

## A REVIEW ON BIO-BASED CONTROL OF POST-HARVEST DISEASES

**Abstract:** *Bacillus subtilis* non-pathogenic beneficial bacteria, promotes plant growth, disease resistance and tolerance to abiotic stresses. It produces bioactive substances with antibiotic properties and induces physiological features in plant metabolism without adverse effects on the environment or human health. *Bacillus subtilis* has been used to treat various postharvest diseases during handling, transportation and storage of various fresh fruits and vegetables. It is the first microorganism patented as a postharvest bio control agent for Brown rot of stone fruits, improving the post-harvest physiology of various fruit/vegetables. *Bacillus* strains AG1 and H110 have been shown to be effective against Vine wood fungal pathogens and post-harvest pathogens. They have been shown to reduce symptoms of Anthracnose in fruit caused by fungal pathogens *Colletotrichum gloeosporioides* and *C. acutatum* and White rot caused by *Botryosphaeria dothidea*. Endophytic *Bacillus* strains have been developed that can colonize plant tissues and live in the same ecological niches as pathogens, thus preventing post-harvest diseases and improving preservation during storage. *Bacillus* strains induce auxins, cytokinins, gibberellins, ABA, JA and SA in plants, which can stimulate plant growth under stressful conditions. Endophytic bacteria can induce ISR against pathogens and abiotic stressors, extending the shelf life of stored fruits and vegetables. Microbial antagonists can be applied after harvest to control fruit and vegetable diseases, but a single microbial strain cannot prevent all fruits/vegetables from decaying during storage. Combining diverse antagonistic microorganisms with diverse microbial activity and combining various bio-controlling characteristics can prevent post-harvest decay on fruits/vegetables.

**Keywords:** *Bacillus subtilis*, bio control agent, postharvest diseases, Endophytic bacteria, Microbial antagonists.

### 1. INTRODUCTION

“A Food and Agriculture Organization estimate that about 45% of all fruits, vegetables, roots, and tubers harvested are lost due to disease incidence. During storage, the majority of this loss is caused by pest infestations and pathogens (bacteria, fungi, and insects), unfavourable storage conditions, water loss, scarification and sprouting. Despite their effectiveness in preventing post-harvest decay, chemical fungicides and/or food preservatives can be hazardous to humans, animals, and the environment” [2]. “Due to the toxicological risk of residual chemicals in food products, their use in the post-harvest period has been restricted to a few registered chemicals, and some European countries have prohibited their use completely” [3]. “A novel, efficient, environmentally friendly, bio-safe approach to reducing post-harvest food losses would be highly desirable as food and environmental issues become more relevant, along with the need for energy conservation through green technologies and organic products. Research-led alternatives to synthetic fungicides and/or food preservatives for controlling post-harvest diseases could be biologically based products derived from beneficial strains, such as plant growth-promoting bacteria (PGPB). Plant metabolism is altered by these products, resulting in systemic resistance and prolonged shelf life without adverse effects on plants, humans or the environment” [4,5,6].

“PGPBs are nonpathogenic beneficial bacteria that promote plant growth, disease resistance, and tolerance to abiotic stresses. These organisms may be found living autonomously in soil or colonizing the rhizosphere, phyllosphere and plant interior tissues (endophytes)” [4,7,8,9,10]. “An interesting PGPB is *Bacillus subtilis*, a member of the genus *Bacillus* spp. one of the most attractive natural plant protection agents. *Bacillus* spp. are generally recognized as safe microorganisms for food applications by the FDA. They are similar to many pathogens and produce a variety of bioactive substances with antibiotic activity. *Bacillus* spp. also induced various physiological aspects of plant metabolism without adverse effects on the environment or human health” [6,11]. “Endospores of this organism survive dynamic physical and chemical treatments, such as heat, desiccation, organic solvents, and UV radiation, thus triggering defense responses even under adverse conditions” [12,13]. “This process enables the preparation and storage of *Bacillus*-based biological products, which serve as powerful bioactive components against pathogens. In the literature, it is well documented that *Bacillus* strains protect plants from biotic (pathogens, pests) and abiotic stressors (drought, salinity, extreme temperatures, toxic metals, etc.)” [5,7,9,12]. “A wide range of biologically active compounds are synthesized by *Bacillus* spp., including antibiotics, siderophores, lipopeptides, enzymes, and 1-aminocyclopropane-1-carboxylate deaminase. They are known to affect the regulation of phytohormone biosynthesis pathways, modulate ethylene levels in plants and influence the emission of volatile organic compounds (VOCs) and the launch of host plants' systemic resistance/tolerance” [14,15,16,17].

As a result of handling, transportation, and storage of a wide variety of fresh fruit and vegetables [5,18,19]. *B. subtilis* has been used to treat many postharvest diseases. During postharvest time, *B. subtilis* suppresses gray mold (*Botrytis cinerea* and *B. mali*) pathogens in strawberries, pears, apples, and tomatoes. In the study of *Bacillus* microbial antagonists, it was found that they possess considerable potential for improving vegetables/fruit sets, resistance to postharvest diseases and tolerance for temperature fluctuations. Additionally, they reduce mechanical injuries caused during transport, unloading, packaging, and storage of products [20,21]. Their role in controlling postharvest disease and the underlying mechanisms regulating fruit and vegetable storage quality remain largely unknown. However, they play a vital role in plant growth, development, and health under normal and stressful conditions.

## 2. Post-harvest loss reduction

*Bacillus subtilis* was the first microorganism patented as a bio control agent for brown rot of stone fruits after harvest [22]. The use of antagonistic microorganisms, such as *Bacillus* spp., to improve the post-harvest physiology of various fruit/vegetables began to emerge as time passed, increasing their ability to resist post-harvest diseases and unfavorable storage conditions, thereby prolonging shelf life and ensuring nutritional quality. In melon fruits (*Cucumis melo* L.), *B. subtilis* EXWB1 suppresses post-harvest diseases caused by *Alternaria alternata* by 77.2%. Also thought to have a positive effect on melon fruit surfaces and wounded tissues, as EXWB1 inhibited *A. alternata* hyphae. *B. subtilis* EXWB1 suppresses ethylene production by 72.3% and decreases respiration rates by 26.1% and 71.9% of infected and non-infected melons, respectively after harvest [18]. By suppressing ethylene biosynthesis, *B. subtilis* EXWB1 may have delayed the development of rot in melon fruit. In a similar manner, EXWB1 treated fruit while maintaining turgor pressure. During storage, it reduced weight loss, increased sugar content by 36.7%, and increased titratable acidity near fresh fruit. In another study of different bacteria viz., *B. subtilis*, *B. pumilus*, *B. cereus*, *B. megaterium* and *Agrobacterium radiobacter*; *B. subtilis* and *A. radiobacter* were most effective in controlling the post-harvest citrus fruit disease caused by *Penicillium digitatum*. On consistent evidence, *B. pumilus* and *B. amyloliquefaciens* suppress the growth of gray mold caused by *Botrytis cinerea* on pear and tomato crops [24]. Also found that *Bacillus* strains (*B. pumilus* B19, *B. subtilis* 1J, *B. cereus* B16, *B. subtilis* B11, and *B. cereus* B17) controlled Gray mold in apples caused by *Botrytis mali* [9], demonstrating inhibitory effects in dual culture samples ranging from 13.6 to 74%, in cell-free metabolite tests ranging from 12.3 to 87%, and in volatile experiments ranging from 11 to 53%.

*B. subtilis* AG1 has also been shown to be effective against vine wood fungal pathogens such as *Phaeoacremonium aleophilum*, *Phaeoconiella chlamydospora*, *Verticillium dahliae*, and *Botryosphaeria rhodina* [26]. According to a study, *B. subtilis* produces antibiotics and volatile organic compounds (VOCs) to suppress post-harvest pathogens like *Rhizopus stolonifer* (soft rot), *Botrytis cinerea* (gray mold) and *Colletotrichum* spp. (Anthracnose) in Berries (*Fragaria* x *Ananassa*). In vitro, *B. subtilis* SK1-2 was found to have a high antagonistic activity against *Botryosphaeria dothidea*, *Diaporthe actinidiae* and *Botrytis cinerea* was effective at controlling Kiwifruit post-harvest rot [19]. In addition to its strong antifungal activity against *Botryosphaeria dothidea*, *B. subtilis* 9407 also significantly reduces Apple fruit ring rot [27]. Fungi such as *Aspergillus niger*, *Botryodiplodia theobromae* and *Penicillium oxalicum* cause fungal rot after harvest in *Dioscorea* fruit. It has been shown that postharvest *Botrytis cinerea* infection of apple trees was reduced by 80% when *B. subtilis* strain GA1 was applied after pathogen inoculation [28]. To prevent fading (30 days at 5 °C) and to maintain proper quality in Litchi fruits were treated with *B. subtilis*. In all cases of treatment with *B. subtilis* bacterial cells, no change in fruit taste was observed [20]. It was found that *B. subtilis* CF-3 strains retained 65% of their fruit quality after 36 days stored at 10 °C, which was a significant improvement over the non-treated controls [29]. Citrus fruit bacterial decay caused by *Penicillium digitatum* and *P. italicum* was reduced by *B. subtilis* [30].

Several studies suggested that *B. subtilis* H110's inhibition of pathogens is influenced by its ability to produce antagonistic proteins and natural competition for nutrients and space [31]. It has been shown that *B. subtilis* can control fungal rot in Citrus [30] as well as *Monilinia fructicola* infection in Peaches and Cherries [22,32]. A processing method using *B. subtilis* strains APEC170 and *Paenibacillus (Bacillus) polymyxa* APEC136 reduced symptoms of Anthracnose in fruit caused by the fungal pathogens *Colletotrichum gloeosporioides* and *C. acutatum* and White rot caused by *Botryosphaeria dothidea* [33]. *Rhizopus stolonifer* and other pathogens, such as *Monilinia fructicola*, *Cephalothecium*, *Rhizoctonia*, and *Alternaria* were effectively controlled by *B. subtilis* SM21 in Peach fruit [34]. During storage, the development of Anthracnose in Avocado fruit rot complex (*Dothiorella/Colletotrichum*) was significantly reduced by co-application of *B. subtilis* and commercial wax Tag, enriched at different concentrations [35]. Incubation of fruit in water containing bacterial cells yielded similar results. *B. subtilis* Ch-13 has been shown to reduce bacterial antagonist colonization on Potato tuber surfaces during cold storage and during the growing season [36]. In cold storage, microbial preparations can be used to intensify the adaptive immune response of Potato tubers, resulting in more than double the defense response. *B. subtilis*-based microbial preparation increased potato tuber peroxidase activity, phytoalexin production and ascorbic acid concentration by 2.4, 3.1, and 1.3 times, respectively, compared with a control preparation. A mixture of *Pseudomonas fluorescens* Pf1, *Bacillus* sp. EPB10 and *Bacillus* sp. EPB56 was applied to Banana fruit (*Musa* sp.) to reduce *Fusarium oxysporum* development [37].

## 3. Endophytic activity

Endophytic *Bacillus* strains have been developed as a key part of bio-control, since they colonize plant tissues and live in ecological niches similar to pathogens. Consequently, they can survive without external environmental influences while conferring economically useful properties on host plants [17,38]. Endophytic bacteria (*B. subtilis* 26D) were introduced before planting or during the vegetative phase to protect plants from certain defects. The effects were sustained over a prolonged period, resulting in better preservation during storage [39]. A combination of *B. subtilis* 10-4 and salicylic acid (SA), combined with anti-stress activity, improved plant growth [40]. In comparison to non-treated tubers, tubers treated with *B. subtilis* 10-4 and SA were less infected by pathogenic

micromycetes *Aspergillus*, *Penicillium* and *Alternaria* and they had fully faded *Cladosporium*, *Fusarium*, and *Mucor*. The tubers treated with both *B. Subtilis* 10-4 and SA maintained antifungal activity for 30 days after storage, indicating *B. subtilis* prolonged protective effect. Additionally, the co-application of endophytic *B. subtilis* and SA during storage is more effective at bio-controlling potato diseases compared to *B. subtilis* alone [41,42]. Furthermore, SA can serve as a pre-harvest and post-harvest control strategy in addition to improving nutritional quality and extending fruit/vegetable shelf life. In addition to reducing chilling injury and decay, SA delays ripening and enhances fruit and vegetable health benefits by improving disease resistance and antioxidant activity. Therefore, when bacterial antagonists are applied to harvested fruits and vegetables during storage, either alone or in combination with other natural regulators, the defense response in plant tissues is enhanced. Bioactive components can be developed to extend crop longevity and maintain nutritional quality while maintaining crop longevity. The development of preparations based on antagonistic bacteria is hindered by a lack of knowledge about the interactions between *Bacillus* spp.-host plants-pathogens.

#### 4. Inducing Systemic Resistance in hosts

In harvested fruits and vegetables, *Bacillus* spp. suppress disease development through the synthesis of fungicidal compounds as well as indirectly by launching multiple defense response mechanisms. Phytohormones such as SA, ABA, JA, ethylene and CLPs regulate these indirect mechanisms as they form ISR and SAR (in whole host plant organisms) [41]. Several bacteria induce auxins, cytokinins, gibberellins, ABA, JA, and SA [43,44,45,46]. *Bacillus*, *Azospirillum*, *Pseudomonas*, *Brevibacterium* and *Lysinibacillus* are among the strains that synthesize ABA in plants, especially under stressful conditions. A binary system developed by ABA-deficient mutants of Tomatoes, flacca and sitiens stimulated their growth under drought stress and induced ABA accumulation by inoculating them with *Azospirillum lipoferum* (strain USA59b). Tomato plant growth is heavily dependent on maintaining ABA levels under normal conditions and under stress, as evidenced by *Bacillus megaterium* synthesizing ABA [3]. Plants synthesize and catabolize hormones and their precursors by phytopathogenic bacteria. The Rice rhizosphere bacteria isolated from rice can dispose of ABA for the promotion of tomato plant growth via an ABA-dependent mechanism. Under PGPB influence, plants undergo endogenous hormonal shifts. Both beneficial bacteria as well as pathogens produce phytohormones. Pathogen phytohormones suppress host defenses, whereas PGPB phytohormones optimize plant hormonal balance. Aside from initiating protective mechanisms against pathogens, PGPB phytohormones can also act as growth promoters.

The *Bacillus* strains produce VOCs with low molecular weights (typically less than 300 grams), which can diffuse over long distances by diffusion through the air and soil pores [49]. The VOCs redistribute endogenous auxins tissue-specifically and decrease the ABA content in *Arabidopsis*. They also activate the ISR against pathogens and abiotic stressors. To enhance resistance to powdery mildew, *B. subtilis* UMAF6639 stimulates SA and JA-dependent protective reactions in melon. Other bacteria utilize SA as a signaling molecule to initiate defense responses [51]. By inhibiting respiration during harvest, post-harvest technologies manipulate harvested product metabolism. In addition, ethylene is a key regulator of ripening and senescence of fresh fruit and vegetables. In addition, overproduction of ethylene reduces shelf life and accelerates senescence after harvest [52,53]. Several studies have suggested that PGPBs synthesizing ACC-deaminase modulate ethylene levels in plants, preventing harmful stress responses and promoting disease tolerance [54,55,56]. In plants, ACC-deaminase-producing bacteria significantly reduced ethylene production, preventing the inhibition of plant growth caused by a variety of stress factors. Flooding, anoxia, drought, salinity, heavy metals, organic contaminants, fungal and bacterial pathogens and nematodes inhibit plant growth. By applying bacteria that deaminase produce ACC during storage, fruits/vegetables extended their shelf life and aging process by reducing the level of ethylene.

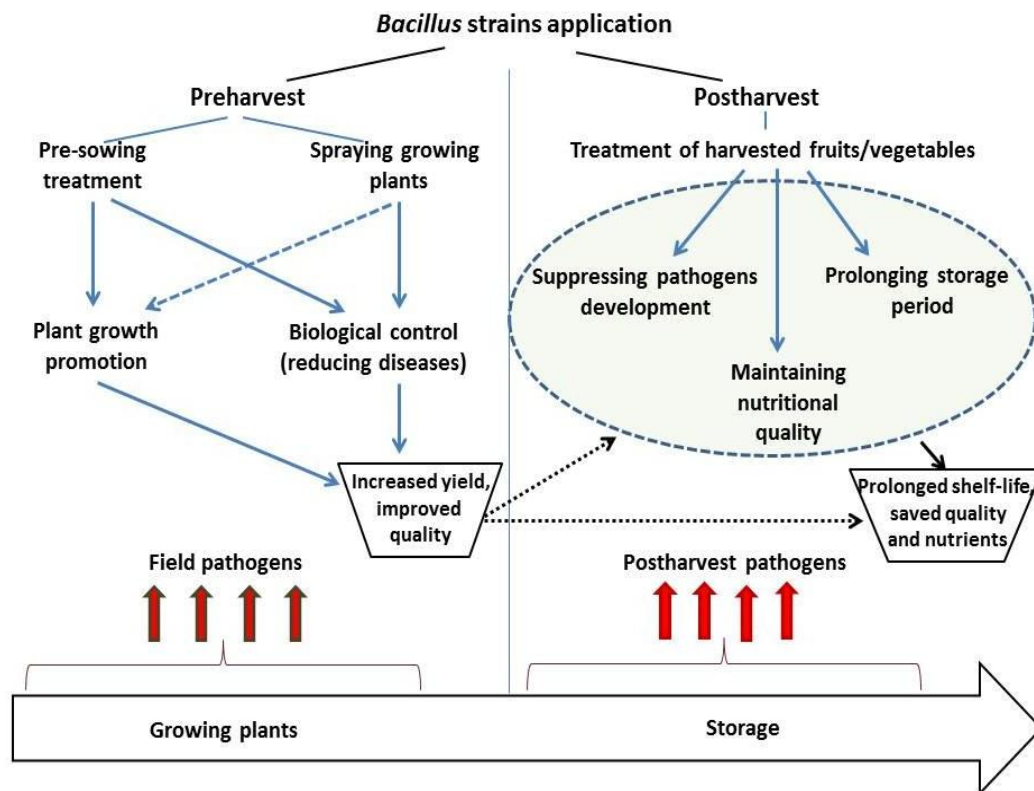
Researchers have found that microbial antagonists also release antifungal compounds that contribute to host defense mechanisms. In this way, fruit and vegetable diseases can be prevented biologically. As a result of its production of iturin and fungicin, *B. subtilis* triggers plant gene expression of phenylpropanoid metabolism genes, which leads to ISR. Furthermore, the SAR pathway could be implicated in LPS' induction mechanisms for the defense system, which are triggered by ROS generated by SA. It was shown that *B. subtilis* strain 168 producing surfactin and fengycin enhanced tomato and bean plant resistance by activating lipoxygenase enzymes [57], which produce JA, a key component of plant resistance. It was found that surfactin induced ISR in beans, melon, tomato, tobacco, and grapes, while fengycin induced protective responses in potatoes, tomatoes, and tobacco. *B. amyloliquefaciens* strains containing surfactin induced ISR in *Brassica napus* against *Botrytis cinerea* [58] and *B. amyloliquefaciens* FZB42 induced ISR in lettuce against *Rhizoctonia solani* [59]. A protective reaction is triggered by mycosubtilin in grape plants. A recombinant strain of *B. amyloliquefaciens* FZB42 that is deficient in surfactin synthesis, fengycin synthesis, and bacillomycin D synthesis could not improve lettuce resistance to *Rhizoctonia* [59], while ISR is induced by BBG111 starin from *B. subtilis*. *R. solani* causes hypersensitivity and cell death in rice rhizospheres due to the secretion of fengycin and surfactin by the rhizosphere. Through JA/ethylene, ABA, and auxin-dependent defense signaling, immune responses prevent the growth and development of pathogens in early stages of pathogenesis [61]. During genetic shuffling, a *B. amyloliquefaciens* strain was created that synthesized 8.3 times more fengycin than the strain ES-2-4 isolated from *Scutellaria baicalensis* Georgi, which displayed high biocidal activity against pathogens [62]. Studies have shown that *Bacillus* spp. accumulate phytoalexins (scoparone and scopoletin) that help fruits and vegetables prevent postharvest decay by eliciting defense mechanisms. Storage at

18°C with microbial products derived from *B. subtilis* (Ch-13 strain) significantly increased peroxidase activity, ascorbic acid concentration, and phytoalexin production.

Endophytic bacteria induce ISR in plants that can be preserved for long periods of time, making it effective against pathogens [41]. A rapid accumulation of ROS and H<sub>2</sub>O<sub>2</sub> occurs immediately following infection onset, resulting in unregulated redox-sensitive transcription factors and PR genes. It was found that *Pseudomonas putida* LSW17S induced the rapid accumulation of transcription PR genes and the production of H<sub>2</sub>O<sub>2</sub> in Tomato plants infected with *P. syringae* pv. tomato DC3000 inhibited pathogen growth [63]. It was found that *Rhizopus stolonifer* growth was reduced on Peach fruit treated with *Bacillus cereus* AR156 and *B. subtilis* SM21. The activity of their protein products was associated with producing H<sub>2</sub>O, overexpression of 1,3-glucanase and phenylalanine-ammonium lyase genes and the overexpression of chitinase genes. In pepper seedlings infected with *Pythium aphanidermatum* [64]. Activating peroxidases and polyphenol oxidases catalyzes lignin biosynthesis in *P. subtilis* BSCBE4 and *P. chlororaphis* PA23. There are several strains of *Pseudomonas fluorescens* [65], *Trichoderma viride* [Tv1 and Tv13 strains] and Bs16 that protect plant tissues against pathogens by producing peroxidases, polyphenol oxidases, and phenylalanine-ammonia lyases, as well as peroxidases and polyphenol oxidases [65]. ROS can be crucial for the priming effect of ISR induced by endophytic bacteria. Hypersensitivity to foreign substances occurs when bacteria prime the host genome. Plants can become more resistant to pathogens and insects by activating their cellular mechanisms of protection more quickly and more strongly. This can persist for quite some time. It has been suggested that plant DNA methylation status changes as a result of such priming in response to bacterial infection [66]. There is still a lack of understanding of the undelaying protective mechanisms *Bacillus* induces in fruit and vegetables against pathogens.

## 5. *Bacillus* strains application

Potential microbial antagonists are capable of suppressing pathogens in fruits and vegetables when applied properly. However, the effectiveness of these antagonists depends both on the characteristics of the strain and how they are applied. As a general rule, microbial agents can be used pre- or post-harvest, depending on the purpose [67].



**Figure 1.** Scheme of *Bacillus* strains application strategies for diseases management of harvested fruits/vegetables during storage.

### 5.1. Pre-harvest application

A pathogen usually infects fruit and vegetables and lives in plant tissues without causing symptoms, but these latent infections can cause significant losses when they develop during storage. A number of studies have found that microbial inoculants, particularly *B. subtilis*, can reduce stress-induced defects and increase crop yields during storage [29,68]. The potato tuber growth, development and yield were positively influenced by potato tuber strains 10-4 and 26D. A study conducted with inoculated tubers found that they were less likely to develop pathogenic micromycetes such as *Aspergillus*, *Penicillium* and *Alternaria* less likely to develop *Cladosporium*, *Fusarium*, and *Mucor* [42] compared to non-inoculated tubers. A pre-harvest application of microbial agents is often effective in controlling post-harvest disease in fruit and vegetables [69,75]. By using *B. subtilis* under field conditions, the microbial antagonist colonizes the Apple fruit surface before harvest. Consequently, the post-harvest pathogens *Penicillium expansum* and *Botrytis cinerea* are effectively controlled in apples [76]. Generally, infections occur shortly before harvest. As a result of congenial conditions, these infections may not appear at harvest, but may become apparent after harvest, especially if pathogens are able to develop. Pathogens such as *B. cinerea*, *Monilinia fructicola*, *Sclerotium rolfsii* and *Geotrichum candidum* can cause late-onset infections [77]. The application of antagonistic microorganisms prior to harvest can help fruit surfaces colonize during storage and protect them from pathogens [69]. The low survival rate of microbial antagonists in the field makes this approach generally not commercially feasible, despite some success.

### 5.2. Post-harvest application

Fruit and vegetable diseases can be controlled through the application of microbial antagonists after harvest. Various preparations containing microbial antagonists are available for spraying on harvested fruit and vegetables or as solutions [70,71]. However, a single microbial antagonist cannot prevent all fruits/vegetables from decomposing while they are in storage or after harvest. In order to enhance the protection of biological preparations,

manufacturers use a variety of bacteria strains to enhance the protection of them [72,73,71]. It is difficult to select a single microbial strain that has a broad spectrum of activity against a wide variety of pathogens [72,73,71]. A variety of bacteria are used in 'Companion' (Growth Products Ltd., USA), and in 'Bactril' (Biopharmatec, Russia), which contains *B. subtilis* GB03, *B. subtilis* MBI600 and *Bradyrhizobium japonicum*. *Bacillus* spp. can also be considered as an integral part of an integrated disease management approach, in addition to other biological and physical approaches. Combining diverse antagonistic microorganisms with diverse microbial activity and combining various bio-controlling characteristics can use polymicrobial mixtures used to control multiple post-harvest diseases [74]. An enhancer effector can enhance the effectiveness of microbial antagonists in preventing fruit/vegetable decay after harvest. It is possible to combine microbial antagonists with wax agents during pre- and post-harvest periods. There are many examples of calcium chloride, calcium propionate, sodium bicarbonate, ammonium molybdate, sodium carbonate, potassium metabisulphite, SA, etc. The bioefficacy of microbial agents may be enhanced by combining them with physical methods such as curing or heat treatment [72,74]. A growing body of knowledge exists about microbial antagonists (*Bacillus* spp.), biologically active compounds, and induced resistance, making it likely that effective formulations, application methods and combinations with additional approaches will be developed in order to enhance their additive and synergistic effects [2].

## 6. Conclusion

*Bacillus* species can improve the post-harvest physiology of fruits and vegetables by enhancing their resistance to different pathogens, leading to a longer storage period and a longer marketing life, as well as maintaining their nutritional value and freshness. By using *Bacillus* strains (particularly endophytic), a bio-control agent can prevent postharvest decay. Post-harvest bio control with these microbial antagonists seems promising. While it is an eco-friendly method of reducing food losses during storage, its effects on post-harvest physiology and preservation under pathogenic infection are not well understood and further research is needed.

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