Indian Journal of Agricultural Sciences **90** (8): 1389–96, August 2020/Review Article
https://doi.org/10.56093/ijas.v90i8.105900

Disease management in horticulture crops through microbial interventions: An overview

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Received: 9 March 2020; Accepted: 9 June 2020

ABSTRACT

Horticultural crops-based nutritional security is now a buzz word to Indian agriculture. Significant level of progress has been made towards the management of some pandemic pathogens affecting the production economics of horticultural crops. Some of these pathogens are : *Fusarium oxysporum, Ralstonia solanacearum, Pythium* spp., *Phytophthora* spp, *Xanthomonas* spp., *Colletotrichum* spp., *Pencillium* spp , *Alternaria* spp etc. Many soil-borne diseases as well as post harvest fungi are effectively addressed through a variety of bioagents belonging to predominant microbial genera represented by *Trichoderma* spp., *Bacillus* spp., *Gliocadium* spp. and fluorescent *Pseudomonads*. Our thumping success with different microbial bioformulations showed an effective control of both pests and diseases in major crops of northeast India comprising tea, banana, turmeric, ginger, in addition to bacterial wilt of *Bhoot jolokia,* tomato, hydroponic lettuce and *Rhizoctonia*- induced root rot management of chilli and patchouli. These area wide and crop wide results put forth a renewed claim in favour of developing a vibrant organic horticulture in years to come. All these issues are analysed highlighting the future use of rhizospheric and endophytic plant beneficial microbes as dominant communities.

Key words: Horticultural crops, Microbial interventions, Organic horticulture, Post-harvest diseases, Soil borne diseases

Horticultural crops have carved an indelible mark on the daily dietary requirement as an alternative source of nutritional security to the extent that the per capita consumption of cereal crops have reduced remarkably. Horticulture is ably recognized to have the potential to raise the farm income, provide livelihood security and earn foreign exchange, considering as much as 37% contribution of horticulture out of the total export of agricultural commodities (NHB 2020). The importance of horticulture in day-to-day life is like a panacea, made unexpected strides in recent past, thereby, surpassed the food grain production by nearly 30 million tons, which in coming years is expected to make some bigger strides. India's horticulture production registered a 1% rise during 2018-19 to record 314.67 million tons from 25.87 million ha area (NHB 2020). Despite, such revealing success, quality production coupled with low productivity and profitability in several horticultural crops are still a cause of serious concern. Of them, outbreak of diseases account for maximum damage to crops.

Most of the growers basically rely on chemicals for disease management as a quick remedy. But development of resistance against chemicals by pathogenic microbes coupled with environmental and public concern over hazardous chemicals have posed newer challenges to disease management of horticultural crops. The world trend in recent times is moving towards chemical residue free fruits, vegetables and tea plantations (Bora *et al.* 2016a). The production without chemical pesticides, technology based on natural resources has immense potential wherein exploring plant beneficial microorganisms is one such novel approach (Sharma *et al.,* 2012). Significant progress has been made worldwide in research for identification of efficient and aggressive biocontrol agents and exploited in disease management programmes due to their persistent nature in soil, phyllosphere, rhizosphere, high multiplication rate, easiness in handling and mass multiplication without any adverse effects on non-targeted organisms (Bora *et al.* 2016b). Voluminous literatures on rhizospheric antagonists are available which signifies its implications on plant health management in horticultural crops. Many microbes residing inside plants have (Popularly known as endophytes) caught researchers' attention in recent times

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due to their ability to suppress plant pathogens (Bora *et al.* 2019).

Microbial bioagents : The background

Both rhizospheric and endophytic microbes hold greater promise as sustainable option for plant health management in horticultural crops. Some of the plant beneficial microbes used as bioagent facilitate crop production through disease suppression as well as enhanced plant growth. *Trichoderma* spp. fluorescent Pseudomonads, *Bacillus* spp. etc. are established as plant growth promoter and commercially used as growth enhancer too (Bora *et al.* 2013). Horticultural crops including vegetables, fruits and medicinal plants harbor many endophytic microbes and many of them have been reported to possess antimicrobial properties. Compant *et al.* (2011) reported that bacterial genera *Pseudomonas* and *Bacillus* isolated from the interior of flowers, fruits and seeds of grapevine showed antagonistic property against pathogenic microbes.

Microbial biological control agents (BCA) provide protection to plants through different modes of action, viz. direct antagonism against the pathogens and priming host defense against pathogenic attack (Conrath *et al.* 2015). This has been resulted from a microbe-plant cross talk that involves an array of bioactive metabolites produced by the antagonist (Woo *et al*. 2006; Alfano *et al.* 2007). BCAs 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, Singh *et al.* 2014).

Fluorescent *pseudomonads* such as *Pseudomonas fluorescens* and *P. putida* have been reported to exhibit biocontrol efficacy against a wide range of phytopathogens. They are aggressive colonizers of the rhizosphere of various crop plants and their antagonistic activity is attributed to antibiotics, siderophore production and ability to compete for nutrition or space beside*s* producing an array of antifungal metabolites and lytic enzymes (chitinase and glucanase) that inhibit the mycelial growth of pathogenic fungi (Bora and Dek*a* 2007, Bora *et al.* 2016a). *Bacillus* spp. also produce a wide range of antibiotics effective against many plant pathogens. These bacterial bioagents are effective against many bacterial and fungal pathogens (Bora and Bhagabati 1999, Bora *et al.* 2019). *Trichoderma* spp. (*T. viride, T. harzianum, T. koningi, T. hamatum, T. polysporum* and *T. longibrachiatum*) are reported successful against many soil borne diseases, employing an array of mechanisms, viz. hyperparasitism, competition for nutrients, antibiosis and induction of host defense mechanism in plants (Harman *et al.* 2004).

The plant benefial microbes offer priming to the host plant through induced systemic resistance (ISR) regulated through salicylic acid independent mechanisms. However, PGPR triggering an SA-dependent type of ISR resembling pathogen induced SAR have also been reported. *P. fluorescens* strain WCS374 applied in the greenhouse grown radish indiced ISR and suppressed Fusarium wilt disease and also increased yield in radish (Pieters *et al.* 2014).

Microbial interventions and disease management

Fruit crops: Some of the destructive fruit diseases causing huge losses to the fruit industry worldwide include apple scab, mango malformation, guava wilt, fire blights, *Fusarium* wilt of banana, brown rot of stone fruits, crown gall, downy and powdery mildews etc. (Singh 1996, Bora and Deka 2014). Significant progress have been made for biological management of these diseases through BCAs like *Trihcoderma* spp., *Gliocladium* spp*, Verticillum* spp., *Bacillus subtilis*, *Agrobacterium radiobacter., Pseudomonas* spp. (Rishbeth 1988). Successful management of crown gall pathogen *A. tumifaciens* through microbial strategy is a classic success story. The excellent control of bacterial crown gall disease of grapevine, pome and stone fruits have been achieved worldwide by using strain of K84 and its derivates K 1026 of *Agrobacterium radibactor* (Bell *et al.* 1995, Thind 2017). These strains produce Agrocin 84 that kill *A. tumefaciens* and prevents the disease from further spread.

Microbial antagonists are more suited for control of soil borne pathogenic genera (*Pythium, Phytophthora, Rhizoctonia, Fusarium* and *Sclerotium*), where *Trichoderma* spp and *Gliocladium* spp., *P. fluorescens* are the most extensively used. *T. harzianum, T. viride, T. koningii*, *Pseudomonas putida* and *Mycothecium roroidum* provided an effective control of *Phytophthora* infestation in citrus (Menge 1993). Various fungal antagonists, *Ophisotoma* spp, *Chaetomium* sp, *Aureobasidium* sp and *Phoma* spp. inhibit the growth of the apple scab fungus (Outmet *et al.* 1997). While other antagonists such as *B. subtilis* and *T. koningii* have been also used for controlling apple canker (Lima *et al.* 1994).

Fusarium wilt in banana is a major concern for banana industry due to the devastating nature of the pathogen *F. oxysporum* f. sp. *cubense.* Management of Race 1 of the pathogen have been successfully addressed by several workers using strains of *Trichoderma* spp (Baruah *et al.* 2018). Anuratha *et al.* (1990) reported management of Moko disease, another disease of banana caused by bacterial pathogen *R. solanacearum* through application of *P. fluorescens* and *B. subtilis*. Citrus canker caused by *Xanthomonas citri* pv. c*itri* is considered as another economically important disease as it affects the marketability of fruits. *T. viride* and *P. fluorescens* based formulation showed significant disease reduction (72%) with enhanced fruit yield when applied as soil application and foliar spray (Saikia 2018). *B. subtilis* and *P. fluorescens* significantly lowered the disease symptoms of citrus mal secco disease (*Phoma tracheiphila*) and the stem inoculated sour orange seedlings maintained higher population of these BCAs of the host plant (Wilhelm *et al.* 1998). Satisfatory progress has been made in addressing apple scab (*Venturia inaequalis)* management through microbe-based strategies. Burr *et al.* (1996) reported that isolate of *Pseudomonas syringae* (isolate 508) prevented conidial germination and effectively suppressed apple scab pathogen to a level comparable to the fungicide captan. Efficacy of another antagonistic isolate, *Cladosporium cladosporioides* H39 was demonstrated by Kohl (2015) under field condition of organic apple orchard where 48-92% reduction in apple scab incidence was observed.

Besides fungal and bacterial bioagents, AM Fungi are also being explored against many fruit diseases. In an experiment papaya seedling treated with AM fungi *Glomus mosseae* and *G. deserticola* significantly suppressed the foot rot caused by *Pythium aphanidermatum* and also enhanced the seedling growth (Olawuyi *et al.* 2013). Combined treatment of melon seedlings by *Glomus intraradices* and *T. harzianum* provided a greater control against *Fusarium* wilt as compared to *T. viride* treated alone (Medina *et al.* 2016).

Vegetable crops: Ecofriendly management of vegetable disease through microbe based strategy has been quite successful and being practised in organic and IDM practices. Bacterial wilt (*R. solanacearum*), fungal wilt (*Fusarium* spp.), damping off and root rot (*Pythium* spp, *Phytopthora* spp, *Rhizoctonia solani*), anthacnose (*Colletotrichum* spp) etc. are few examples of diseases with successful management through different bioagents under field condition (Bora and Deka 2007). Bioagents have been explored against *R. solanacearum,* a pathogen enlisted among top 10 economically important bacterial pathogen with unusually wide host range that includes solanaceous vegetables. Bora *et al.* (2016c) reported formulation of *P. fluorecens* and *Trichoderma* spp. combination to reduce percent wilt incidence with increased yield attributes in brinjal and chilli. Few new and uncommon microbes, viz. *Chrysobacterium indologenes, C. luteola, Ralstonia pickettii, Streptomyces rochei* are reported to show inhibitory effect against the pathogen (Yuliar *et al.* 2015). However, their mode of action and risk assessment needs further investigation. Hydroponically grown vegetables, also face pathogenic challenges that can be addressed through bioagents. Khan *et al.* (2018) reported that consortia of *T. viride* and *P. fluorescens* could significantly reduce root rot incited by *F. oxysporum* f sp. *lectucae* with enhanced growth parameters in hydroponically grown lettuce. Utilisation of different microbial bioagents for successful control of various diseases of vegetable crops have further been summarised (Table 1).

Spices, plantation and medicinal crops: With the growing demand of organic spices, tea, coffee etc., biocontrol has become inevitable as disease management in these crops. *Trichoderma* spp. are widely used against many diseases of spice crops including ginger, turmeric, pepper, cardamom, cumin, turmeric etc (Chattopadhyay *et al.* 2018). *T. harzianum* (Mass multiplied on wheat bran preparation in 1:1 ratio and applied at 50g per vine) was reported promising solution for the management of foot rot in black pepper (Jahagirdar and Siddaramaiah 2000). In tea, microbial bioformulations are predominantly used against diseases and insect pests. Some successful application of bioagents in medicinal, spices and other horticultural crops have further been enlisted (Table 2).

Post-harvest diseases

Exploiting the immune capacity of plants has been described as a plausible route to prevent post-harvest diseases, since plants have evolved the capacity to sensitize their immune system for a better expression of induced defence mechanism (Conrath *et al.* 2006). Induced post-harvest resistance through the use of safer chemicals from biological origin, and physical means have also been reported (Petriacq *et al*. 2018). Quite strikingly, however, more than a third of fruit and vegetables fails to reach the customer due to infections with pathogenic microbes (Oerke *et al.* 2004). Post-harvest losses can dramatically impact fruit production and quality (Romanazzi *et al.* 2016), resulting in losses of an average of 22.5% of the yield in developing countries. This phenomenon is known as priming of defence

Table 1 Biological control agents used against some popular diseases of vegetable crops

Vegetable	Disease and pathogen	BCA	Reference
Tomato	Fungal wilt (*F. oxysporum* f.sp. *lycopersici*) Bacterial wilt	*Trichoderma* spp. *Bacillus amyloliquefaciens*	Someshwar *et al.* (2013) Wei *et al. (* 2011)
Tomato	Early blight (*Alternaria solani*)	*T. virid*	Roopa *et al. (* 2014)
Chilli	Fruit rot (*C. dematium*) Root rot *(R. solani)*	T. viride T. viride and B. subtiis	Belge *et al. (* 2009) Ahmed *et al*. (2003)
Brinjal	Vertcilium wilt	*B. cereus*	Li *et al.* (2008)
Okra	Root rot (*R. solani*)	*P. fluorescens*	Adhikari *et al.* (2013)
Cucumber	Damping off (*Pythium ultimum*)	*B. subtilis* *P. fluorescens*	Khabbaz and Abbasi (2014)
French bean	Anthracnose *(Colletotrichum lindemuthianum)*	*T. viride, T. harzianum* and *P. fluorescens*	Amin *et al.* (2003)
Cauliflower	Black rot *(Xanthomonasa campestris* pv. *campestri*s)	*Bacillus* spp	Luna *et al*. (2002)

Table 2 Successful bioagents against major diseases of spices, plantation and aromatic crops

Crop	Disease and pathogen	BCA	Reference
Ginger	Rhizome rot *(Pyuthium aphanidermatum)*	*T. viride* and *P. fluorecens*	Sarma (2000)
	Bacterial wilt (*R. solanacearu*)	*T. harzianum* and *P. fluorescens*	Bora *et al.* (2016 a)
Cardamom	Clump rot (*P. vexans, R. solani*)	*Trichoderma* spp	Reddy (2014)
	Phytophthora rot (*Phytophthora* spp.)	*Trichoderma* spp.	Thomas (2003)
Black pepper	Foot rot (*Phytophthora capsici*)	*T. viride, T. harzianum* and *Glomus fasciculatum*	Dhanapal and Thomas (2009)
Fennel	Damping off and seedling rot (*F. oxysporum* f. sp. *solani, R. solani*, *Pythium* spp)	*Trichoderma sp.* and *Bacillus subtilis*	Gebily (2015)
Vanilla	Root rot (*Phytophthora meadii)*	*T. harzianum* and *P. fluorecens*	Reddy (2010)
	Wilt (*F. oxysporum* f. sp. *vanillae*)	*T. harzianum* and *P. fluorescens*	Athul *et al.* (2012)
Tea	Grey blight	*T. viride, B. subtilis* and *P. fluorescens*	Deb *et al.* (2017)
	Phomopsis canker *(Phomopsis theae)*	*Gliocladium* virens and *T. harzianum*	Ponmurugan and Baby (2007)

and is understood as an adaptive part of induced resistance (Mauch-Mani *et al.* 2017).

Devastating post-harvest fungal pathogenic genera such as *Penicillium* and *Botrytis* cause post-harvest fruit decay in considerable proportions. *P. digitatum* and *P. italicum* are responsible for green mould (or green rot) in citrus fruit and *P. expansum* cause blue mould which significantly affects orchard fruits (Kumar *et al.* 2018). The mildews caused by *Plasmopara viticola* and *Erysiphe necator*, and rots caused by *A. alternate*, also result in considerable post-harvest decay of grapes and tomatoes, respectively. The infections with *Clavibacter michiganensis* sub sp. *Sepedonicus* causing ring rot in potato and subsp. *michiganensis*, causing bacterial wilt and canker of tomato (Eichenlaub and Garteman 2011) causing quite a substantial economic losses are reported. The other diseases, such as bacterial spot in tomatoes and peppers caused by *Xanthomonas axonopodis,* also result in serious damage to the fruits (Basim *et al.* 2004) Yeast cultures are the most preferred option of biocontrol of post-harvest diseases against fungal pathogens (Wilson and Wisniewski 2003). The strains such as *Pichia membranefaciens* resulted in the up or down regulation of 25 proteins, which included antioxidants and PR proteins in peach fruit (Chan *et al*. 2007). Similarly, strains of *Aureobasidium pullulans* and *Cryptococcus laurentii* induced resistance in cherry tomatoes and peach, respectively, through the activation of host antioxidants metabolism. In addition, induced resistance activity triggered by *A. pollutants* has been linked to the accumulation of GLU, CHI and PAL enzymes (Ippolito *et al.* 2000). The bacteria, *Bacillus cereus* was proven effective to control anthracnose disease caused by *Colletotrichum acutatum* in loquat fruit through induced production of phenolic compounds and H_2O_2 (Wange *et al.* 2014). Priming-based induced resistance has several benefits: i. it does not trigger a direct activation of defences therefore does not incur in major costs in growth and development; ii. it provides broad-spectrum resistance; and iii. it is long-lasting and can reach the fruiting stage (Petriacq *et al.* 2018).

Microbial bioformulations and disease management: Our experiences of quality biopesticides with aggressive strains and their survival during storage are two key factors governing success of biological control in organic agriculture as well as IDM Programmes in conventional agriculture. Assam Agricultural University, the pioneer in biopesticide research in NE India greatly contributed to microbe mediated pest management through development of a few bioformulation, viz. Bioveer *(T. viride),* Biofor Pf2 *(P. fluorescens+ T. viride),* Biozin PTB (*P. fluorescens+ T. harzianum + B. brevis*)*,* Biotime (*P. fluorescens+ T. viride+ Metarhizium anisopleae*)*,* Biozium *(T. harzianum),* Biogreen-5 (*P. fluorescens+ T. viride+ B. subtilis + M. anisopleae + B. thuringiensis*)*.* A bioformulation carrying antagonists as well as entomopathogens could be more sustainable and cost effective solution from the farmer's perspective as it would address both insect pests and disease problem simultaneously. Compatibility assay of different antagonistic microbes and entomopathogens such as *T. viride, P fluorescens, Bacillus* spp and *M. anisopleae* have been carried out at Assam Agricultural University (Bora and Deka 2007, Deuri 2013*,* Khan *et al.,* 2016). Based on these studies we developed the consortia formulations and different field studies established that combination of different bioagents observed the higher magnitude of disease reduction as compared to single bioagent.

Microbial bioformulations developed at AAU, Jorhat have been found successful against some pests and diseases of economically important horticultural crops in Assam and NE India, *viz.* banana (Dutta 2015), tea (Deb *et al.* 2018), turmeric and ginger (Deuri 2013, Lalfakawma *et al*. 2014). Sarkar (2015) used aggressive strains of four different bioagents *Trichoderma viride, T. parareesei, M. anisopliae* and *B. thuringiensis* against *R. solanacearum* causing bacterial wilt of *Bhoot jolokia* (*Capsicum chinense*) and recorded significant reduction of wilt incidence (83.87%) with highest yield (9.84 t/ha). Record of yield enhancement as well as suppression of bacterial wilt incidence in *Bhoot jolokia* (*Capsicum assamicum*) using consortia of *Trichoderma* along with some PGP microbes was also made by Bora *et al*. (2016a), bacterial wilt in tomato by Nath *et al.* (2016) and Bora *et al.* (2016b). Bora *et al*. (2016c) also recorded *Rhizoctonia* root management in chilli (*Capsicum frutescens*) using *Trichoderma*-based microbial consortia. Khan *et al.* (2016) used microbial consortia containing *T. viride* against wilt causing pathogen *Fusarium oxysporum* f. sp. *lactucae* in hydroponically grown lettuce (*Lactuca sativa*).

Gogoi *et al*. (2017) observed that soil application of Biofor-pf-2 (containing *T. viride and P. fluorescens*)@ 100 g/pot showed least root rot incidence (44.40%) of patchouli caused by *Fusarium solani* as compared to inoculated control (100%). Seed treatment of vegetable seeds with nano-bio formulation of *Trichoderma asperellum* and chitosan NP suppressed the growth of *F. oxysporum, Sclerotium rolfsii* and *Rhizoctonia solani* as compared to recommended chemicals (of patchouli caused by *Fusarium solani*).

Future line of research

Microbes from different genera of rhiospheric and endophytes have shown some precision in disease management. However, root colonization efficiency of effective microbes should also be further studied to increase their effectiveness as bioinoculants via. consortia inoculants as priority research. The major thrust area in years to come would be: concentration on development of novel formulation, understanding the impact of environmental factors on population dynamics of bio-control agents, mass production of bio-control microorganisms and use of biotechnology and nano-technology in improvement of bio-control mechanism and strategies and development of a multi-utility bioformulation by integrating micronutrient supplements. Micronutrients can enhance disease suppression indirectly by stimulating indigenous populations of antagonistic microorganisms (Huber 2007) and directly by activating systemic acquired resistance in host plant systems (Dordas 2007). Exploration of antagonists and bio-fertiliser agents as consortia can significantly reduce crop diseases in field along with enhanced growth and yield (Srivastava *et al*. 2010).

The researches around the world are showing concern about the probable risks of biopesticides also, correlating the facts that biopesticides showing toxic property against target pests may also transfuse these properties to plant physiology and biochemistry. Therefore, toxicological studies of microbial biopesticides are now essential through accumulation of biological characteristics and chemistry data of the microbes used for preparation of biopesticides.

Role of AMF in providing an additional resilience to rhizosphere's ability of carbon accredition within rhizosphere and associated development of plant's antioxidant profile as a defense mechanism (Wu *et al*. 2013) should divert the research studying strong mycorrhizal dependency of fruit crops. Rhizosphere specific AMF-based microbial consortium would add a new dimension in providing newer options for raising the productivity potential of fruit crops as well as enhancing plant immune system to add the required physiological preparedness to the plant fight against pathogens. Researches are using different approaches for screening rhizobacteria to select effective PGPR including promotion of root/shoot growth under genotobiotic condition, *in-vitro* production of plant growth regulators/biologically active substances and assessing of deaminase activity of the rhizobacteria (Zahir *et al*. 2003). Fungal biofertilizer agent arbuscular mycorrhizal applied along with *T. harzianum* based formulation could lead to a significant reduction of disease along with enhanced growth and yield of crops like tomato in field (Srivastava *et al*. 2010).

A system-based approach emphasizes the incorporation of different practices nvolving: (i). Prevention of introduction and spread of pathogens; (ii.) Reduction of incoculum to the point, manageable under natural system; (iii.) Promotion of disease suppressive soil microbial communities and (iv.) Use of an integrative approach to interfere with life cycle of pathogens and minimising the disruptive actions of pesticides/biopesticides. These approaches must go hand-in-hand while addressing various diseases in horticultural crops in order to maintain sustainable production of zero risk horticultural crops in the days to come.

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