread commercial application has been associated to a number of factors, including unbalanced performance, lack of eradicative potential, lack of resources, varying regulatory requirements in different nations which restricts their widespread adoptability and usage and issues associated with the environmental safety. In order to improve the potential of microbial antagonists, research has been directed to combine their use with other alternative approaches.

CONCLUSION

Post-harvest application of synthetic chemicals has been the traditional scheme for the management of postharvest diseases. The increasing concern for health hazards and environmental pollution due to chemical use has necessitated the development of eco-friendly strategies for the control of postharvest diseases of perishable fruits. Management of postharvest diseases by microbial antagonists has been manifested to be most suitable strategy to alternate the chemicals which are either being banned or recommended for limited use.

The recent approach of postharvest disease management in fruits and vegetables is based on the knowledge of natural process of the antagonist pathogen interaction. Although there are several success stories in commercialization of antagonist against post-harvest pathogens. In the controlled environment of a postharvest system, there are specific opportunities to apply microbial antagonists as delivery system. In future, it may be possible to use only strains adapted to postharvest conditions and introduce targeted genes for antagonistic activity as needed. However, if the similar pace follows, the use of microbial antagonists for the control of postharvest diseases of fruits will be greatly expanded in the future and will definitely become a globally adopted practice.

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