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Horticulture

A Review on Prospects of Pre-harvest Application of Bioagents in Managing Post-Harvest Diseases of Horticultural Crops

Elangbam Premabati Devi*[1], Deepshikha[1], Bimla Kumari[1] and Shivani[1]

[1]Department of Plant Pathology, College of Agriculture, G.B. Pant University of Agriculture and Technology, Pantnagar-263 145, Uttarakhand, India

*Corresponding author: epremabati35@gmail.com

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Abstract

The reduction of post-harvest food losses is a critical component of ensuring future global food security. For controlling post-harvest losses of fruits and vegetables biocontrol measures play an important role in ensuring an effective and safe alternatives to synthetic chemicals. Biological control of pre- and post-harvest diseases has been one of the most extensively studied alternatives and appears to be a viable technology. Post-harvest application of biocontrol agents has been reported since very early to control post-harvest diseases, but it has one major limitation that it cannot control the latent infection that starts from the field itself during the flowering stage, fruit maturity stage, wounds develop at harvesting and transporting the produce. For these pre-harvest applications of biocontrol agents can be an appropriate strategy for fruits and vegetables that subject to several ways of damage in post-harvest handling till storage.

This review deals with the importance of pre-harvest application of biocontrol agents for controlling the post-harvest diseases of fruits and vegetables along with the several ways to increase the efficacy of biocontrol agents with others integrated control measures.

Highlights

- Post-harvest food losses is a critical component in the integrated resource management.
- Pre-harvest applications of biocontrol agents is emerging as an appropriate strategy for fruits and vegetables.

Keywords: Post-harvest diseases, chitosan, *Trichoderma harzianum,* antagonist*, Cryptococcus laurentii,* biocontrol agents

Fruits and vegetables are the most perishable agricultural produces, and their post-harvest decay is a major challenge throughout the world. In the industrialized countries, it is estimated that pathogens decay about 20– 25% of the harvested fruits and vegetables during post-harvest handling and storage (Sharma *et al.* 2009). Losses of fresh fruits and vegetables after harvest are more in underdeveloped countries where it ranges from 20 to 50 % (Sudheer *et al.* 2007).

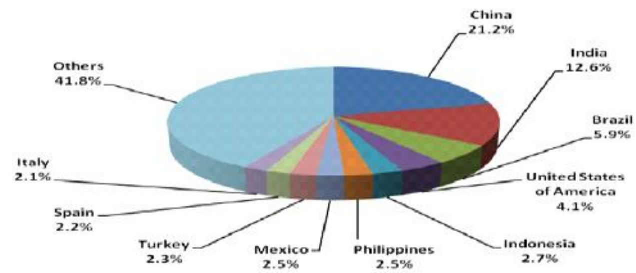
Globally, India is the second largest producer (after China) of both fruits and vegetables in the world with 88.97 and 162.89 MT respectively in 2013-14 (Department of Agriculture and Cooperation, India) but due to the absence of modern cold storage facilities and lack of proper food processing units, suffers post-harvest fruits and vegetable losses worth ₹ Two lakh crore every year (Assocham study titled Horticulture Sector in India: State Level Experience) State-wise analysis indicated that West Bengal is India's leading horticulture producing state with over 27,000 tonnes of fruits and vegetable produced across the state annually (Hand Book on Horticulture Statistics 2014). Unfortunately, this is also the state that stands first by incurring post-harvest losses worth over 13,600 crores annually. Gujarat ranks second in post-harvest fruit and vegetable losses thatare as high as 11,400 crores, followed by Bihar (₹ 10,700 crores), Uttar Pradesh (₹ 10,300 crore) and Maharashtra (₹ 10,100 crore).

Several factors influence the post-harvest losses in fruits and vegetables that include losses due to physical, physiological, mechanical and unhygienic conditions, lack of proper storage conditions, refrigerated facilities and diseases and pests, etc. While harvesting to handling for storage till marketing several wound pathogens are known to infect the produced that destroy the keeping quality, quantity ultimately economic losses. post-harvest decay of fruits and vegetables occur either between flowering and fruit maturity or during harvesting and subsequent handling and storage. Wound pathogens mainly cause post harvest infections that occur through surface wounds inflicted during harvest and handling. Even fruit that appears healthy when harvested may cause significant losses during storage due to the quiescent and latent infections in the field, and these latent infections become amajor factor for decay during transportation or storage of cherry tomato fruit (Sharma *et al.* 2009).

Among the control measure chemical method is one of the common practices due to its efficacy and low cost. But it has some residual effects on the produce that leads to environmental pollution, health hazards to the consumers and development of some resistance strain of pathogens against some chemicals. So, the global trend is shifting towards reduced pesticide use in agriculture in general and in post-harvest in particular. Thus, Biological control has emerged as an alternative method for post-harvest disease management. As wound-invading necrotrophic pathogens are vulnerable to biocontrol, antagonists can be applied directly to the targeted area (fruit wounds) which significantly reduces fruit decays (Janisiewicz *et al.* 2002).

Two basic approaches have been reported for using the microbial antagonists against the post-harvest diseases of fruits and vegetables (Sharma *et al.* 2009). The use of microorganisms which already exist on the produce itself i.e. naturally some beneficial microorganisms are already present on fruit surface that are known as naturally occurring antagonist (Wilson *et al.* 1989). Monaco *et al.* 2009, has reported tomato against *Botrytis cinerea* by using naturally occurring antagonists. And another is those that can be artificially introduced against post-harvest pathogens like Botrytis rot of strawberry with *Trichoderma* spp. (Tronsmo and Denis 1977) and brown rot of stone fruit by *Bacillus subtilis* (Pusey and Wilson 1984) which are more effective in controlling post-harvest diseases.

In this review, we will be focussing on the impact of post-harvest and pre-harvest application of biocontrol agents in controlling post-harvest diseases of fruits and vegetables so that safe and effective alternative control measures will be reported against present day synthetic fungicides.



Source: Economic Survey 2013-14, Ministry of Finance and Handbook of Statistics on Indian Economy 2014, RBI

Mechanism of action of Biocontrol agents

It includes antibiosis, parasitism, production of lytic enzymes, the competition of nutrients and space, induction of host resistance. Biocontrol agents have more efficient utilizing uptake system for the substancesso they exist more competition for micronutrients than the pathogens. *Pichia guilliermondii* inhibits *Botrytis cinerea* by competing for nutrients at the wound site (Chalutz *et al.* 1991). Biocontrol agents also compete with the pathogen for the physical occupation of the site and thereby reduce the root colonization. *Pichia guilliermondii* has the ability to induce defence mechanism in host and raised the levels of enzyme phenylalanine ammonia lyase (PAL). β 1-3 glucanase can degrade chitin present in cell wall of the pathogen directly and indirectly release oligosaccharide and elicits defence reaction. Chitinase leads to hydrolyse β 1-4 linkage in chitin that are responsible for inducing resistance to the host against the pathogen. *Trichoderma* has the role of lytic enzymes including glucanases, chitinases and proteinases for reducing disease incidence (Mortuza 1999).

Some bioagents can control different pathogens at a time with their multiple mechanisms. The efficacy of *Trichoderma* strains was effective in inducing systemic resistance in tomato by increasing the activity of chitinolytic and glucanasese enzymes in the leaves upto 14th day (Saksirirat *et al.* 2009). *Bacillus subtilis* B-30 inhibited the growth of *Moniliania fructicola* causing peach brown rot disease on culture medium by theproduction of antibiotic iturin (Gueldner*et al.*1988). *Pseudomonas syringae* strains ESC-10 and ESC-11 produced syringomycin and effectively controlled green and blue molds of citrus caused by *Penicillium digitatum* and *P. italicum* respectively. The bacterium *Pseudomonas cepacia* inhbits *Botrytis cinerea* and *Penicillium expansum* growth in apples by the production of the antibiotic, pyrrolnitrin. Several bio-control strains are known to produce multiple antibiotics that can suppress one or more pathogens. *Bacillus cereus* strain UW85 is known to produce both zwittermycin and kanosamine (Pal and Gardener 2006) that helps to suppress diverse microbial competitors at a time. But BCAs may act on the post-harvest pathogen through more than one mechanism. *Aureobasidium pullalans* control *Botrytis cineria and Penicillium expansum* in apples by enhancing the activities of β-1-3 gluconase, chitinase, peroxidase along with the competition for nutrients and space.

Application of Biocontrol agents

Biocontrol agentscan be appliedin two different ways, i.e., pre-harvest application, and post-harvest application.

Post-harvest application of microbial agents

post-harvest application of bioagents is commonly used method for the control of post-harvest diseases. For example, Apple blue mold by *Pseudomonas syringae* (Janisiewicz, 1987), *Pseudomonas cepacia* (Janisiewicz and Roitman, 1998), gray mold of apple by *Pichia guilliermondii* (McLauhlin *et al.* 1990), green mold of citrus by *Pichia guilliermondii* (Chalutz and Wilson 1991), green mold of citrus by *Bacillus subtilus* (Singh and Deverall, 1984), sour rot of citrus by *Pichia guilliermondii* (Chalutz and Wilson 1991), mucor rot of pear by *Cryptococcus laurentii* (Roberts 1990). But healthy produce when harvested may harbour latent and quiescent infections capable of causing significant losses during storage (Jarvis 1994). Latent infections had reported in stone fruits (Northover and Cerkauskas, 1994; Wittig *et al.* 1997), apples (Biggs 1995), avocados, mangoes, bananas, papayas, citrus fruits (Eckert and Ogawa 1985) andgrapes and strawberries (Snowdown 1990).

But the post-harvest application of bioagents has one limitation that they are not able to control latent infection or quiescent infection and incipient infection that are caused by wounds during handling operations. Many investigators have given strong evidence that before harvesting the crops infection starts in the field itself, and some pathogens infect at flowering stages and remain as latent pathogens until storage time. After harvesting some biochemical changes have been take places inside the produce that activates latent pathogens and decays fruits and vegetables during transportation and storage of the commodities and also becoming critical factors for economic losses of produce. Hence, argued that pre-harvest application of microbial antagonist are often effective for the control of post-harvest diseases (Ippolito and Nigro 2000).

Pre-harvest application of microbial agents

Pre-harvest application is done to pre-colonize the fruit surface with the antagonistic microbes so that wounds inflicted during harvesting can be colonized by the antagonists before colonization by the pathogens. Then, they slowly multiply their numbers before the arrival of pathogens, and when pathogens came they displace the pathogens from the wound sites. Thus, they not only reduce the post-harvest diseases but also control latent infection that develops from the field and reduced decay in storage conditions. So, field applications of microbial agents may avoid the limitation of post-harvest application of bio-agents as antagonistic micro-organisms enable a pre-emptive colonization, which can protect fruit from subsequent infections. Field application of bio-agents may result in early colonization of fruit surfaces offering protection to the fruits against infection by post-harvest pathogens entering through wounds caused by improper handling.

Pre-harvest application of antagonistic microorganisms can suppress the pathogen at the source, which may reduce harmful microorganisms on infection and may protect the environment and human health (Ippolito *et al.* 2005; Tian *et al.* 2004). Biological control agent, *Bacillus spp.* Can be effective when antagonists are applied as pre-harvest treatments to control leaf and fruit diseases such as Cercospora leaf spot on groundnuts caused by *Cercospora arachidicola* (Knudsen*et al.* 1987), powdery mildew and anthracnose of mango caused by *Oidium mangiferae* and *Colletotrichum gloeosporioides* (Korsten *et al.* 1992), rust on beans caused by *Uromyces phaseoli* (Baker *et al.* 1985), and charcoal rot on potato caused by *Macrophomina phaseolina* and *Botryodiplodia solanituberosi* (Thirumalachar *et al.* 1997). The pre-harvest spraying with 108 CFU ml^{-1} *Pichia guilliermondii* is effective in controlling the post-harvest decay of cherry tomato fruit.

Moreover, the inhibitory effect is positively correlated with spraying frequency. Pre-harvest spraying with *P. guilliermondii* has no significant influence on the main quality attributes of cherry tomato fruit and it induces theplant defence responses activities of POD, PAL and β-1,3-glucanase (Yan Zhao *et al.* 2011). Lima *et al.* 1999 studied the biological potential of *Aureobasidium pullulans* (isolate L470) against grey mold of table grapes was evaluated by spraying BCAs on field before harvest and again applied at 5days before harvest provided sustained protection against *Botrytis cineria*.

Yeast antagonists appear to be very well adapted to growth and colonizing fruit surface and especially fruit wound sites that are important traits for pre-harvest application. The yeast *Candida sake* CPA-1 was effective for the control of blue mold caused by *Penicillium expansium* in apples when applied as pre-harvest spray (Teixido *et al.* 1998).Pre-harvest applications with microbial antagonists like *Gliocladium roseum* Bainier (Sutton *et al.* 1997), *Trichoderma harzianum* (Tronsmo and Denis, 1977; Kovach *et al.* 2000) and *Epicoccum nigrum* Link (Larena *et al.* 2005) have achieved successes in post-harvest disease control of strawberries. Three strains of *Trichoderma harzianum* T39 (Trichodex), T-161, T-166 were evaluated under field conditions for the control of strawberry anthracnose disease. All the strains were effective in higher dosage at 0.8% in reducing the severity. Field application at the flowering time reduced the fruit rot of strawberry during post-harvest storage by 64 to 72% by the yeast antagonist *Metschnikowia friucticola* (Karabulut *et al.* 2004). Korsten, 1993 and Korsten *et al.* 1995, reported effective control of post-harvest decay from anthracnose, stem-end rot, and *Dothiorella*– *Colletotrichum* fruit rot complex when using pre-harvest *Bacillus subtilis* as field sprays. Canamas *et al.* 2008 reported that pre-harvest application of different concentrations of *Pantoea agglomerans* was effective against *Penicillium digitatum* during storage of oranges.

Foliar application of *Trichoderma atroviride* LU 132 (10^7/ml)at 2 days just before harvesting of strawberry on the development of *Botrytis cinerea* was effective (Dauglas, 2005). Waffa *et al.* 2012, had applied the antagonist *Pseudomonas florescence* twice, one at flowering time and another at seven day intervals. Twice applications of antagonist were effective in reducing the percent disease incidence of gray mold of strawberry as compared to control and fungicide treatments. And the yield was also increased when antagonist was applied. Application of *Burkholderia spinosa*as a foliar spray in weekly intervals for nine repetitive times suppresses the abundance of *Aspergillus* spp., *Fusarium* spp. And several other unidentified bacteria, fungi and yeast spp. Thatdwell on banana leaves (Silva *et al.* 2014). Pre-harvest spraying of *Trichoderma harzianum* 5R was found to be effective for managing the post-harvest decay of table grapes (Sawant *et al.* 2010).

Characteristics of an ideal antagonist for pre-harvest application in the field

Resistance to environmental stress

The antagonists must be able to tolerate low nutrient availability, UV radiation, desiccations, rapid climate changes (Leibinger *et al.* 1997) and the presence of agrochemicals (Kohl and Fokkema 1998). Filamentous fungi and yeast are more effective for field application than the bacteria as they are less tolerable to very harsh environment conditions but in the presence of high humidity or free water bacteria can control disease completely as they require free water for colonization (Fokkema and Shippers 1986; Andrews 1992). Thus, frequent application of bacterial antagonist may be required for pre-harvest application in the field (Fokkema 1993). Exposure to the high amount of U.V. rays directly effects the antagonist longevity but bacteria and yeast that have extracellular polysaccharides that can formed slime layer and capsules with pigment formation (Dickinson 1986). In some cases low doses of U.V rays promotes the population of bacterial and yeast antagonist in disease controlling of post-harvest diseases of peach, citrus fruits, tomato, carrot, strawberry, onion, apple, pepper (Tripathi *et al.* 2013).

Attachment to host surface

Only the persistence attachment would contribute to better colonisation and help to avoids dislodging due to the wind, rain, and water level fluctuation (Dickinson 1986). Yeast produces slime layer so they get easily adhere to leaves, fruits etc. (Bashi and Fokkema 1976). Magnesium and calcium reduce the electrostatic repulsion forces that present on plant surface so provides better adherence of antagonists to the fruits surface (Fletcher 1980).

Fruit surface colonisation

Antagonist should have the ability to colonise and to survive on target host tissue. So it requires a high level of competitive capability. There are reports that poor commercial success of bio-control agents is probably due to their unsatisfactory ability to colonise the host surface (Ippolito *et al.* 2000). If colonisation occurs and survival is high only one or few application of antagonist may be enough to protect the fruit over time and that would provide a good control of post harvest disease. The intensity of competitive interactions among coexisting microbial populations will be affected by the degree of niche overlap that exists between them (Cristiansen and Loeschcke 1990). Therefore, effective biocontrol agents for pre-harvest application should have the trait of efficient resource requirement similar to the pathogens (Ippolito *et al.* 2000).

Increasing the efficacy of Biocontrol agents (BCAs)

The efficiency of the microbial agents may be enhanced by the addition of chemical supplements, using mixture of bioagents, physiological and genetic manipulation. Using the mixture of different antagonists that have no inhibitory effect on them will be more effective as they broaden the spectrum of activity of the antagonist. They also help in increasing their effect like better utilisation of substrate that results in acceleration of growth rate, removal of inhibitory substances produced by other BCAs, the formation of amore stable microbial community that may exclude other microbes including pathogens. Thus, enhance efficacy provides a way in reducing the application rates and also mixing the compatible antagonist enables to combine the various BCAs without genetic engineering. But, there should be no antagonism between BCAs, only those have positive interaction or mutualism that allows more effective utilisation of resources will be effective.

Integration with fungicides

An integration of biocontrol agents with a reduced amount of fungicide provides fewer levels of residues on marketed products and also results in the slower development of resistant pathogenic strain. But for an effective method there should be compatibility among the biocontrol agents and the fungicide. Lima *et al.* 2008, have reported the combined use of BCA with fungicides for the control of *Botrytis cinerea* in citrus. Pre-harvest applications of *Bacillus subtilis* as field sprays integrated with copper oxychloride or benomyl consistently reduced the severity of avocado black spot *Pseudocercospora purpure* (Korsten *et al.* 1997). It was also shown that the integrated treatment is consistently more effective over time and location as compared with commercial fungicides and has the greatest potential for acceptance by growers (Korsten *et al.* 1997). Lima *et al.* 2008 mentioned integrated used of bioagents with fungicides, i.e. *Cryptococcus laurentii* with thia-bendazole against *Penicillium expansum* of pear (Chand *et al.* 1997), *Cryptococcus laurentii* LS28 with thiabendazole against *Botrytis cinerea* of apple (Lima *et al.* 2006), *Pseudomonas syringae* with cyprodinil against *Penicillium expansum, Botrytis cinerea and Monilinia* of Apple (Erampalli *et al.* 2006), *Cryptococcus laurentii* and *Areobasidium pullulans* with benomyl against *Botrytis cinerea* and *Penicillium expansum* of apple (Lima *et al.* 2003) etc.

Biocoating

Wisniewski *et al.* 2007, have examined yeast biocoat antagonist, *Candida saitoana* in controlling several post-harvest pathogens of apples, oranges, lemon and were effective in reducing fruit decay. So, application of biocoat (biocoating of yeast with chitosan salt) and biocure reduced the diseases as they helpsin formation of biofilm and parasitizing pathogen hyphae with the production of some lytic enzymes, glucanase activity and ultimately induction of resistance responses against the pathogens.

Integration with Chitosan

The antifungal activity of chitosan inhibits fungal pathogens by halting the growth of the pathogen or by inducing marked morphological changes, structural alterations and as an biological elicitor, chitosan can induce fruit resistance and defence related responses. Chitosan and its derivatives, including glycolchitosan, were reported to inhibit fungal growth and to induce host-defense responses in plants and also in harvested commodities (Allan *et al.* 1997). Combinition of 0.2% glycolchitosan with the antagonist *Candida saitoana* was more effective in controlling green mold of oranges and lemons, caused by *P. digitatum*, and gray and blue molds of apples than either treatment alone (El-Ghaouth *et al.* 2000). The protective treatments of combined applications of chitosan and *Pseudomonas syringae* 48SR2 showed significantly improved green mold control and compensated for the modest protective activity of chitosan treatment (Claudia, 2008).

The pre-harvest application of *Pseudomonas fluorescens* FP7 plus chitin at thepre-flowering stage was durably effective against anthracnose of mango both in the field and in post-harvest storage. Besides higher levels of phenolics, peroxidase, PAL and polyphenol oxidase have contributed to induce resistance in mango trees against anthracnose, that resulted in better mango fruit quality and greater yields (Vivekananthan *et al.* 2006). Biocontrol activity of antagonistic yeasts has more capacity of rapid colonization in the fruit wounds by ensuring its efficient competition for nutrition with the pathogens thus inhibiting the early pathogenic process of the pathogen (Janisiewicz *et al.* 2002). Meng *et al.* 2008 studied the integration of pre-harvest spray with biocontrol yeast *Cryptococcus laurentii* and post-harvest chitosan (0.1%) coating treatment and reported it to be a promising management strategy for decay control and quality maintenance of table grapes.

Integration with Adjuvants

Zhang *et al.* 2005, has examined the yeast antagonist *Cryptococcus laurentii* for the control of *Botrytis cinerea* of pear in combination with adjuvant 2% $CaCl_2$. Adjuvant $CaCl_2$ helps in reducing the gray mold disease development as they help to reduce the electrostatic repulsion force between the leaf surface that results in even distribution of yeast cell in the surface of the fruits and ultimately the effective yeast cell concentration present in the surface that compete for the nutrients uptake. The yeast directly attached to the hyphae of the pathogen leading to the formation of concave appearance thus reduces the disease development. Calcium chloride significantly improved the efficacy of *Aureobasidium pullulans* in pre-harvest table grape applications and the activity lasted until the end of storage (Ippolito *et al.* 1998). The addition of $CaCl_2$ (2% w/v) to the formulation of the yeast biocontrol agent, *Candida oleophila*, enhanced the ability of yeast to protect apples against post-harvest decay(Wisniewski *et al.* 1995).Besides, sodium carbonate, sodium bicarbonate and sodium silicate also improved the effectiveness of pre-harvest applications of *A. pullulans*.

Conclusion

From the above-describedstudies, it has been concluded that the pre-harvest application of bioagents as afoliar application not only control the post-harvest diseases that develop in the storage condition but also prevents the infection by the latent pathogen that start from the field itself which cause decay at storage time. The pre-harvest application of bioagents able to precolonise the fruits surface and then multiplies their numbers before the arrival of the pathogen. So when the pathogen arrives they displace the pathogenthus able to control the further development of pathogens and resultsin reducing these verity of the disease. The time of application of bioagents should be managed that the antagonists would have more time to colonise and to saturate the fresh wound before the arrival of pathogens.

With the pre-harvest application of bioagents helps in reducing the inoculums at harvesting time. Regarding the quality of fruits and vegetables which includes texture, firmness, appearance, colour, flavour, total soluble solids, ascorbic acids, nutritional value and acceptability are not significantly changed by the pre-harvest application of bioagents. Applying mixtures of these antagonists in the orchard may be useful for controlling post-harvest decays that would be effective by their several mechanism of action. The integration of different treatments could benefit by their synergic effects and improve the efficacy of each method otherwise alone treatment would not be effective.

Antagonist that has the capacity of high population density remains effective throughout the post-harvest period and would be effective in managing the further pathogen development. Thus, antagonists are at the right place and right moment by the pre-harvest application for the control of post-harvest diseases. So, as researchers, we have to challenge the several opportunities for developing safe and effective alternatives to present-day synthetic fungicides for managing the post-harvest diseases of horticultural crops by pre-harvest application of bioagents.

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