CHAPTER

14

Microbial formulation approaches in postharvest disease management

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14.1 Introduction

And so, from hour to hour we ripe and ripe,
And then from hour to hour we rot and rot,
And thereby hangs a tale.

William Shakespeare

Agriculture sector is the largest source of employment for people all over the world. It is thus the major driver of poverty reduction. Mechanical innovations followed by chemical creativity have transformed agriculture to meet the increasing population growth. Hence, a challenge of agricultural sustainability chronicles all the world economies today (Schreiner, 2005; Bawa, 2019).

Horticultural perishables such as fruits, nuts, vegetables, and spices play a pertinent role in the agribusiness as well as human nutrition. However, the value of these commodities can be measured at every stage along the chain, that is, from the farm to the final buyer/during agricultural production, postharvest, and processing stages in the commodity channel (Fig. 14.1). At every stage it is packed, processed, and transported, which incurs qualitative and quantitative losses (Kader and Rolle, 2004). Qualitative losses relate to changes in appearance, texture, nutritional value, freshness, edibility, flavor, and are hence very difficult to assess than the quantitative losses.

The Indian Ministry of Food Processing Industries estimated a loss of 23 million tons of grains, 12 million tons of fruits, and 21 million tons of vegetables in 2014. This amounts to

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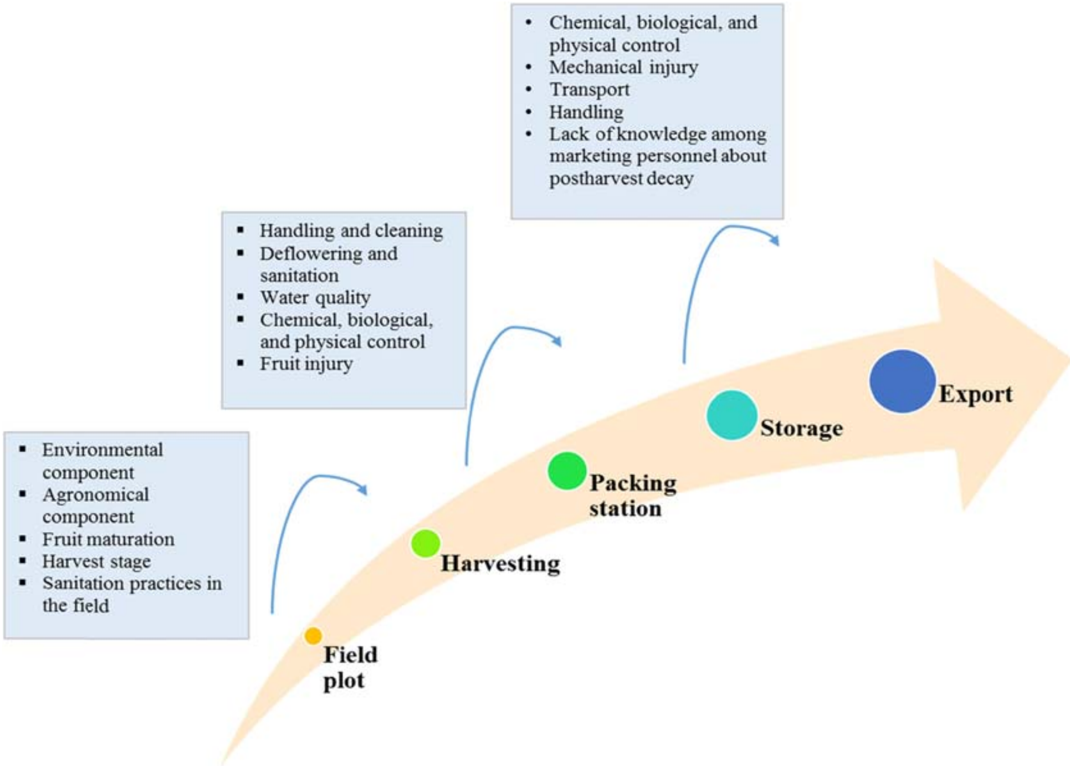

FIGURE 14.1 Diagram representing the stages during commodity channel and key factors that influence the quality and quantity of the farm produce.

10.6 billion USD (Ganesh et al., 2016). Even though highest postharvest losses were recorded in developing nations of Asia and Africa, according to the Food and Agriculture Organization of the United States (FAO Report, 1997), food loss is not restricted in developing economies. The global postharvest loss is nearly 30% for cereals, 40% for crops among other perishable items contributed by world's leading powers (Ministry of Finance, 2019). The 2011 report of FAO documented that with respect to the amounts of globally produced fruits and vegetables, 15% and 50% are lost due to postharvest decay, mainly due to fungal pathogens (FAO, 2011).

The Central Institute of Post-harvest Engineering and Technology, Ludhiana, India, reported that about 16% of fruits and vegetables valued at 6 billion USD were lost between 2012 and 2014 in the Indian agrobusiness. It was also reported that a major share of the budget was spent on cold storage facilities (https://pib.gov.in/newsite/printrelease.aspx?relid = 136922).

Hence, there is a necessity to develop better strategies to meet the demand of postharvest management.

In the present scenario, registered chemicals available in market for postharvest losses are prone to environmental and health hazards, making it prohibited in some countries (Wisniewski et al., 2016). Widespread and prolonged interaction of fungicides showed resistance toward pathogens. Therefore requirement of an alternative solution has become an utmost need of society (Marcet-Houben et al., 2012). A number of strategies have been evolved including biocontrol agents for postharvest disease management, but microbial formulation has been widely accepted at industrial and consumer levels due to their environment-friendly, cost-effective, user-friendly, and higher tolerance properties.

14.2 Postharvest disorders caused by biotic factors

Malfunction of physiological conditions of crops, fruits, and vegetables due to abiotic stresses such as temperature, relative humidity, moisture level, and pH makes a conducive environment for pathogen attack. Most postharvest pathogens invade through the cuts and wounds or when the host defense is weak. They are also selective in their host tissues and pH. Fungal pathogens prefect in an acidic environment and hence, infect citrus fruits that generally have pH less than 4. Hence, there aren't any major commercial reports on bacterial postharvest diseases. Conversely, bacterial infections are quite common in vegetables that generally have a pH of more than 5.

Postharvest pathogens can be grouped into two categories (Mincuzzi et al., 2020):

Latent pathogens: They infect the farm produce in the preharvest stage viz., during blooming or fruit development. They remain dormant until a postharvest favorable condition allows their reactivation. Example: *Botrytis* sp., *Alternaria* sp.

Wound pathogens: As the name suggests, these pathogens need an entry site in the form of a lesion, injury, or other abiotic damages to penetrate the host, in the pre- or postharvest stage. Example: *Aspergillus* sp. and *Penicillium* sp.

Common postharvest decay pathogens infecting fruits:

14.2.1 Penicillium rot

Penicillium rots are the most significant and widely reported infections in pome and citrus fruits. The key threat of this pathogen is due to their powdery spores. It usually infects the fruit from its stem end. *Penicillium digitatum* and *Penicillium italicum* are the two most common pathogens in this category. After infecting a fruit, there happens plentiful sporulation that results in a completely white fruit covered with greenish blue spores. The fungal pathogens produce abundant quantities of ethylene culminating in rapid senescence of adjacent fruits. Synthetic chemical mixtures containing limonene, ethanol, acetaldehyde, and CO_2 at particular levels could also *stimulate* the growth of *P. digitatum*.

Blue mold is a common terminology applied to refer several *Penicillium* species that cause postharvest decay. In study by Yin et al. (2017), blue rot fungi from pome fruits such as stored apples and pears were identified using a suite of molecular DNA markers. The pathogens were identified as *Penicillium expansum*, *Penicillium solitum*, *Penicillium carneum*, and *Penicillium paneum*. Every species of *Penicillium* contributing to the blue rot is different although they seem similar morphologically. For instance, *P. expansum* is more dangerous since it produces more toxins such as ochratoxins, patulin, citrinin on apples and pears than its cousin *P. solitum*. This puts forth the need to develop and implement a fast yet reliable method for differentiating between two species (Pitt et al., 1991).

Green mold that includes *P. digitatum* is widely observed in India. In contrast with blue mold, the green mold infection grows rapidly at higher temperatures (25°C–30°C) through wounds. Its mitigation begins at the preharvest stage using benomyl spray.

14.2.2 Aspergillus rot

This fungus penetrates fruits through microwound and bruises. Fruits infected with Aspergillus rot appear black. Hence, it is also called black mold. It spreads very fast at higher temperatures 30°C–35°C. This fungus is temperature specific. Cold storage (7°C–10°C) of farm produce usually helps to avoid sporulation and decay (Mincuzzi et al., 2020). Fruit decay due to *Aspergillus niger* is common in India.

14.2.3 Rhizopus rot

This troublesome parasitic pathogen follows the "nesting strategy" of infection. It infects its neighboring fruits with mycelium. Unlike Penicillium rot and Aspergillus rot, Rhizopus rot spreads rapidly at 15°C (Ladanyia and Ladaniya, 2010). One of the deadliest postharvest pathogens in this category is *Rhizopus stolonifer* and only infects mature fruits (Gonçalves et al., 2010). It is known to infect nectarine and stone fruits mainly at wound sites at harvest and packing stage. Its spores are abundant in the atmosphere (Qing and Shiping, 2000).

14.2.4 Brown rot

This latent infection is caused by *Monilinia laxa* (Aderhold & Ruhland) Honey, *Monilinia fructigena* (Aderhold & Ruhland) Honey, and *Monilinia fructicola* (Winter) Honey. This rot majorly affects peaches, nectarines, apricots, sour and sweet cherry, apples, and pears. It infects the plant at the field stage although most of the losses are in the postharvest stage (Martini and Mari, 2014).

14.2.5 Crown rot

This is a complex infection resulting from a cocktail of fungal pathogens. The microorganisms commonly contributing to crown rot are *Musicillium theobromae*, *Colletotrichum musae*, *Ceratocystis paradoxa*, *Lasiodiplodia theobromae*, *Nigrospora sphaerica*, *Cladosporium* spp., *Acremonium* spp., *Penicillium* spp., and *Aspergillus* spp., as well as many *Fusarium* species. This postharvest disease affects bananas. The infection begins at harvest in the peduncle region and is hence called crown rot. However, the symptoms develop gradually during shipping and ripening. The fungal complex brings about softening and darkening of the crown region and finally results in detachment of the fruit. It affects the fruit quality due to necrosis and early ripening because of ethylene produced by mycelia of the fungus *C. musae* (Lassois and de Bellaire, 2014). The postharvest decay symptoms caused by *L. theobromae* are called diplodia stem-end rot. The fungus commonly occurs on citrus fruits grown in hot, humid, and tropical regions. The economically important fruit, mango is also affected by this postharvest disease (Diskin et al., 2019). The rot develops from latent infections. Postharvest, the fungus reactivates and infects the fruit through natural openings.

14.2.6 *Botrytis cinerea* rot

The fungus *Botrytis cinerea* (gray mold) takes up the second place in the list of world's top 10 pathogens based on economic importance. It has a wide range of hosts and is hence very easy to find it in pre- or postharvest stage. A study by Williamson et al. (2007) estimated more than 200 dicotyledonous crop species to be hosts for gray mold. There is a huge difference in the global expense of gray mold control and product loss despite the control measures. The crops that are susceptible to gray mold postharvest include artichoke, apple, asparagus, bean, beet, blackberry, blueberry, broccoli, cabbage, carrot, cauliflower, celery, cucumber, currant, eggplant, endive, grape, kale, kaki, kiwi, leek, lentil, lemon, lettuce, onion, pea, peanut, pepper, pear, peach, plum, pomegranate, potato, pumpkin, raspberry, strawberry, sweet cherry, and tomato (Droby, 2007; Romanazzi et al., 2012). Infection may occur at preharvest stage and remain latent. Due to the nesting strategy of infection, the fungus causes extensive spoilage of the produce. The pathogen can sustain itself at storage temperatures as well. However, gray mold infection in grapes is considered beneficial since it contributes positively in production of rare dessert wines. Hence, it is also called "noble rot" (Tosi et al., 2012).

14.2.7 Sour rot

This disease is caused by the yeast such as fungal pathogen *Galactomyces citri-aurantii*. Most of the decay in Nagpur Mandarins during the March−April harvest season is contributed by this rot. Low temperatures below 10°C negatively regulate the fungal growth and hence the infection. The disease develops when the fruit is handled in warm areas during the packaging stage when the surrounding temperature is more than 27°C. As a result of the rapid infection, the fruit becomes soft and starts to stink since the fungus secretes tissue-degrading enzymes.

14.2.8 *Alternaria alternata* rot

This fungal pathogen has been reported all over the world and can proliferate in different environments. It causes postharvest decay in mangoes, black rot in cherry tomatoes, kiwifruit, melons, citrus fruits, figs, and many more. It can infect both fruits and vegetables. Typically, wounded, stressed, and senescent tissues are more susceptible to *Alternaria* infection.

14.2.9 *Thielaviopsis paradoxa, Thielaviopsis basicola* rot

These fungal pathogens are one of the major threats to agronomic produce such as fruits, crops as well as some ornamental plants. *Thielaviopsis paradoxa* infects economically significant farm produce such as pineapple, coconut, sugarcane, cocoa, banana, corn, sweet potato, and many more. The disease caused by *T. paradoxa* is commonly called black rot and is mostly reported in tropical regions (Ploetz et al., 2003). On the contrary, *Thielaviopsis basicola* infection, also called as black root rot targets ornamental foliage plants, greenhouse plants, and field crops such as peanut, tobacco, carrot, and bean. In a case study carried out by Eshel et al. (2009), it was reported that the fungus *T. basicola* could contaminate farm-produced carrots during the postharvest stage through wounds and brushing.

14.2.10 Diplodia stem-end rot

The postharvest decay is caused by fungus *L. theobromae* and commonly occurs on citrus fruits grown in hot, humid, and tropical regions. The economically important fruit, mango is also affected by this postharvest disease (Diskin et al., 2019). The rot develops from latent infections. Postharvest, the fungus reactivates and infects the fruit through natural openings.

14.3 Control strategies for postharvest diseases

A lot of conventional methods are still practiced in order to maintain the quality of harvested fruits, vegetables, and crops. These include deflowering, maintaining water quality standards and sanitation at packing stations, proper environmental controls during storage and shipping of the farm produce (Bastiaanse et al., 2010). Some control strategies mentioned in Table 14.1 have been reported to have considerable potential for mitigation of postharvest decay.

Nevertheless, commercial synthetic fungicides are the preferred choice for agricultural sector specialists and farmers to control the postharvest decay. This strategy was introduced back in 1960s since the discovery of benzimidazole derivatives (Lapeyre de Bellaire and Nolin, 1994). Eventually, many industries such as Syngenta, Bayer Crop Science, and BASF introduced into the agro-market various fungicides to control postharvest decay caused by gray mold (http://www.frac.info). Even though fungicide treatment can reduce postharvest losses, the effectiveness decreases with emergence of resistant strains of pathogens. There has also been a consistent public demand internationally to reduce the use of chemical pesticides due to their severity on the consumer's health. A high-risk pathogen, *B. cinerea* that has been often reported worldwide developed resistance to the commonly used benzimidazole fungicides. The mutants that emerged were resistant to myriads of fungicides. As a result, many countries revoked or restricted the use of certain synthetic fungicides (Myresiotis et al., 2007; Dayan et al., 2019). The Fungicide Resistant Action Committee (FRAC) has proposed guidelines that suggest a limited number of applications per year (Hermann and Stenzel, 2019). Hence, there is a considerable interest in developing alternative methods to eliminate fungicide treatment (Mari et al., 2003).

14.4 Microbes' role in controlling postharvest disease

The potential of microbes in plant growth and development is well known, but the role of microbes to inhibit growth of phytopathogen that causes postharvest disease is needed to be explored. Postharvest disease causes greater losses to crops. These diseases could be successfully controlled with application of chemical fertilizers and pesticides. But, they pose a constraint to plants and human health. It also leads to the development of resistant strains of

TABLE 14.1 Summary of commercially practiced postharvest control strategies, their benefits and drawbacks.

S. no	Control strategy	Technique	Benefits	Drawbacks	References
1.	Hot water treatment/hot water rinsing and brushing	Disinfecting fruits and vegetables with hot water 44°C–48°C and brushes for <5 min.	Inhibits fungal spore germination and subsequent growth. Pesticide-free method.	Fruit peel injury, heat damage.	Porat et al. (2000), Obagwu and Korsten (2003), Jing et al. (2010)
2.	Ultraviolet C (UV-C) irradiation (254 nm)	Low doses of UV-C (2.5 kJ/m^2).	Clean, safe, and sustainable. Pesticide-free method.	High doses (> 5 kJ/m^2) may cause fruit damage. Change in metabolic profile of the fruit.	Brown et al. (2002), Stevens et al. (2004), Charles et al. (2008), Terao et al. (2015)
3.	Gaseous treatment	Application of fumigants in storage houses using "Closed Loop System" in the United States, SIROFLO, SIROCIRC in Australia, and PHYTO EXPLO in Europe. The gases used are methyl bromide, phosphine (FUME, FRISIN), sulfuryl fluoride (ProFume), propylene oxide, carbonyl sulfide, ethyl formate, hydrogen cyanide, carbon disulfide, methyl iodide, ozone, and carbon dioxide.	High speed of action, penetrative.	Toxic, ozone-depleting potential. Change in fruit quality and residues, corrosive, emergence of resistant pathogens, propylene oxide is flammable.	Navarro (2006), Venta et al. (2010), Liu (2008)
4.	Synthetic fungicides	Fungicide dip solutions are prepared in large tanks and are used on pack lines. Examples: Imazalil, thiabendazole, pyrimethanil, prochloraz, guazatine, benzothiazole.	Easy to use, high potency.	Generation of toxic waste, hazardous waste disposal is expensive, emergence of resistant pathogens. Environmental and human hazard.	Smilanick et al. (2008), FAO Report (1997), Altieri et al. (2004)
5.	Biological control: antagonistic bacteria and yeast	The common mechanisms effective against postharvest decay are competition for nutrients and space, siderophores, parasitism, formation of biofilms, and initiation of resistance. The currently registered postharvest biocontrol agents are Aspire (Ecogen, USA) containing *Candida oleophila*, strain I-182, BioSave 110 (EcoEcience, USA) containing saprophytic strain of the bacterium *Pseudomonas syringae*.	Genetically stable, effective at low concentrations, environment friendly, cost effective.	Limited spectrum of activity and efficacy, application complexity.	Mari et al. (2003), Bélanger et al. (2012), Carmona-Hernandez et al. (2019)
6.	Generally recognized as safe and natural compounds, plant volatiles	Application of aqueous extracts of various plants, chitosan, plant secondary metabolites such as limonene, camphone with fungicidal potential.	Environment friendly, cost effective, limited health hazard.	Laborious production process, continuous use may be harmful.	Leuba and Stossel (1986), Ali et al. (2016)

pathogens (Buchholz et al., 2018). Hence, there is a need for an eco-friendly treatment that could be an alternative to pesticides and chemical applications (Brzozowski et al., 2016). In some cases, antagonistic microorganisms also help in integrated disease management strategies to avoid crop quality loss. The mechanisms used by microbes for postharvest disease management involve siderophores, biological control, hydrolytic enzymes, antibiosis, biofilm formation, and induction of plant resistance (Köhl et al., 2019).

An enormous number of microorganism-based formulation including yeast, bacteria, and fungus involved in pathogen control have adopted an array of mechanism such as production of volatile organic compounds (VOCs; Di Francesco et al., 2018), antagonistic activities (Calvente et al., 1999; Castoria et al., 2001; Rajendiran et al., 2010; Wu et al., 2019). These microbial attributes induced resistance in crops and fruits by enhancing phenolic content and antioxidant enzymes (Romanazzi et al., 2016; Yang et al., 2017). Use of microbes in terms of bioformulation ultimately enhanced the plant health because interaction of plant microbe in rhizosphere promoted sustainable growth of host plant, altered soil atmosphere, and protected crop from phytopathogen (Table 14.2; Jones et al., 2019). Most commonly used fungi in bioformulation are *Metarhizium anisopliae* and *Beauveria bassiana*. The synergetic interactions of microbes with phytosanitary products cause mortality of the target pathogen, ultimately becoming a tool for deployment in integrated pest management (IPM; Bukhari et al., 2011). A *Bacillus* sp.—based formulation having remarkable antimicrobial activity against myriad of pathogens viz., *Botryosphaeria dothidea, R. stolonifer, B. cinerea, Botrytis mali, P. digitatum, Phytophthora infestans, Fusarium oxysporum* by producing secondary metabolites such as macrolactin, bacillaene, mycosubtilin, surfactin, fengycin, and iturins has been reported by many researchers (Wu et al., 2019; Cozzolino et al., 2020; Tian et al., 2020). Another advantage of *Bacillus* sp. over other formulations is endospore formation and survival in extreme conditions (higher and lower temperature, irradiation, pH, chemical pressure).

Yeast, a single cell microbial flora also plays a key role in postharvest pathogen control. Various yeasts have been reported earlier for showing proficient antagonistic activity against pathogens. *Aureobasidium pullulans* has been shown as antagonist by producing extracellular enzymes by directly interacting with fungal hyphae (Castoria et al., 2001), while some strains of *A. pullulans* produce lipopeptides (LPs). These are low-molecular-weight VOCs able to reduce brown rot disease by strengthening cuticle and membrane protein synthesis (Di Francesco et al., 2018). Likewise, according to Zheng and Chen (2009), *Cryptococcus laurentii*, a biocontrol yeast upregulated the expression of protein responsible for postharvest pathogen resistance. Yeasts are also able to elicit pathogen-triggered immunity in fruit by upregulating defense responsive genes, in response to bacterial flagellin exposure at cell surface(Zhao et al., 2020b). Various microbial formulations are being used for commercial practices for controlling broad and narrow range of postharvest pathogens (Calvo-Garrido et al., 2019), but there should be a regulation for optimization and IPM strategies for standard application.

Table 14.2 shows list of postharvest diseases, casual organism, and their antagonist microorganism that can effectively inhibit postharvest diseases.

14.5 Bioformulation

Bioformulation is a mixture of bioactive ingredients of beneficial microbial strains in a form of formulated product with inert (inactive) substances (Stamenković et al., 2018). It can be

TABLE 14.2 List of bioformulation used for postharvest loss.

S. no.	Formulation	Source	Effect	Crop	Reference
1.	Fungal formulation	*Aureobasidium pullulans* and *Candida vanderwaltii*	Fungicide against *Botrytis cinerea* and *Rhizopus stolonifer* at varied temperature	Strawberry	Lima et al. (1997)
2.	Leafy formulation	*Ocimum kilimandscharicum*	Insect pest	Maize and sorghum grains	Jembere et al. (1995)
3.	Natural product formulation	Salicylic acid + putrescine	Fruit decay	Murcott mandarin fruit	Ennab et al. (2020)
4.	Natural product formulation	25 mg/L kojic acid and 10 g/L calcium chloride (KA + $CaCl_2$)	Inhibit off-odor production	Broccoli florets	Yan et al. (2020)
5.	Bacterial formulation	*Bacillus amyloliquefaciens* (VB2) and *Bacillus subtilis* (AP)	*B. cinerea* infection after harvesting	Rose	Nakkeeran et al. (2019)
6.	Bacterial formulation	Bacillus and salicylic formulation 0^3–10^8 CFU/mL and 0.05 mM, respectively	Resistance to *Phytophthora infestans* and *Fusarium oxysporum*	Potato tuber	Lastochkina et al. (2019)
7.	Organic formulation	Methyl jasmonate	Phenolic content enhances	Plum	Karaman et al. (2013)
8.	Yeast formulation	*Rhodotorula glutinis* and *Cryptococcus laurentii*in combination with 238 mmol/L sodium bicarbonate or 15 mmol/L ammonium molybdate	Against blue mold	Jujube fruits	Wan et al. (2003)
9.	Bacterial formulation	Bacterial strain B106 1×10^8 CFU/mL	*Colletotrichum musae*	Banana	Fu et al. (2010)
11.	Yeast formulation	*R. glutinis* 1×10^8 CFU/mL	Gray and blue mold	Apple	Zhang et al. (2009)
12.	Yeast formulation	*Meyerozyma guilliermondii* with methyl jasmonate	Blue mold decay	Apple	He et al. (2020)
13.	Yeast formulation	*Pichia membranifaciens* 5×10^8 CFU/mL	Rhizopus rot	Nectarine	Qing and Shiping (2000)
14.	Bacterial formulation	*Bacillus* sp. w176	Green mold	Citrus	Tian et al. (2020)
15.	Bacterial formulation	*Pseudomonas fluorescens*	*Penicillium italicum*	Oranges	Wang et al. (2020)

(*Continued*)

TABLE 14.2 (Continued)

S. no.	Formulation	Source	Effect	Crop	Reference
16.	Yeast formulation	*Cryptococcus podzolicus* with β-glucan	Blue mold	Pears	Zhao et al. (2020a)
17.	Yeast formulation	*Sporidiobolus pararoseus* Y16 and 1 mM glycine betaine	Apple blue mold	Apple	Abdelhai et al. (2019)
18.	Bacterial formulation	*Stenotrophomonas rhizophila* 1×10^8 cells/mL	*Colletotrichum gloeosporioides*	Mango	Reyes-Perez et al. (2019)
19.	Yeast formulation	*P. membranifaciens* + 20% (w/v) trehalose, 2% (w/v) sodium glutamate, 5% (w/v) polyvinyl pyrrolidone, and 20% (w/v) skim milk (vacuum dried)	Enhanced fruit viability	*Citrus sinensis*	Zhang et al. (2020)
20.	Yeast formulation	*Hanseniaspora uvarum* with β-aminobutyric acid	Gray mold and black rot	Kiwifruit	Cheng et al. (2019)
21.	Yeast formulation	*Saccharomyces cerevisiae* EBY100	Fruit decay by *B. cinerea*	Tomato	Zhao et al. (2020b)
22.	Yeast formulation	*A. pullulans* strains L1 and L8	*Monilinia laxa*	Peach	Di Francesco et al. (2018)
23.	Bacterial formulation	*Bacillus ginsengihumi*	*Botrytis* bunch rot	Grapes	Calvo-Garrido et al. (2019)
24.	Bacterial formulation	*B. amyloliquefaciens*	Brown rot	Crops	Gotor-Vila et al. (2017)
25.	Bacterial formulation	*Burkholderia spinosa*	Banana anthracnose	Banana	Ray et al. (2011)
26.	Yeast formulation	*Pichia anomala*	Penicillium rots	Citrus	Liu et al. (2017)

defined as use of microorganism with synergistic effects as a substitute for pesticides to improve soil fertility, plant growth, and phytopathogen suppression (Arora et al., 2010; Bargaz et al., 2018). Basically, various types of formulations are used in the world but two types of bioformulations are commonly available, solid and liquid (Fig. 14.2; Mishra and Arora, 2016).

Uses of microbes in bioformulation or emulsion are secure, safe, and reliable (Bulgari et al., 2019a). Various microbes have been used in bioformulation such as *Bacillus cereus* and *Pseudomonas rhodesiae* enhanced plant vegetative growth (Kalita et al., 2015). In industry, microbes are used in emulsion for their beneficial effect such as prevention of contamination of phytopathogen. Many studies reported use of microbes in emulsion such as *Bacillus subtilis* (Felix et al., 2019), *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* (Ly et al., 2006), *Acinetobacter*, *Pseudomonas aeruginosa*, *Pseudomonas carboxydohydrogena*, and *Alcaligenes latus* (Huang et al., 2010).

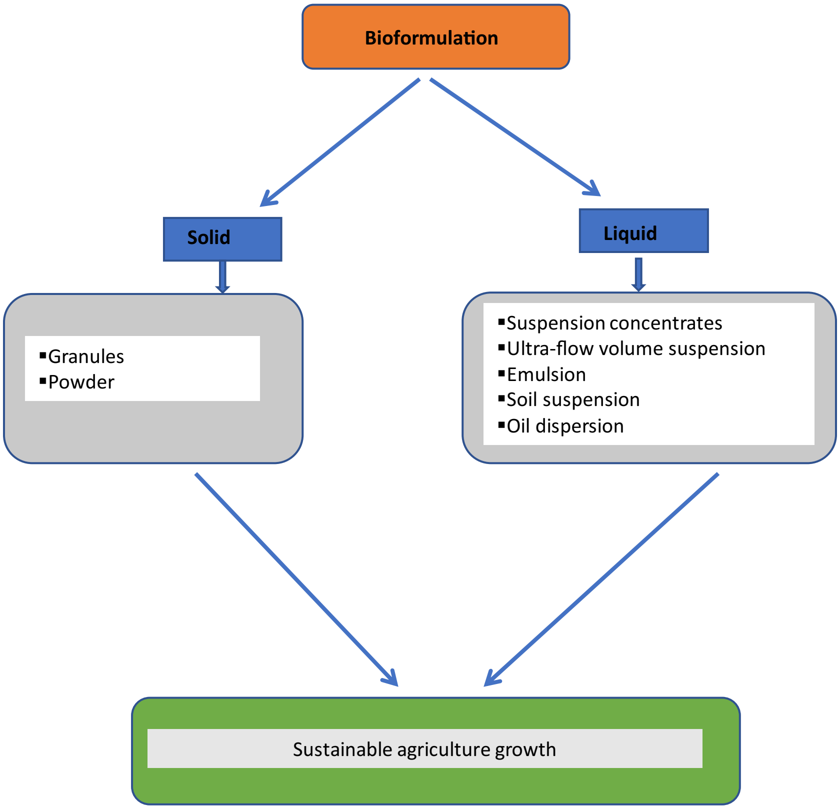

FIGURE 14.2 Types of bioformulations commonly used to control pathogens.

14.6 Volatile organic compounds or antimicrobial secondary metabolites produced by antagonists as direct pathogen control

VOCs or antimicrobial secondary metabolites produced by microbial antagonists can play a major role in showing antagonistic behavior against postharvest pathogens. Many of the VOCs have been purified from the biocontrol agents and their mechanisms against pathogens have been elucidated (Table 14.3).

14.7 Action mechanism of bioformulations

There are several mechanisms employed by the microbes involved in bioformulations to control postharvest diseases. Recent advances in techniques of microbiology, molecular biology, bioinformatics, and biochemistry have provided an insight into the multiple mechanisms employed by microbes to combat postharvest diseases. Several mechanisms including mycoparasitism, antibiosis, competition for iron and nutrients, secretion of

TABLE 14.3 List of secondary metabolites, their sources and applications.

S. no.	Secondary metabolites	Source	Application	References
1.	T22-azaphilone	*Trichoderma harzianum* 22	Biological control	Keswani et al. (2017)
2.	Genistein	*Bradyrhizobium japonicum*	Increase nodules numbers and dry matter	Muñoz et al. (2014), Morel et al. (2016)
3.	Hesperitin and naringenin	*Rhizobium leguminosarum*	Increase number of nodule and plant dry matter	Morel et al. (2016)
4.	6-Pentyl-2 Hyran-2-one (6-PP)	*T. harzianum*, *T. viride*, *T. koningii*, *T. atroviride*	Biological control, plant-growth promotion	Keswani et al. (2017)
5.	Butyrolactone	*Streptomyces* sp.	Morphological differentiation and secondary metabolites	Van der Meij et al. (2017)
6.	Paenilarvins	*Paenibacillus* sp.	Biocontrol	Hertlein et al. (2016)
7.	Iturin	*Bacillus amyloliquefaciens*	Biocontrol activity	Dunlap et al. (2019)
8.	Fengycin	*Bacillus subtilis*	Antifungal activity	Meena and Kanwar (2015)
9.	2,5-Diketopiperazines	*Pseudomonas aeruginosa*	Quorum sensing, plant-growth promotion	Alshaibani et al. (2017)
10.	Trichodermin	*T. harzianum*	Pharmaceutical compounds and plant-growth regulators	Contreras-Cornejo et al. (2018)

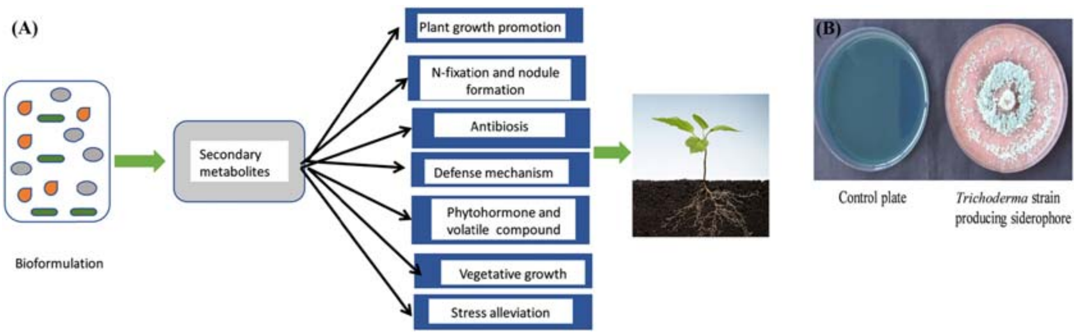

FIGURE 14.3 (A) Mode of mechanisms of bioformulations against postharvest pathogens. (B) Plate showing production of siderophore from *Trichoderma* sp.

antimicrobial compounds, and induction of induced systemic resistance (ISR) in crops play an important role during tripartite interactions of plant–pathogen and microbes for biotic stress amelioration and postharvest of crops (Fig. 14.3A). A detailed discussion with examples is provided in the following section.

14.7.1 Competition for space and nutrients

Nutrients including carbohydrates, amino acids, vitamins, and minerals are vital for growth of a pathogen and so is the space. The space and nutritional resources present in plant as host during tripartite interactions are limited (Spadaro and Droby, 2016; Dukare et al., 2017). A microbial agent that competes with the pathogen for nutrient and space and wins the battle can be an effective biocontrol agent against postharvest diseases. A number of effective biocontrol agents for control of postharvest diseases including fungi and yeast compete and exclude out their pathogenic counterpart because of their fast growth rate and ability to utilize resources effectively (Carmona-Harnandez et al., 2019).

Tryfinopoulou et al. (2020) in their studies against *Aspergillus carbonarius* in grapes/juice found yeast very efficient to inhibit the growth of *A. carbonarius* by competition for space and reducing the production of toxin ochratoxin A. Yeasts are also a successful competitor for sugar and nitrogen as observed by Spadaro and Gullino (2004), where strong inhibition of radiolabeled glucose use was observed in *B. cinerea* while competing with yeast *Sporobolomyces roseus.*

Pichia caribbica supplemented with vitamin C upregulated proteins involved in the glucose metabolism, thereby competing successfully with pathogen *P. expansum* for carbon source to inhibit blue mold decay in harvested apples (Yang et al., 2020).

14.7.2 Production of siderophores and competition for iron

Siderophores are low-molecular-weight (500–1000 Da) compounds secreted by many fungi and bacteria to chelate ferric ions from surroundings by forming stable ferric complexes and transporting them to the cells (Wilson et al., 2016). Iron is a major nutrient required by all pathogens to sustain successfully. Bioformulations producing siderophores mobilize, limit, and deplete the iron source to pathogen inducing imbalance in iron homeostasis and ultimately death of the pathogens (Glick, 2012).

Pseudomonas fluorescens is a well-known biocontrol agent for control of postharvest diseases (Wang et al., 2019; Carmona-Harnandez et al., 2019) owing to the production of siderophores. Wang et al. (2019) demonstrated efficient control of green mold caused by *P. digitatum* on oranges by application of bacteria antagonist *P. fluorescens*.

Trichoderma sp. often used as a potent postharvest disease control agents is also a prolific producer of siderophores (Fig. 14.3B; Köhl et al., 2019). Many synergistic applications of combing *Trichoderma* sp. and *P. fluorescens* have also been developed against postharvest diseases. *Metschnikowia citriensis* was shown to deplete iron and thus inhibiting the growth of *Geotrichum citri-aurantii* while controlling sour rot of postharvest citrus fruit (Kurtzman and Droby, 2001).

Yeast was also developed as an efficient postharvest disease management and a prolific producer of siderophores (Mukherjee et al., 2020). Antagonistic action of siderophores from *Rhodotorula glutinis* upon the postharvest pathogen *P. expansum* was assessed by Calvente et al. (1999).

14.7.3 Production of volatile secondary metabolites and organic compounds (VOCs)

Some of the biocontrols used in the management of postharvest diseases are able to produce antifungal and antibacterial secondary metabolites and VOCs, and other biological activities (Table 14.3; Costa et al., 2019; Kamat et al., 2020).

Trichoderma sp. is known to produce a number of antifungal secondary metabolites including peptibols, terpenoids, and alkaloids (Kumari et al., 2019). Li et al. (2018) in their studies demonstrated volatile compound−mediated recognition and inhibition between *Trichoderma* biocontrol agents and *F. oxysporum*.

Earlier, antagonistic effect of two peptibols producing isolates of *Trichoderma harzianum* was observed against postharvest pathogens of tomato (*Lycopersicon esculentum*; El-Katatny et al., 2011).

Bacillus sp. is well known for production of VOCs against fungal pathogens. The main bioactive compounds produced by *Bacillus* sp. are LPs, enzymes, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, and exopolysaccharides (Lastochkina et al., 2019). Iturin A produced by *B. amyloliquefaciens* PPCB004 was found to be the main component involved in inhibition of postharvest fungal pathogen (Arrebola et al., 2010).

Yeasts have also gained an equal importance by producing antimicrobial VOCs in postharvest disease management. Several killer toxins produced by yeasts (K1, PMKT) kill the pathogen by forming membrane pores but are immune to their own class of toxins (Spadaro and Droby, 2016).

14.7.4 Mycoparasitism and antibiosis

By producing lytic enzymes, fungal biocontrol agents can directly kill the pathogenic fungus by destructing the fungal cell wall (Kumari et al., 2017a). Biocontrol agents used in postharvest disease management are able to release lytic enzymes including glucanase, chitinase, cellulase, which are able to break the fungal cell wall. Chitinase produced by many biocontrol fungi and yeasts is able to break b-1−4 linked subunits of acetylated amino sugar *N*-acetylglucosamine while glucan acts as a filling material and constitutes about 50%−60% of the total cell wall (Spadaro and Droby, 2016).

Similarly, antibiosis is the secretion of potent antibiotics by bacteria and actinomycetes, which directly kills the pathogen. *Bacillus*, *Pseudomonas*, and *Streptomyces* sp. are some of the bacterial species known to produce antibiotics (Dukare et al., 2017). Blasticidin-S was the first antibiotics used to control rice blast disease in Japan, which was isolated from *Streptomyces griseochromogenes* (Law et al., 2017). Earlier, Smilanick et al. (1993) had demonstrated role of antibiotics producing *Pseudomonas* sp. to control postharvest brown rot of nectarines and peaches.

14.7.5 Induction of disease resistance in crops and fruits

Biocontrols used during postharvest disease management are able to induce systemic resistance in the plants in general and wounded area in particular. If applied earlier, before occurrence of disease, they are able to generate a more profound effect on plant immune systems. Microbial biocontrols are known to induce priming, systemic acquired resistance, ISR, and hypersensitive reactions (Köhl et al., 2019). Enhancement of secondary metabolite production, activation of mitogen-activated protein kinase signaling pathways, production of phytoalexins, and strengthening of plant cell wall are some of the mechanisms employed by microbial biocontrols to elicit plant immune response to alleviate biotic stresses (Shoresh et al., 2010; Conrath et al., 2015).

Petriacq et al. (2018) in their review summarized the role of induced resistance in controlling fruit decay diseases. Shoresh et al. (2010) studied alleviation of ISR of plants after treatment with fungal biocontrol agents. Hase et al. (2008) studied involvement of jasmonic acid signaling in bacterial wilt disease resistance induced by biocontrol agent *Pythium oligandrum* in tomato. *P. fluorescens* strains WCS374r and WCS358r induced protection in both wild-type *Arabidopsis* and SA-nonaccumulating NahG plants without activating pathogenesis-related gene expression by inducing ISR (Van Wees et al., 1997).

14.7.6 Change in reactive oxygen species production profile in plants

Microbial biocontrols are known to interfere with reactive oxygen species (ROS) profile of plants in postharvest disease management after their application. ROS can be a double-edged sword, which is manipulated by microbial biocontrols to enhance the immunity of host plants during tripartite interactions (Kumari et al., 2017b; Dixit et al., 2016). In early plant defense response, ROS level is increased by biocontrol agents, thereby increasing the immunity of plants against pathogens while in compatible plant–pathogen interactions, biocontrols help to lower down the ROS level to cope up with the oxidative stress (Dukare et al., 2017).

Yarrowia lipolytica induced PPO, POD, PAL, APX, GLU, and CAT activities of grapes to ameliorate oxidative stress to control postharvest decay of table grapes caused by *Penicillium rubens* (Wang et al., 2019). β-Glucan induced PPO, POD, and CAT activities of pears treated with *Cryptococcus podzolicus*, which helped to overcome tomatoes infected with *Ralstonia pseudosolanacearum* (Zhao et al., 2020a). Castoria et al. (2003) demonstrated role of antagonistic yeast to make the postharvested fruit more tolerant to ROS against pathogen *R. glutinis.* Antagonistic yeast enhanced H_2O_2 accumulation in apple fruit exocarp around infected area enhancing the defense response of citrus flavedo to postharvest pathogen (Macarisin et al., 2010).

14.7.7 Quorum sensing and biofilm formation

Quorum sensing and biofilm formation can help biocontrol agents to successfully colonize and adhere to the plant surface for their better thriving in comparison to pathogens. Many biocontrol agents secrete small diffusible molecules such as quinolone, indole, and γ-amino butyrate as their mode of communication to each other (Crépin et al., 2012). This phenomenon is well described for phenazine-producing *Pseudomonas* sp. (Köhl et al., 2019).

Though this phenomenon has not been studied in much detail in tripartite interactions of plant–pathogen and microbial biocontrol during postharvest disease management, some reports suggest in favor of this. Lastochkina et al. (2019) described biofilm and surfactin components play an important role in biocontrol efficacy of *Bacillus* sp. Efficacy of antagonistic yeasts was also attributed due to quorum sensing and biofilm formation (Freimoser et al., 2019).

14.7.8 Change in "ome" of plants

Biocontrol agents can modulate the proteome, metabolome, transcriptome, lipidome, and secretome of plant and pathogens during tripartite interaction of plant–pathogen and microbial biocontrol. *Saccharomyces cerevisiae* successfully inhibited the growth of pathogen *Fusarium graminearum* by modulating proteome and transcriptome of pathogen (Zhao et al., 2019). *Trichoderma atroviride* during interaction with *B. cinerea* and *Phytophthora capsici* regulated transcription of various genes involved in cell wall degradation (Reithner et al., 2011). Hershkovitz et al. (2013) observed differential expression of more than 250 genes involved in antagonist–pathogen interaction, genes related to transmembrane, multidrug transport, and amino acid metabolism in *Metschnikowia fructicola* during interactions with *P. digitatum* and grapefruit peel.

Though many mechanisms are employed by biocontrols against pathogen during management of postharvest diseases, they are often interrelated. An in-depth study finding their correlation can provide further insight into the mechanistic aspects of postharvest biocontrol agents. Several important aspects including the microbiome composition on the plant surfaces, interaction of antagonistic microbes with epiphyte and endophyte of plant, cultivar of plant, and the disease resistance developed in pathogens also play a vital role in successful application of bioformulations to control postharvest diseases.

14.8 Commercial production of bioformulations to control postharvest diseases

Though several successful reports of antagonist biocontrols are reported to control postharvest diseases, very few successful examples are there which has crossed all the barriers from lab to land, and has reached to the stage of commercial production. The biocontrol product has to undergo a large-scale production maintaining the similar efficacy in large-scale pilot production (Droby et al., 2009) to reach to land from lab. Their biosafety and efficacy evaluations are some of the measures that need to be guaranteed before their large-scale commercial application. A good bioformulation should have the following characteristic features to sustain in the market for commercial production.

1. High efficacy against postharvest pathogens
2. Sustainable release of the active ingredient(s)
3. Nontoxic toward host
4. Increased shelf life
5. Stability and wide host range

Table 14.4 describes some of the successful examples of bioformulations produced commercially and available in market under different brand names.

TABLE 14.4 List of bioformulations available commercially across the globe.

Name of bioformulation	Microbial ingredient	Company	Effective against pathogen	Target plants	Reference
Kalisena SD and Kalisena SL		Ms Cadila Pharmaceuticals Ltd., IARI	Soilborne pathogens	Crops	Newsletter of IARI
None	FAT3 and FAT6	PDBC, Bangalore	Multiple pathogens	Chickpea, tomato	Wahab (2009)
Avogreen	*Bacillus subtilis*	S. Africa	Cercospora spots	Avocado	Korsten and Bornman (2004)
Bio-Save 10 LP	*Pseudomonas syringae*	Jet Harvest Solutions USA	*Botrytis*, *Penicillium* spp.	Citrus fruits	Korsten and Bornman (2004)
BlightBan A506	*Pseudomonas fluorescens* A506	Nufarm	Russet-inducing bacteria	Tomato, potato, peach	https://nufarm.com
Galltroll	*B. subtilis* K84		*Agrobacterium tumefaciens*, crown gal disease	Fruit, nut	Mehnaz (2016)
Candifruit	Candida sake	IRTA/Sipcam-Inagra, Spain	*Botrytis*, *Penicillium* spp.	Pome	Mehnaz (2016)
Aspire	*Candida oleophila*	Ecogen, USA	*Botrytis*, *Penicillium* spp.	Pome	Mehnaz (2016)
Shemer	*Metschnikowia fructicola*	Bayer, The Netherlands	*Botrytis*, *Penicillium*, *Rhizopus* spp.	Pome, grape, potato	Mehnaz (2016)
TrichoderMax	*Trichoderma asperellum*	Europe	Soilborne diseases	Broad host range	Mehnaz (2016)
Cedomon	*Pseudomonas chlororaphis*	Europe	*Ustilago nuda*	Barley	Mehnaz (2016)
Bioscrop BT16	*Bacillus thuringiensis*	Europe	Lepidopteran larvae and beetles	Citrus, cauliflower, deciduous fruit trees, horticultural brassicas, olives, pepper, banana, and tomato	Mehnaz (2016)
Biovaccine	*Trichoderma viride*	BioPower, Sri Lanka	*Pythium*, *Rhizoctonia* and *Fusarium* spp.	Multiple host range	Mehnaz (2016)
Biotilis	*B. subtilis*	Biopesticide	Multiple pathogens	Multiple host range	Mehnaz (2016)
BioKuprum	*Chaetomium cupreum*	Biopesticide	Rust, blight, rots	Multiple host range	Mehnaz (2016)

14.9 Hurdles during commercialization of bioformulations and measures employed to increase the transition from lab to land

Though bioformulations are an economic and eco-friendly way to combat postharvest pathogen attacks, their commercialization needs lot of efforts from researcher, government, and policy makers.

14.9.1 Stress-tolerant microbes

The major hurdle faced during commercialization of bioformulations is to maintain the efficacy of microbial component from lab to land. There are several kinds of stresses faced by the microbes in field conditions which are important to overcome to be a good bioformulant to control postharvest diseases.

Stress-tolerant microbes or synergistic application of antagonistic microbes can play an important role in maintaining the efficacy of antagonistic microbes in field conditions. Many *Trichoderma* strains have been isolated and developed, which can tolerate drought and salt while acting as an excellent biocontrol (Hirpara et al., 2017; Ndolo et al., 2019). Synergistic application of microbes has shown to help horticultural crops under abiotic stress conditions (Bulgari et al., 2019b).

14.9.2 Increasing shelf life and efficacy of antagonistic microbes

Increasing shelf life of antagonistic microbes in field and stressed condition is a major hurdle to commercialize the product. Selection of stress-tolerant microbes can be good criteria for their successful commercialization. Their genetic stability and the colloidal release of bioformulations provide them an edge for their successful commercialization (Martin et al., 2017). Edible coating of fruits and crops with polymers to control water loss and exchange of gases can also increase the shelf life of biocontrol agents (Martin et al., 2017).

The form in which biocontrol agents are prepared also affects the shelf life of antagonistic microbial agent. In general, dry powder forms of microbes have a better shelf life than the liquid form (Usall et al., 2009). However, solid formulations also have their own drawbacks, owing to their viability loss due to constant dehydration−rehydration process. Many biologically available coating materials such as milk and whey protein, maltodextrins have been reported to be used to protect microbes from dehydration (Martin et al., 2017).

Certain measures have also been employed to increase the efficacy of biocontrol agents. Combining vitamin C, salicylic acid, and lysozyme with the potent antagonistic microbes are some of the examples of strategy developed to increase the efficacy (Dukare et al., 2017).

14.9.3 Sustained release of volatile organic compounds or microbes in field

When antimicrobial secondary metabolites or VOCs are used to control postharvest diseases, the major hurdle encountered is their solubility in water and their sustained release on the plant surface. Most of the VOCs being small organic molecules face the problem of solubility in hydrophilic environment. Nanotechnology has become a common term used along with agriculture coining the new term agri-nanotechnology (Mishra et al., 2014; Kumari et al., 2017c).

Active antimicrobial ingredients encapsulated in nontoxic and degradable polymers such as sodium alginate and chitosan allow them to release slowly and confer them hydrophilicity (Soh and Lee, 2019). Chitosan has been studied for its triple actions including elicitation, coating, and antimicrobial properties to control postharvest decay in fruits and vegetables (Romanazzi et al., 2018). A combined antimicrobial nanoparticle and microbial formulation approach is also helpful for pivotal success and commercialization of the products (González-Estrada et al., 2019). Recently, Housseiny and Aboelmagd (2019) studied that nanoencapsulated naringinase produced by *Trichoderma longibrachiatum* ATCC18648 can work efficiently against citrus juice debittering.

14.9.4 Preharvest versus postharvest application of microbial formulations

Selecting the appropriate timing and mode of application is a prerequisite for successful antagonistic application of microbial bioformulations.

Some of the pathogens are carried along with fruits, vegetables during their harvest from field to storehouses to increase their susceptibility toward diseases. Preapplication of bioformulations does not only prevent the diseases but also strengthen the first line of defense in plant system (Kumari et al., 2017b). Vivekananthan et al. (2006) demonstrated in their studies that preharvest application of *P. fluorescens* and a strain of *B. subtilis* in mango field enhanced disease resistance and fruit yield. However, in some cases, preharvest application is reported to be less effective than postharvest applications (Carmona-Harnandez et al., 2019). It becomes very necessary to study the mechanism and mode of application of each bioformulation prepared and standardize them for their successful commercial applications.

14.9.5 Safety evaluation of microbial bioformulations

For any product development, it is an essential prerequisite to test their bioaccumulation and toxicity on the native microbiome and the end users. A product can only be considered as safe when it does not harm the environment, nor does it has any cytotoxic potential on healthy animal cells. Stable bioformulations derived from *Xenorhabdus stockiae* PB09 have controlled mushroom mite diseases *Luciaphorus perniciosus* Rack successfully without hampering the health of nontargeted organisms (Namsena et al., 2016). However, on a deeper screening, many biocontrol agents or their VOCs have been observed to be causative agents of human or animal diseases or some are very potent enough to interfere with the native microbiota (Keswani et al., 2019). *Bacillus gobisporus* used as an excellent antagonist against a wide range of pathogenic fungi is known as opportunistic human pathogen (Ghosh et al., 2003; Keswani et al., 2019).

To check the stability, ecotoxicity and cytotoxicity of bioformulation products are of utmost importance before their production on a large scale for their successful commercial applications.

14.9.6 Policy making, regulation, and promotion of bioformulation uses

To commercialize and promote uses of bioformulations, it is necessary that strong inputs should be provided by government, policy makers, and funding agencies.

In India, Indian Council of Agricultural Research (ICAR), Central Integrated Pest Management Centres, Council of Scientific and Industrial Research (CSIR), Department of Biotechnology (DBT), and Department of Science & Technology (DST) are putting their constant efforts to commercialize the application of antagonistic microbes. The programmes such as National Biocontrol Network Programme (NBNP) by DBT, which are running resulted in identification and mass production of more than 30 biopesticides. Continuous workshops and symposia are organized by ICAR and other agencies regarding importance and commercialization of biocontrol agents during postharvest disease management. Pilot plant set up and economical support during that process should be provided by governing bodies.

Further, there is also a need of spreading awareness about bioformulations to farmers who are the end users of the product. Awareness campaigns should periodically be organized by the government to promote uses of eco-friendly and economical bioformulation approaches for storage pest managements. Development of bioformulation technologies and the technology transfer to companies for product development should be encouraged by policy makers.

14.10 Conclusions and future aspects

Postharvest diseases put a significant pressure on the yield and quality of crops and horticulture. Biocontrols have been developed as an eco-friendly and economical strategy to combat postharvest pathogens to overcome financial and mental burden on farmers. The multiple mode of actions employed by them to mitigate the biotic stresses make them favorable antagonistic agents against postharvest pathogens. Some of the bioformulations and VOCs have been produced successfully on a large scale and commercialized, though most of them still have to travel a long path to write their successful stories. Synergistic application of bioformulations, supplementing them with proper elicitors, policy making and regulations by funding agencies, government bodies and promotion of their uses are some of the parameters that can be worked upon to increase the safe use of bioformulations. Recent development in "omics" technologies can be employed to gain a mechanistic insight into their mode of actions against postharvest pathogens. Further development on better biocontrol strategies displaying broad-spectrum activities in a cost-effective manner can provide a ray of hope to a better future for both bioformulation technologies as well as farmers.

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