

Harnessing the potential of plant beneficial microbes for disease management in fruit crops

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Received, June, 2024; Revised accepted, August, 2024

ABSTRACT

Fruit crops, more particularly the perennial fruit crops have always been challenged by different phytopathogens and the changing dynamics of pathogens and vectors in the backdrop of climate change has aggravated the disease scenario. The report of tropical race 4 of Fusarium oxysporum f. sp. cubense of banana in different parts of the world, citrus greening devastating the world citrus industry has raised the warning bells to search for novel and environmentally benign technologies. In recent times, several efforts have been made to address the pre and post harvest diseases to overcome or to bridge the gap for the failure of many conventional management approaches wherein, plant-associated microbes are considered as a potent candidate for next-gen technologies. The role of rhizospheric and endophytic microbes in disease management and abiotic stress resilience is undoubtedly accepted and broad-spectrum bio-formulations have shown their efficacy against a wide range of fruit crops. Understanding the tripartite interaction between pathogens, hosts, and bioagents in regulating defense arsenals and/or disrupting the invading pathogen is still an unresolved mystery. Post-harvest disease management using microbes is still in its infancy, although different groups of microbes viz., Bacillus spp, Pseudomonas spp., and some yeasts have shown promising results. Microbial secondary metabolite-based cell-free technologies, use of AMF and nano bio fungicides, etc. could sustain the fruit industry by preventing or managing the major phytopathogens bothering productivity and international trade.

Keywords: Biotic stress, Disease resistance, Fruit crops, Microbial antagonists, Post-harvest disease

INTRODUCTION

Fruit crops have long been cultivated worldwide, both commercially and as homestead amateur plants contributing a significant portion of farm income. Fruit crops with a production of 675 million metric tons worldwide including popular fruit crops like apples, citrus, bananas, grapes, etc. offer nutritional security options. India alone contributes 112.62 million metric tones from an area under cultivation of fruits standing at 7.04 million hectares and earns a foreign revenue of Rs. 8178.22 crores/ 986.32 million USD from exporting fruits (APEDA, 2024). However, the fruit industry faces different challenges including biotic stress caused by plant pathogenic microbes causing significant yield loss (Ahmed *et al.*, 2021). Fruit crops, mostly being perennial in nature witness a horde of pathogens causing diseases in different parts of plants viz., leaves, fruit, stem, and rot resulting in compromising the quality and quantity of produce in both pre-harvest and post harvest stage. The persistence of pathogen inoculums in soil and plant parts aggravates diseases over a

period which makes disease management difficult in perennial fruit crops (Swagataet *al.*, 2021; Bora and Bora, 2022). History has several evidence of the importance of fruit crop diseases (Boraet *al.*, 2021). The famous Fusarium wilt menace in Gros Michel banana wiping away the cultivar before the mid-1900s by *F. oxysporum* f. sp. *cubense* (Foc) warranted a new source of resistance and management strategy (Ploetz, 2015). Further, the emergence of the tropical race 4 of Foc has threatened banana cultivation in several countries including India (Kumari *et al.*, 2023). Phytophthora diseases and greening in citrus are two major diseases, the entire citrus industry has been struggling with throughout the world (Das *et al.*, 2023; Handique *et al.*, 2024),s besides citrus canker disease (Nasreen *et al.*, 2020), and gummosis, mango anthracnose, guava wilt, apple scab, fire blights of peach and pears, banana Fusarium wilt and bunchy top, brown rot disease of stone fruits, bacterial blight and heat rot of pomegranate, crown galls in apple, grapes, stone fruit, powdery mildew and downy mildew of grapes etc. are some examples

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of diseases significantly affecting fruit industry worldwide (Bora and Bora, 2020a ; 2020b). Several fungal genera viz., *Rhizoctonia*, *Colletotrichum*, *Fusarium*, *Phomopsis*, *Botrytis*, *Phytophthora*, *Aspergillus*, *Penicillium*, and bacterial genera *Ralstonia*, *Agrobacterium*, *Erwinia*, etc. have been associated with different pre and post-harvest diseases in fruits (Taye *et al.*, 2023). Further emerging virus diseases such as diseases like citrus viruses (psoriasis, Tristeza, exocortis, ring spot, xyloporosis, stubborn, vein yellow, mosaic), banana (bunchy top, mosaic, infectious chlorosis), grapes (leaf roll, stem pitting, fan leaf, yellow mosaic, vein bending.), apple (mosaic), pineapple wilt virus, mulberry (mosaic, yellow net vein, ring spot, dwarf), plum line pattern virus, plum, and peach mosaic etc. also worsen the scenario. Several new viruses have also been reported in the last 5 years, exhibiting novel genomic features (e.g., Fabavirus, Luteovirus) that challenge the management of plant viruses due to rapid evolution (Maliogka *et al.*, 2018; Saikia *et al.*, 2022). Trans-boundary movement of infected fruit and plant material through international trade, use of infected propagating material and bud wood, vector borne nature of plant viruses are some factors posing serious threat in aggravating or introduction of new pathogens. Therefore, the strong intervention in disease management is a priority and especially residue free fruit production is a global agenda and research priority in modern horticulture.

PLANT BENEFICIAL MICROBES FOR FRUIT CROPS

The productions of fruits without chemical have received growers and scientists' attention considering the increasing demand for organic or residue free fruits. Fruits being consumed raw, mostly treated with harsh insecticides/fungicides pose serious chronic health hazard. In recent time, technology based on natural resources has been a priority wherein plant-beneficial microorganisms are considered as the most suitable alternative (Srivastava *et al.*, 2023; Bora *et al.*, 2019). Globally, a wide range of plant-beneficial microbes have been screened and identified from the rhizosphere, endosphere, and phyllosphere as aggressive and efficient biocontrol agents against many fruit crop pathogens and many have been exploited in

disease management programs due to their persistence in soil, high multiplication rate, ease in handling and most importantly due to lack of phytotoxicity and adverse effects on ecosystem. Better sustainability in crop health management in fruit crops involves integrating the plant beneficial microbes including BCAs, PGPRs into holistic crop management programmes, which in turn will contribute to eco-safe and health-friendly crop production sustainably. Microbial biological control agents (BCA) protect plants through different modes of action viz., priming host defense against pathogenic attack, and direct antagonism to the pathogens (Bora *et al.*, 2019; Saikia *et al.*, 2022). Various BCA like *Pseudomonas* sp., *Bacillus* sp., *Trichoderma* sp., *Verticillium* sp., etc. have been established as valuable allies in this pursuit, offering unique biological properties that can benefit crops in multiple ways. (Bora *et al.*, 2022, Bora and Bora, 2021; Rahman *et al.*, 2023). The microbe-plant cross-talk involves an array of biochemical pathways and bioactive metabolites produced by the BCAs (Alfano *et al.*, 2007). Fungal bioagents like *Trichoderma* spp. interact directly with the pathogen by hyperparasitism, antibiosis, production of antimicrobial secondary metabolites, or indirectly through competition for nutrients and space (Bora *et al.*, 2013).

The major back through in biological control of fruit crop diseases was achieved by *Agrobacterium radiobacter* strain K84 against crown gall pathogen *Agrobacterium tumefaciens* wherein K84 could inhibit the pathogen through bacteriocin (Agrocin 84). This success story encouraged researchers to search for efficient microbe-mediated disease management practices. Another group of PGP bacteria Fluorescent *pseudomonads* such as *Pseudomonas fluorescens* and *P. putida* have also been reported to exhibit biocontrol efficacy against diseases of fruits like sweet cherry, grapes (Esitken *et al.*, 2006). They are aggressive rhizosphere colonizers with antagonistic activity attributed to siderophores production, antibiosis, antifungal lytic enzymes, and ability to outcompete pathogens depriving nutrients and space (Bora *et al.*, 2016). Another bacterial genus *Bacillus* also has promising results against many pathogens and being gram-positive in nature the genus shows efficacy in a wide range of soil and climatic conditions. *B. subtilis*, *B. amyloliquifaciens*, and other species

produce a wide range of antimicrobial metabolites such as PAL, peroxidase, and β -1,3-glucanase antibiotics acting directly against plant pathogens including post harvest rots and pests besides inducing host defense through elevated defense enzyme activity (Bora *et al.*, 2024a, b). Li *et al.* (2023) established the efficacy of *Bacillus* spp. against *Botryosphaeria dothidea* causing ring rot of apples. *Trichoderma* spp., the most widely used fungal BCA (*T. harzianum*, *T. viride*, *T. hamatum*, *T. koningi*, *T. polysporum*, *T.*

parareesei and *T. longibrachiatum*) are reported successful against many soil-borne diseases, employing an array of biocontrol mechanisms for against plant pathogens (Harman *et al.*, 2004, Rahman *et al.*, 2021, 2023). Management of Fusarium wilt of Foc TR4 through *T. reesei*-based formulation in India is another breakthrough (Damodaran *et al.*, 2023) wherein antimicrobial metabolites also induced immunity in Cavendish banana.

Table 1: Effect of different plant beneficial microbes on biotic stress and growth attributes of fruit crops

Sl. No.	Fruit crop	Disease and associated pathogen/biotic stress	Microbes involved	Growth and yield response parameters	Reference
1.	Apple	Anthraco nose (<i>Colletotrichum</i> spp.)	<i>Bacillus subtilis</i> GYUN-2311	Fruit yield and nutrient composition of soil	Heo <i>et al.</i> (2024)
2.	Banana	Banana Fusarium wilt (<i>Fusarium oxysporum</i> f. sp. <i>cubense</i>)	<i>Trichoderma</i> spp. <i>Bacillus vallismortis</i>	Growth, yield, and rhizosphere microbial properties	Damodaran <i>et al.</i> (2023) Baruah <i>et al.</i> (2024)
3.	Apricot (<i>Prunus armeniaca</i> (L.))	Brown rot (<i>Monilinia fructicola</i>)	<i>Bacillus</i> (OSU-142)- <i>Pseudomonas</i> (BA-8)	Growth, yield, and leaf nutrient composition	Kaymak <i>et al.</i> (2010)
4.	Mango (<i>Mangifera indica</i> L.)	Anthraco nose (<i>Colletotrichum gloeosporioides</i>)	<i>Azotobacter chroococcum</i>	Seedling diameter and number of leaves	Sharma <i>et al.</i> (2011)
5.	Apple (<i>Malus domestica</i> Borkh.)	Anthraco nose (<i>Colletotrichum</i> spp.)	<i>Azotobacter chroococcum</i> - <i>Pseudomonas striata</i> - <i>Trichoderma viride</i> <i>Bacillus mycoides</i> - <i>B. polymyxa</i> , <i>Trichoderma harzianum</i> - <i>Azotobacter chroococcum</i> - <i>Pseudomonas fluorescens</i>	Germination, root growth, and pest incidence	Jayant <i>et al.</i> (2012)
6.	Nagpur mandarin (<i>Citrus reticulata</i> Blanco)	Brown rot and gummosis (<i>Phytophthora</i> spp)	<i>Pseudomonas fluorescens</i>	Shoot weight, root weight and rhizosphere microbial properties	Wu <i>et al.</i> (2012)
7.	Grape (<i>Vitis vinifera</i> L.)	All kinds of biotic stress	<i>Pseudomonas fluorescens</i>	Root development	Ma <i>et al.</i> (2017)
8.	Banana (<i>Musa acuminata</i> L.)	All kinds of biotic stress	<i>Azospirillum</i> sp.	Height and girth of the pseudo stem, leaf area, and yield	Sivasakthivelan <i>et al.</i> (2023)
9.	Passion fruit (<i>Passiflora edulis</i> Sims.)	All kinds of biotic stress	<i>Azotobacter</i> sp.- <i>Azospirillum</i> sp. - <i>Trichoderma</i> sp.	Plantlet growth and yield	Dubey <i>et al.</i> (2019)
10.	Walnut (<i>Juglans regia</i> L.)	All kinds of biotic stress	<i>Pseudomonas chlororraphis</i> - <i>P. fluorescens</i> - <i>Bacillus cereus</i>	Plant height, shoot, and root dry weight	Bertani <i>et al.</i> (2021)
11.	Citrus	Citrus canker (<i>Xanthomonas citri</i> pv. <i>citri</i>)	<i>Trichoderma viride</i>	Fruit yield and quality	Saikia <i>et al.</i> (2020)

While addressing diseases, the plant nutrition component is mostly overlooked (Srivastava and Bora, 2023). The effect of biotic and abiotic stress on fruit crops leads to compromised quality and quantity of yield. Nutrient deficiency often predisposes plants to several primary and secondary pest and disease infestations. Growing crops with enhanced resilience against both biotic and abiotic stresses, hence require the introduction of beneficial microbes with multiple functional traits. It is evident from robust literature that most BCAs, besides suppressing pathogens, help in nutrient acquisition and growth promotion. *Trichoderma* spp., *Pseudomonas* spp., and *Bacillus* spp. were reported to be efficient Phosphorous, Zinc solubilizers, siderophore, and IAA producers by many researchers which resultantly add on to the growth and yield of many crops (Bertani *et al.*, 2021; Bora and Bora, 2008a; 2008b ; 2010 ; Bora *et al.*, 2013). *Pseudomonas* and *Bacillus* were recorded to enhance growth, yield, and leaf nutrient composition in cherries (Esitkan *et al.*, 2006) and peaches (Ortíz-Castro *et al.* 2008).

Besides BCAs, arbuscular mycorrhizal fungi, popularly known as AMF are also of great interest to researchers owing to their multiple benefits. AMF as symbiont are close allies of many plant species including fruit crops and benefit plants with supply of vital nutrients such as phosphorus and water through their extensive networks of hyphae. Research evidence has established AMF effective against phytopathogens either directly or via enhanced resistance to pathogens (Liu *et al.*, 2007). Berdeni *et al.* (2018) recorded that root colonization in apple by two AMF *Rhizophagus irregularis* and *Funneliformis mosseae* could reduce the apple canker intensity caused by *Neonectria ditissima* besides improving growth, flower and fruit production and plant and soil nutritional status. Cheng *et al.* (2020) observed stimulation of signal transduction related genes against *Phytophthora parasitica* infection in trifoliolate orange upon inoculation with *Funneliformis mosseae* for a period of 14 weeks. Many such reports on success of AMF against fruit crop pathogens identify this group as possible add on to microbe mediated disease

management.

The performance of some plant beneficial microbes against disease and growth promotion in fruit crops are summarized in Table 1.

ADDRESSING POST_HARVEST PATHOGENS

Post -harvest diseases are a major contributory factor for significant loss during storage and transportation and can dramatically impact the quality and quantum of fruit production, resulting in an average yield loss of 22.5% in developing countries (Romanazzi *et al.*, 2016). Quite strikingly, more than 30% of harvested fruits and vegetables fail to reach consumers due to post harvest pathogenic microbes (Oerke and Dhene, 2004; Bora *et al.*, 2016). Devastating post-harvest pathogenic fungi such as *Penicillium* (*Penicillium expansum*, *P. digitatum* and *P. italicum*). *Botrytis cinerea* and *Aspergillus* cause fruit decay in considerable proportions, whereas *P. digitatum* causes 90% of fruit decay in all citrus fruits. In citrus, *P. digitatum* and *P. italicum* cause green mold disease in citrus fruit, and in apples, *P. expansum* causes blue mold disease significantly affecting orchard fruits (Kumar *et al.*, 2018). *Colletotrichum* spp. inciting anthracnose in mango, banana, and strawberry is also considered a major threat to fruits after harvest. Diseases caused by such fungi appear in the fruit after harvest, although many pathogens initiate infection during the pre-harvest stage.

Fungicides are the most commonly used management strategy against post-harvest decay but, there is a greater risk of pesticide residue through direct exposure. Exploiting the in-built plant immunity has been considered a plausible route to address post-harvest pathogens since plants have well-designed defense cascades for identification and suppression the pathogens through various biochemical and structural armors (Conrath *et al.*, 2006). Induced resistance after harvest through the use of safer chemicals and BCAs has also been recorded (Petriacq *et al.*, 2018). This defense priming is understood as an adaptive part of induced resistance (Mauch-Mani *et al.*, 2017).

Table 2: Successful bio control agents against post-harvest diseases of fruit crops

Sl. No.	Fruit crop	Disease with pathogen	Microbes involved	Reference
1.	Apple (<i>Malus domestica</i>)	Gray mold(<i>Botrytis cinerea</i>)	<i>Candida oleophila</i>	Liu <i>et.al.</i> (2013)
2.	Citrus	Green mold (<i>Penicillium digitatum</i>)	<i>Aureobasidium pullulans</i>	Sperandio <i>et.al.</i> (2015)
3.	Peach and plum	Brown rot(<i>Monilinia fructigena</i> and <i>M. fructicola</i>)	<i>Debaryomyces hansenii</i>	Grzegorzczak <i>et.al.</i> (2017)
4.	Apple (<i>Malus domestica</i>)	Blue mold (<i>Penicillium expansum</i>)	<i>Pseudomonas</i> spp	Hernandez-Montiel <i>et.al.</i> (2019)
5.	Strawberry (<i>Fragaria ananassa</i>)	Post-harvest Anthracnose (<i>Colletotrichum fragariae</i>)	<i>Streptomyces</i> sp. <i>Bacillus</i> sp. <i>Trichoderma</i>	Li <i>et.al.</i> (2021)
6.	Citrus (<i>Citrus</i> sp.)	Green mold (<i>Penicillium digitatum</i>)	<i>harzianum</i> - <i>Bacillus</i> spp.- <i>Pseudomonas fluorescens</i>	Poveda <i>et.al.</i> (2022)
7.	Strawberry (<i>Fragaria</i> sp.)	Gray mold (<i>Botrytis cinerea</i>)	<i>Bacillus subtilis</i> - <i>Clonostachys rosea</i>	Song <i>et.al.</i> (2022)
8.	Mango (<i>Mangifera indica</i> L.)	Post-harvest Anthracnose (<i>Colletotrichum fragariae</i>)	<i>Streptomyces griseoviridis</i> - <i>Bacillus subtilis</i>	Ciofini <i>et.al.</i> (2022)
9.	Peach (<i>Prunus persica</i>)	Brown rot (<i>Monilinia</i> spp.)	<i>Pichia guilliermondii</i> - <i>Bacillus subtilis</i> - <i>Streptomyces</i> spp.	Li <i>et.al.</i> (2023)

Recent interest in microbial bioagents as a meaningful approach to postharvest disease management has identified many potent microbes (Wisniewski and Wilson, 1992; Bora *et al.*, 2020). Microbes are being used with two basic approaches viz., i) the use of beneficial microflora already existing on fruit surfaces and ii) introduction of new bioagents against post-harvest pathogens. Our knowledge on exploring naturally occurring microbiome of mixed population is meagre, however, use of known BCAs as pre- and postharvest treatment has been well documented. The use of microbial BCAs against postharvest pathogens holds greater promise owing to certain conditions, viz., i. controlled environment for predictable response, ii. Well defined site of BCA inoculation, iii. Cost -effectiveness and iv. Short-duration trial and response (Bora *et al.* 2021; 2022). Therefore, value chain of microbes from isolation, screening, identification of efficient antagonists and developing delivery mechanism should receive a prioritized attention (Bora and Rahman, 2022). Some of the studies on the use of BCAs against postharvest diseases such as *Pseudomonas syringae* against blue mold, *Pseudomonas cepacia*, *Cryptococcus* spp., *Pichia guilliermondii* against graymold in apple; *Pichia guilliermondii*, *Bacillus subtilis* against green mold and sour rot; *Bacillus subtilis*

against and stem end rot in citrus; *Pseudomonas gladioli* against blue mold and grey mold, *Cryptococcus laurentii*, *C. flavus*, *C. albidus* against mucor rot in pear; *Bacillus subtilis* against brown rot in peach and *Trichoderma harzianum* and *Pichia guilliermondii* against graymold in grape have been widely reported using a range of microbial antagonists (Wisniewski and Wilson, 1992; Bora *et al.*, 2021). Interestingly, the exact mechanism of action of most of the antagonists during post-harvest stage is not yet clear. However, the basic mechanisms such as site exclusion, direct antagonism may work equally on fruit surface. Most of the studies on post harvest diseases are still based on dual culture assay and the delivery mechanism or products are miniscule in number (Sharm *et al.*, 2020). There is another challenge in the application method is the stability of bioagents due to the acidic environment on most fruit surfaces. Different kinds of salts as additives has been studied and such microbe based technologies needs a large-scale validation to benefit the different stakeholders including consumers to safeguard their health.

EPILOGUE

With the advent of multi-omic based technologies, the early detection and molecular

identification of pathogens along with the close relatives and evolutionary shift associated with fruit crops, precision disease management has become comparatively easier. A diverse group of plant beneficial microbes have been identified as possible alternatives to chemical fungicides against pre and post harvest diseases. Major technological breakthroughs have also been achieved against some dreaded diseases of fruit crops. However, diseases like citrus greening and other fastidious bacteria need a management approach using endophytes considering the obligate nature of the pathogens.

Further, post harvest disease management technology is the need of the hour, and microbe-based technologies should be technically validated with an understanding of the mechanistic pathway of antagonism and stability. These approaches need to be cushioned through the development of nano-bio fungicides, precise application of AMF-bioagent inoculants and RNAi-based technologies against viral diseases in tune with an ecologically benign option ensuring residue-free and quality fruits for a sustainable fruit-based industry.

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