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Biological control of foot rot (*Phytophthora capsici* Leonian) disease in black pepper (*Piper nigrum* L.) with rhizospheric microorganisms

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ABSTRACT

Foot rot disease, also known as “quick wilt” caused by *Phytophthora capsici* Leonian in black pepper (*Piper nigrum* L.) often leads to mortality of the infected plants. No cultivated variety is completely resistant, though a few varieties show field tolerance. Plants in all growth stages are susceptible to the disease and the loss incurred will be huge when the plants die. Copper-based fungicides are mainly used for managing foot rot incidence in commercial plantations. Now-a-days organic black pepper has high demand in the consumer markets worldwide. Though fungicides such as copper hydroxide and copper oxychloride are permitted to be used in a restricted scale in organic agriculture, it is always advisable to avoid them as far as possible. Biological control using beneficial and antagonistic rhizospheric microorganisms is a promising alternative for controlling foot rot incidence. Both fungal and bacterial antagonists are used as biological control agents against the disease, and important among them are *Trichoderma* spp. and *Pseudomonas fluorescens* isolates. Biological control with rhizospheric microbial agents when combined with good agricultural practices, including phyto-sanitation, have shown promising results in managing the foot rot disease. Most of the rhizospheric antagonists act as plant growth promoters also. A review on the attempts made on the biological control of foot rot disease with rhizospheric microbial agents is presented here.

1. Introduction

Black pepper (*Piper nigrum* L.), popularly known as the “king of spices”, is one of the oldest and the most popular spices in the world. It is indigenous to the Malabar Coast of India, and its cultivation has spread to several other tropical countries like Indonesia, Malaysia, Sri Lanka, Thailand, China, Vietnam, and Cambodia (Nair, 2004). Black pepper is a plant of humid tropics requiring high rainfall and humidity. The hot and humid climate prevailing in sub mountainous tracts of Western Ghats is ideal for black pepper cultivation (Sivaraman et al., 1999; Thangaselvabal et al., 2008). Black pepper flourishes well in red laterite soil, however, it can be grown in a wide range of soils with a pH of 5.5–6.5 (IISR, 2015).

Foot rot disease caused by the soil borne oomycete pathogen *Phytophthora capsici* Leonian is the most devastating disease of black pepper that affects its production and productivity (Anandaraj and Sarma, 1995; Nair, 2004). Rhizospheric microorganisms, especially bacterial and fungal antagonists have been used as successful biocontrol agents against foot rot disease of black pepper. Selection of antagonists having potential biocontrol ability from the rhizosphere of healthy black pepper plants and screening them under *in vitro* and *in vivo* systems involves several steps before a sound recommendation is made for their use in the field conditions (Anith et al., 2002, 2003). A generalized schematic flow chart that depicts procedures involved in the selection, screening, testing for bio-efficacy and field evaluation with respect to rhizospheric bacteria is given in Fig. 1. The same methodology could be employed for selection of fungal biocontrol agents also. A review of research work done with different rhizospheric microbial biocontrol agents for suppression of *P. capsici* incidence and the strategies employed for foot rot disease management of black pepper is attempted here.

2. Foot rot disease of black pepper

In India, the first case of *Phytophthora* disease incidence in black

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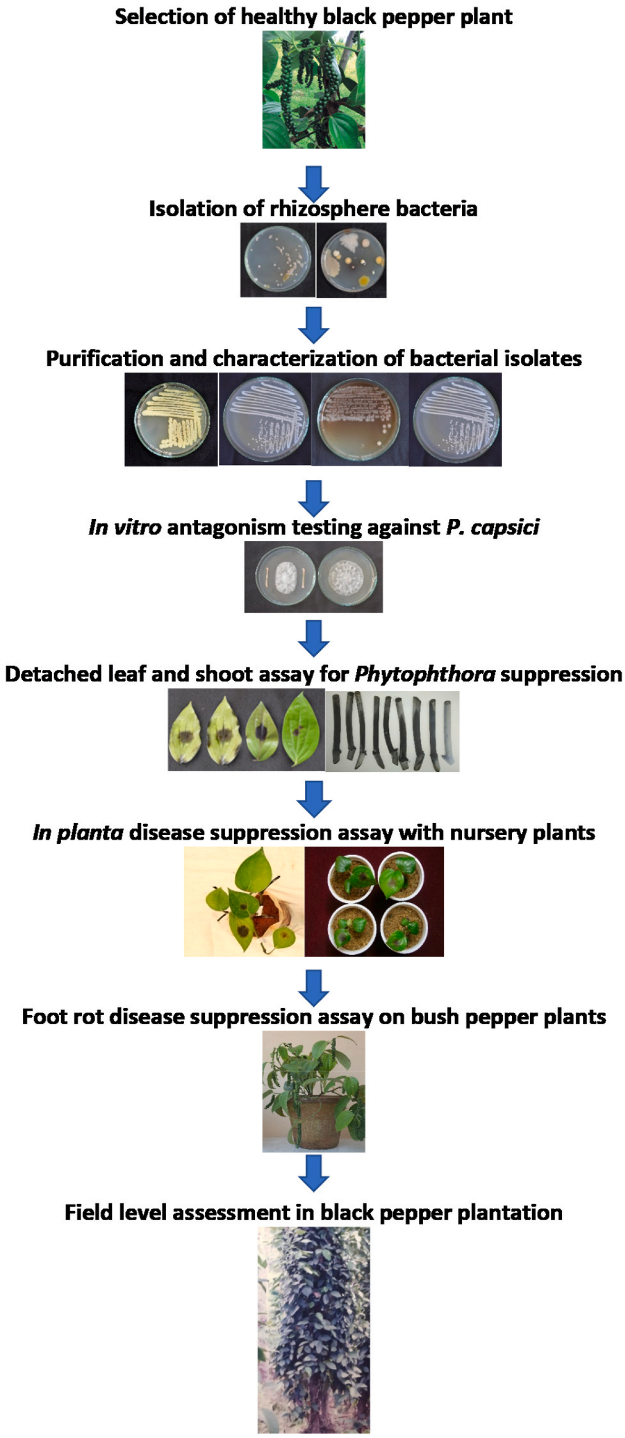

Fig. 1. Steps involved in the development of antagonistic bacteria as biocontrol agents against foot rot disease of black pepper (Reference: Anith et al., 2002, 2003, 2021; Kollakkodan et al., 2021; Paul et al., 2021; Nysanth et al., 2022).

pepper was reported in 1902 (Barber, 1902). Isolation of *Phytophthora* sp from black pepper was reported from Karnataka state in 1929 (Rao, 1929). A further report of the disease incidence was by Samraj and Jose (1966), and *P. parasitica* var. *Piperina* was reported as the incitant. For some time, controversy existed among scientists in the taxonomic positioning of the black pepper isolate, naming it as *P. palmivora* (Holiday and Mowat, 1963) and *P. palmivora* MF4. Later the taxonomic position of the pathogen causing foot rot disease was assigned as *P. capsici* Leonian (Tsao, 1991).

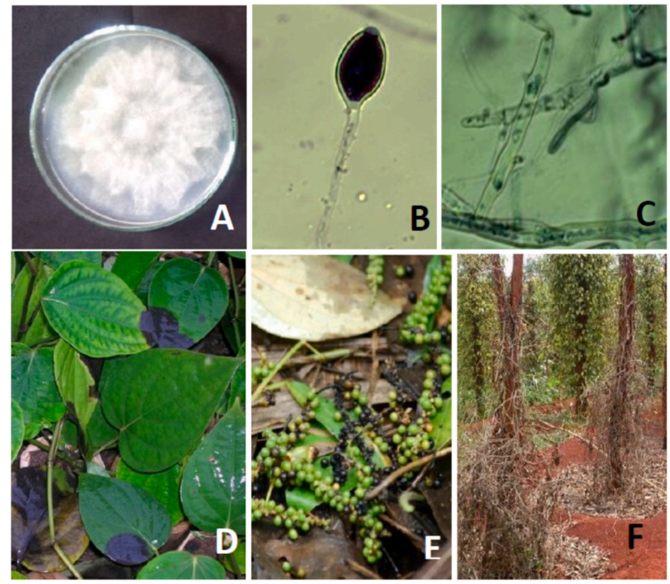

Fig. 2. **A**: Growth of *Phytophthora capsici* on potato dextrose agar. **B**: Lemon shaped sporangium of *P. capsici*. **C**: Hyphae of *P. capsici*. **D**: Foliar infection by *P. capsici* showing lesions. **E**: Infection of *P. capsici* on the berries. **F**: Infected black pepper plants showing typical "quick wilt" symptoms.

The oomycete pathogen, *Phytophthora capsici,* is known to produce different propagules that help in easy dispersal and extended infection. Among different propagules, zoospores generally survive for short periods of time, probably from days to weeks. However, sporangia and hyphae can survive in the soil for 4–8 weeks. Oospores are the primary overwintering propagules and persist for longer periods (Larkin et al., 1995) (Fig. 2). The disease is soilborne and has two important phases: aerial phase and soil phase (Sarma et al., 1991). If the collar region of the plant is affected, mortality of the vines occurs within few days, resulting in "quick wilt" symptoms. Slow decline occurs if infection is confined to the feeder roots (Anandaraj et al., 1994). Foliar infection results in large black to brown coloured water-soaked lesions on the leaves and they fall down subsequently. Similar symptoms appear on the berries too (Fig. 2). The pathogen is passively transported to nearby plants either by soil particles to which they adhere or by latent infection on the roots of runner shoots. The tender runner shoots spreading on the ground or the tender leaves at the base of the vine first get infected by soil splashes resulting in rotting of shoots or lesions on leaves with fast advancing margins. The infection gradually spreads from the lower to the upper regions, in a hopping manner through rain splashes (Ramachandran et al., 1988). The pathogen sporulates profusely and releases zoospores in water drops. The main mode of spread of foliar infection is soil splash or rain splash whereas that of the root infection is soil-water and root contact. During rainy season, slugs act as carrier of inoculum and during off season, termites act as passive carriers of soil inoculum (Sarma and Nambiar, 1982). Foliar infection generally occurs during South-West monsoon period in June–July, but during intermittent rain, root infection would continue even up to November due to persistent soil moisture. The deciding factor on productivity and health of the vine has been reported as root loss to regeneration ratio (Sarma and Nambiar, 1982). The vines sustain with available soil moisture during October–December period despite root degradation. Vine death is noticed during March–April, due to depleted soil moisture levels and degraded root system. Foot rot spreads hastily and is difficult to control once the infection spreads. Infected plants may die within 2–3 weeks during rain and neighbouring plants may be infected within months (Anh et al., 2018). Most of the time, incidence of foot rot disease would cluster around infected vines, and it further spreads to uninfected areas leading to the loss of considerable portions of feeder roots of the nearby plants

(Nambiar and Sarma, 1977).

The occurrence of this devastating disease makes the prediction of black pepper prices at the international level extremely difficult. Yields and quality of pepper are significantly reduced by the disease (Hausbeck and Lamour, 2004). Non-chemical management of the disease using various good agricultural practices, phytosanitation, application of organic amendments and biocontrol measures are strongly recommended for achieving profitable organic production of black pepper. Cultivation of disease-tolerant varieties like IISR-Thevam and IISR-Shakthi is also recommended to mitigate the foot rot incidence (Krishnamoorthy and Parthasarathy, 2011). Removing the source of inoculum by phytosanitary measures is considered to be one of the most important steps in the disease management which reduces the initial load of inoculum. Vines with foot rot symptoms must be uprooted and burned at early stages of its progress (Sadanandan, 2000).

As intensive and clean cultivation favours rapid spread of inoculum through soil water and soil splashes, and therefore minimum tillage practices with maintenance of legume or grass cover in the interspaces during the rainy season can be adopted to prevent the root injury and thus the spread of the disease. Shade regulation is another important operation to ensure adequate light penetration and air circulation to reduce humidity (Anandaraj and Sarma, 1995). Heavy shade contributes to humidity build up, especially during monsoon period, and would support the development and further aggravation of disease. Movement of persons from diseased to healthy plantation is also a cause for disease spread and hence it may be avoided (Anandaraj and Sarma, 1995). In addition, maintaining good drainage, if the chance for water stagnation is more, and application of neem cake are effective in bringing down the pathogen inoculum load in the soil (Sadanandan, 2000). Apart from acting as nutrient source for the plants, neem cake supports the saprophytic growth of antagonistic microflora and thereby supresses the effects of *Phytophthora* and nematodes (Anandaraj and Sarma, 1994). Furthermore, addition of various organic mulches around the base improves the soil texture as well as the growth of antagonistic microflora.

3. Biological control of foot rot disease

3.1. Fungal bioagents for the management of foot rot disease

The most effective fungal biocontrol agents recommended for the management of foot rot of black pepper are *Trichoderma* spp. and arbuscular mycorrhizal fungi (AMF), and they have been developed into successful commercial products. Though many other fungal biocontrol agents are tested and found effective, research works to decipher the fundamental biocontrol mechanisms of them against the foot rot pathogen is very scanty.

3.1.1. *Trichoderma spp.*

Trichoderma spp. are ecologically highly successful fungi and have been used as biological control agents on a commercial scale in agriculture (Knudsen and Dandurand, 2014). Currently, *Trichoderma* spp. are widely used to control plant diseases in sustainable integrated disease management systems and many commercial products of them are available in the market as biopesticides or as soil amendments or as plant growth enhancers (Papavizas, 1985; Chet, 1987; Vinale et al., 2008). *Trichoderma* spp. interact with different plant micro-environments such as root, soil and leaf and are abundantly present in almost all type of soils. They dominate over plant pathogenic fungal community by biosynthesizing a wide array of secondary metabolites which limit their growth. Antimicrobial metabolite secretion (antibiosis) (Harman et al., 2004), hyphal interaction and direct penetration on fungal pathogens (parasitism) (Chet et al., 1998; Harman et al., 2004) and activation of plant defense (induced systemic resistance) (Segarra et al., 2009) are the well documented bicontrol mechanisms in *Trichoderma* spp. Yet another mechanism of *Trichoderma* isolates was identified as competition since they rapidly overgrew the pathogen and penetrate the hyphae of the pathogen (Noveriza and Quimio, 2004). All these mechanisms along with their aggressive colonization characteristics make them the most versatile biocontrol agent among others. Based on the knowledge gathered from a number of previous studies, the potential role of *Trichoderma* spp. in controlling foot rot disease of black pepper has been clearly elucidated and established by several workers.

Foot rot disease incidence can be significantly reduced if all the recommended good agricultural practices are followed in a scientific manner (Sarma et al., 1991). The recommended cultivation practices include use of disease-free planting material and chemical control with copper oxychloride soil drenches and Bordeaux mixture sprays. In addition to this, application of neem cake amended with *Trichoderma* (1 kg/vine) would increase population of antagonistic microflora and reduces root infection. An integrated approach for the management of foot rot of black pepper involving the use of fungal antagonists like *Gliocladium virens* and *Trichoderma* spp. to the soil along with foliar spray of Bordeaux mixture, metalaxyl or phosphoric acid was recommended by Anandaraj and Sarma (1995). These integrated management practices with *T. harzianum* were found to reduce disease incidence from 25 per cent to 15 per cent under field conditions (Sarma and Anandaraj, 1996).

In an effort to develop efficient *Trichoderma* isolates for the management of foot rot disease, Rajan et al. (2002) evaluated five strains of *Trichoderma* under field conditions. Two isolates, *T. harzianum* (T. harz-26) and *T. virens* (Tv-12), showed strong disease suppressing ability. *T virens* (Tv-12), was found proliferating very efficiently in the soil. However, when the mixtures of isolates were applied, no additional reduction in infection was observed. It was assumed to be due to competition among the individual *Trichoderma* isolates which might have nullified effects of the poorly colonizing isolate. An *in vitro* study to understand the enzymatic degradation of the cell wall of *P. capsici* by *Trichoderma* spp. revealed the possible role of β-1,3-glucanases, β-1, 4-glucanases and lipase enzyme produced by these antagonists in inhibiting the mycelial growth of the pathogen (Paul et al., 2005a). Even though the application of *Trichoderma* and *Pseudomonas fluorescens* either alone or in combination reduced the foot rot disease incidence of black pepper under greenhouse conditions, application of *Trichoderma* alone was found more effective under natural condition. Besides, application of *Trichoderma* up-regulated salicylic acid production in the root. Das et al. (2019) made an attempt to evaluate novel strains of fungal bioagents from rhizosphere soil of ginger and black pepper for the effective management of fungal diseases of black pepper and ginger pathogens. Four *Trichoderma* isolates were found to be efficient antagonists of fungal pathogens of black pepper and ginger viz., *Fusarium oxysporum*, *Rhizoctonia solani* and *Phytophthora capsici*. Among the four, *T. harzianum* CH1 exhibited significantly superior biocontrol property.

T. harzianum (CKT isolate) was reported as a promising high temperature tolerant culture as it improved the growth as well as supressed the foot rot disease incidence in black pepper plants grown at elevated temperature up to 41 °C maintained under polyhouse conditions (Vithya et al., 2018). This has relevance as elevation of temperature in pepper growing tracts is reported as a major problem. Development of heat and drought tolerant *Trichoderma* strains, for the use in black pepper disease management, through natural selection or by mutation will be an interesting area of research in the coming days.

An investigation to determine the influence of organic soil amendments commonly used in black pepper plantations on the rate of multiplication *of T. harzianum* used for the control of foot rot in black pepper demonstrated that there is rapid increase in the population density of the antagonistic fungus following the addition of organic media like neem cake, coir pith, farmyard manure and decomposed coffee pulp (Saju et al., 2002). This is a relevant point in the conversion of conventional black pepper production system into the organic system.

Several high-throughput attempts have been undertaken to integrate permissible chemicals used in organic agriculture, *Trichoderma* and organic amendments to manage *Phytophthora* foot rot effectively.

Table 1
Mechanisms of action of bacterial biocontrol agents against the black pepper foot rot pathogen *Phytophthora capsici*.

Biocontrol strain/ isolate	Source	Mechanism of action involved	Reference
Fluorescent pseudomonads: IISR-51, IISR-11, IISR-8, IISR-6, IISR-36, IISR-34	Black pepper roots	Production of siderophores, volatile and non-volatile inhibitory compounds and HCN	Paul et al. (2005a)
Pseudomonas fluorescens IISR-6,	Black pepper roots	Production of pyrrolnitrin (Prn),	Paul and Sarma (2006)
IISR-8, IISR-11, IISR-13 and IISR-51		Pyoluteorin (broad-spectrum antibiotics) and volatiles	
Pseudomonas putida 150-A, 269-A, 199-B, 267- C and 214-D	Black pepper rhizosphere	Production of biosurfactants, including rhamnolipids and cyclic lipopeptides (CLP), disrupt the membrane integrity of *Phytophthora* zoospores	Tran et al. (2008)
Pseudomonas aeruginosa ISRBP 35	Internal tissues of roots and stem of black pepper	Antibiosis, out competition of pathogens and induced systemic resistance	Aravind et al. (2009)
Pseudomonas putida IISRBP 25			
Bacillus megaterium IISRBP 17			
Streptomyces sp (98.1)	Forest soil	Production of antifungal metabolites, chitinase and cellulase	Ramkumar et al. (2015)
Bacillus megaterium (BmBP17)	Black pepper roots	Production of pyrazine, 2-ethyl-3-methyl-Pyrazine, 2,5-dimethyl-pyrazine	Munjal et al. (2016)
Enterobacter cancerogenus DB (2) 7	Black pepper roots	Production of volatile bioactive compounds and diffusible compounds	Toh et al. (2016)
Enterobacter cloacae SB (2) 6 *Enterobacter asburiae* DB (2) 9			
Bacillus velezensis RBDS 29	Black pepper rhizosphere	Production of chitinase, protease, glucanase and other biochemical compounds such as pregn-4-ene-3, 20-dione, 17-hydroxy-6-methyl-, bis (O-methyloxime)] disulfide, methyl 1-(methylthio) propyl, propanoic acid, 2-methyl- decyl ester, benzofuranyl derivative; and propanethioic acid, S-pentyl ester, metronidazole-oh and sulfadiazine	Trinh et al. (2019)

Improved survival of black pepper vines was recorded when integrated management of foot rot of black pepper has been adopted which included application of a mixture of neem cake, *T. harzianum*, garlic and mustard seed extract along with mulching of the wet soil with transparent polythene sheets during the summer periods (Hegde and Anahosur, 1998). Eight percent reduction in foot root disease incidence in black pepper was recorded when vines were treated with neem cake mixed with *T. harzianum* @ 50g vine^{-1} along with areca husk and a subsequent plastic sheet mulching followed by three sprays with Bordeaux mixture (Hegde and Hegde, 2010). A substantial reduction in foliar yellowing (14.9%), defoliation (17.99%), death of vines (5.97%) and improved green berry yield was obtained when the vines were treated with potassium phosphonate (0.3%) as spraying (2 L vine^{-1}) and *T. harzianum* 50 g inoculum per vine with 1 kg of neem cake as soil application (Kumar et al., 2012). Recently, a comparison was made on the efficacy of integrated approaches involving potassium phosphonate @ 0.3% as spray (2 L/vine) + 50g of *T. harzianum* (50 g of commercial inoculum) mixed with one kg of neem cake as soil application (T1) and spraying with Bordeaux mixture (1%) @ 2 L/vine drenching with copper oxychloride (0.1%) @ 3 L/vine (T2) for the management of foot rot disease with farmer's practice (T3: Copper oxychloride @ 0.1% as spray once). It was noticed that treatment T1 and T2 were of equal efficacy in suppressing the disease and were significantly superior to farmer's practice (Rathod et al., 2016). Integration of *Trichoderma* with biological, physical and chemical control strategies as mentioned above helps to avoid routine application of high dose of fungicides, environmental problems and production constraints to a greater extent. The influence of abiotic factors such as pH and soil moisture in maintaining the population and proliferation of *T. harzianum* with respect to the survival and multiplication of *P. capsici* in black pepper system was explicated by Bhai et al. (2010). The study concluded that in order to achieve better biocontrol potential of *Trichoderma*, the soil pH should be maintained at 4.5–6.0 and in the case of nursery for planting material production, the moisture level should also be maintained at 10–15%. These conditions would help to facilitate the growth and proliferation of *Trichoderma* which in turn reduce the population of *Phytophthora* considerably.

Label-free quantitative proteomics employed study elucidated the induction of systemic response in black pepper system mediated by *T. harzianum* against *P. capsici*. Observation on defence response in leaves of black pepper in three different pattern of interactions, black pepper × *T. harzianum* (two-way), black pepper × *P. capsici* (two-way) and black pepper × *T. harzianum* × *P. capsici* (three-way) revealed that induction of resistance proteins occurs only in tripartite interaction. *Trichoderma* induced systemic resistance led to a significant up-regulation in the synthesis of ROS-related and defence-related proteins along with the enhancement of ethylene synthesis, isoflavanoid pathway and lignin synthesis proteins (Umadevi and Anandaraj, 2019).

Application of *T. harzianum* and *Alcaligenes* sp strain AMB 8 in combination or alone could reduce the nursery rot disease in black pepper and had significantly improved the root and shoot growth of plants in the nursery. Moreover, combined inoculation had a remarkable influence on the population dynamics of the fungal or bacterial biocontrol agents (Anith and Manomohandas, 2001). Combined application of *T. harzianum* and *Glomus fasciculatum* was found to be effective in reducing the disease mortality caused by *P. capcisi* (Divya, 2002). Repeated application of talc-based consortium of fungal-bacterial Plant Growth Promoting Microorganisms (PGPM) consisting of *T. viride*, *T. harzianum*, *Pseudomonas fluorescens* and *Bacillus megatherium* to the soil resulted in substantial reduction of foot rot infection caused by *P. capsici* (Rini and Remya, 2020).

Trichoderma spp. also have the ability to survive endophytically in black pepper. Two endophytic fungal isolates, *T. viride* and *T. asperellum* isolated initially from the medicinal plant *Azadiracta indica*, were tested against the foot rot pathogen by Shobha et al. (2019). Both of the fungal strains inhibited the mycelial growth of the pathogen. During *in vivo* disease suppression studies *T. viride* performed better than *T. asperellum*.

The research advancement made in the use of *Trichoderma* as biocontrol agent against *P. capsici* causing foot rot disease of black pepper during the past few years has been remarkable. Growers in the black pepper growing areas of Southern India have been consistently using *Trichoderma* on a prophylactic basis in the plantations for several years and the outcome has been highly encouraging.

Table 2
Mechanisms of action of fungal biocontrol agents against the black pepper foot rot pathogen *Phytophthora capsici*.

Biocontrol strain/isolate	Source	Mechanism of action involved	Reference
Trichoderma harzianum (T. harz-26)	Black pepper rhizosphere	Proliferation and survival in soil and root system, Competition for space.	Rajan et al. (2002)
Trichoderma spp.	Soil	Production of mycolytic enzymes (β-1,3-glucanases, β-1,4- glucanases and lipases)	Paul et al. (2005c)
Trichoderma longibrachiatum *Trichoderma viride*	Black pepper rhizosphere	Mycoparasitism	Vijayaraghavan and Abraham (2007)
Aspergillus flavipes	Continuous cropping soil of pepper	Improving the micro ecology of rhizosphere soil	Wang et al. (2015)
Trichoderma atroviride/ petersenii (KACC 40557)	Korean Agricultural Culture Collection (KACC)	Production of metabolites: Erucamide, Dilaurylthiodipropionate, Dioctyl phthalate, 3-Nitropyrrole and Valeric acid	Bae et al. (2016)
Trichoderma virens (KACC 40929)	Korean Agricultural Culture Collection (KACC)	Production of metabolites: Benzoic acid, 3-(3-hydroxy-3-methyl-1-butyn-1-yl)-, ethyl ester, 1,4-Trans-6-methoxyisocalamenene, Methyl palmitate, Dilaurylthiodipropionate, t-Muurolol, Dioctyl phthalate, α-Calacorene, Valerenal and α-Muurolene, Induction of defense-related genes	Bae et al. (2016)
Diaporthe sp. (BPEF11) *Annulohypoxylon nitens* (BPEF25) *Annulohypoxylon nitens* (BPEF38) *Daldinia eschscholtzii* (BPEF41) *Fusarium* sp. (BPEF72) *Daldinia eschscholtzii* (BPEF73) *Fusarium* sp. (BPEF75) *Ceriporia lacerata* (BPEF81) *Phomopsis* sp. (BPEF83)	Stem, leaves and roots of healthy vines of black pepper	Competition, Antibiosis, Mycoparasitism	Sreeja et al. (2016)
Trichoderma asperellum AFP	Soil	Production of volatile metabolites	Das et al. (2019)
Trichoderma asperellum MC1	Coffee husk		
Trichoderma brevicompactum MF1	Forest soil		
Trichoderma harzianum CH1	Wood waste		
Trichoderma harzianum (MTCC5179)	Microbial Type Culture Collection and Gene Bank (MTCC), India	Induction of systemic resistance	Umadevi and Anandaraj (2019)
Cryptococcus magnus (EPT62)	Leaves, fruits and stems of *Piper* spp.	Antibiosis, production of volatile organic compound, hyperparasitism, and the production of β-1,3 glucanase enzymes	Safitri et al. (2021)

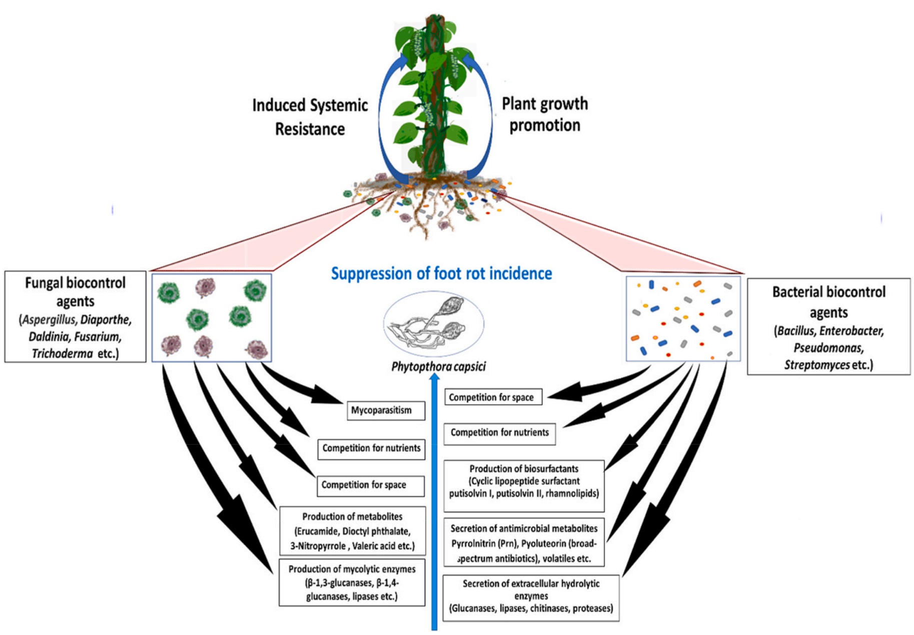

Fig. 3. Mechanisms involved in biological control of foot rot disease in black pepper by rhizospheric fungal and bacterial biocontrol agents.

3.1.2. Fungal biocontrol agents other than Trichoderma

Noveriza and Quimio (2004) conducted an *in vitro* study, and certain mycoflora of black pepper rhizosphere were found to be effective against *P. capsici*. Antagonistic fungi obtained were belonging to fourteen different genera namely, *Mucor, Aspergillus, Trichoderma, Gliocladium, Cunninghamella, Mortierella, Penicillium, Paecilomyces, Geotrichum, Verticillium, Botrytis, Acremonium, Nannizzia* and *Phoma* with dominant mycoflora of three genera, *Penicillium, Paecilomyces* and *Aspergillus*. *Mucor* showed the highest antagonistic activity with percentage inhibition of radial growth of the pathogen by 75.55%. A report on biocontrol efficacy of *Laetiseria arvalis* (Basidiomycota, Aphyllophorales, Corticiaceae) against *P. capsici* was provided by Lokesh et al. (2011). Comparative efficacy of four biocontrol organisms viz., *Trichoderma viride, T. harzianum, Laetiseria arvalis*, and *Bacillus subtilis* against

P. capsici was assessed in a pot culture study. *L. arvalis*, was found superior in suppressing the disease (35.39%) as compared to the bacterial antagonist *Bacillus subitilis* (38.93%). Antagonistic activity of a locally isolated *Aspergillus flavipes* against *P. capsici* was reported by Wang et al. (2015). Inoculation of *A. flavipes* strain into rhizosphere region of both healthy and diseased pepper plants caused drastic decline in the number of bacteria and actinomycetes, whereas remarkable increase in fungal population was noticed. Furthermore, after inoculation, significant increase in soil enzyme activities were also noticed. Based on these results, it was suggested that *A. flavipes* could be developed as a strong biocontrol tool against *P. capsici.*

The use of yeast as potential biocontrol agent is promising but still remains as a less explored area of research. Recently, Safitri et al. (2021) reported epiphytic yeasts obtained from various *Piper* species as effective biocontrol agents against foot rot incidence in black pepper berries. The epiphytic yeast isolates were also found to have the ability to suppress the incidence of foot rot disease in black pepper seedlings through various antagonistic activity namely antibiosis mechanisms, volatile organic compound production, hyperparasitism, and the production of β-1,3 glucanase enzyme.

3.1.3. Endophytic fungi

Since black pepper is vegetatively propagated using the cuttings from runner shoots of the mother plants, efficient stem colonizing endophytes can be made to transfer to the next generation. Abundant occurrence of endophytic fungi has been reported in *Piper* spp. (Munasinghe et al., 2017; Sahil and Nurdiana, 2018). They include even fungi that could produce piperine, the alkaloid produced by the host plant. Sreeja et al. (2016) reported *in vitro* screening of endophytic fungal isolates against *P. capsici*. Maximum mycelial inhibition of 78% was recorded by three isolates BPEF81 (*Ceriporia lacerate*), BPEF83 (*Phomopsis* sp) and BPEF11 (*Diaporthe* sp), followed by 75% inhibition by BPEF73 (*Daldinia eschscholtzii*). Isolates BPEF25 (*Annulohypoxylon nitens*) and BPEF75 (*Fusarium* sp) were equally effective against *P. capsici* (74%). Seven isolates were found positive for antibiosis and four isolates showed mixed interactions (both antibiosis and competition). Mycoparasitism was reported in only one isolate, BPEF83 (*Phomopsis* sp). When assayed for volatile metabolites, after 72 h of incubation maximum growth inhibition was observed by the isolate BPEF38 (34.69%) followed by BPEF25 (31.07%). This work points to the potential of developing endophytic fungi isolated from black pepper as biocontrol agents against the foot rot disease.

Piriformospora indica, the axenically cultivable mycorrhiza like fungus belonging to the order Sebacinales in Basidiomycota, forms mutualistic root endosymbiosis with many plants and improves plant growth and the defensive capacity of several crop plants against bacterial, fungal, nematode and viral pathogens (Athira and Anith, 2020; Daneshkhah et al., 2013; Deshmukh et al., 2006; Lakshmipriya et al., 2017; Sherameti et al., 2008; Varkey et al., 2018; Varma et al., 1999; Weiss et al., 2004). Colonization by the fungus in black pepper and its effects on plant growth have been reported (Anith et al., 2011, 2018). It was also reported that inoculation with the fungus improves growth of bush pepper plants and sets in early flowering. It also improved secondary metabolite production in the berries (Anith et al., 2018). Two bacterial endophytes, *Bacillus velezensis* PCSE10 and *Rhizobium radiobacter* PCRE 10 isolated from the wild relative of black pepper *Piper colubrinum*, and the root endophytic fungus *P. indica* and their combination were tested for plant growth promotion and suppression of *P. capsici* foliar infection in bush pepper plants of the variety Panniyur 1 (Paul et al., 2021). Plants treated with combination of *P. indica* and *R. radiobacter* PCRE10 recorded lowest lesion size (0.43 cm), which recorded 10.41% disease suppression over the pathogen control with the lowest disease index of 0.2. The results also showed that *P. indica* acts as a plant growth promoter and bioprotectant of black pepper.

3.1.4. Arbuscular mycorrhizal fungi

Solarization of nursery mixture and subsequent fortification with AMF, *T. harzianum* and *Gliocladium virens* were found highly effective in producing disease free planting materials in black pepper (Sarma and Anandaraj, 1996). Enhanced crop protection against *P. capsici* due to dual inoculation of arbuscular mycorrhizal fungi and the fungal antagonist, *Trichoderma* sp was also reported by Robert (1998). Anandaraj and Sarma (1995) suggested that the suppressive effect of arbuscular mycorrhizal fungi was due to enhanced root regeneration, nutrient uptake and altered host physiology in mycorrhizal plants. Similar observation was also made by Sarma et al. (1996) and Thanuja (1999). They also reported considerable reduction in root rot, foliar yellowing and defoliation in black pepper due to mycorrhizal symbiosis under field conditions. A similar result was also reported by Thanuja and Hegde (2001) after inoculation with *Glomus. fasciculatum.* There was a negative correlation between the infection of AM fungi on the roots and the intensity of the foot rot disease (Fauziah et al., 2017).

Enrichment of rhizosphere of black pepper in the main field with native, effective AMF strains will have dual benefits in the form of improved plant growth and suppression of foot rot disease incidence.

4. Bacterial bioagents for the management of foot rot disease

Several bacterial antagonists have been tested against the foot rot disease of black pepper. Jubina and Girija (1998) showed that bacterial biocontrol agents could be successfully used for the management of *P. capsici* in black pepper. Anith and Manomohandas (2001) reported the use of an *Alcaligenes* sp along with *T. harzianum* in suppressing the incidence of nursery wilt in black pepper. Though many bacterial bioagents have been tried against the foot rot disease, major success has come with the pseudomonad group of bacteria, especially with strains of the well-known *Pseudomonas fluorescens.*

4.1. Pseudomonas fluorescens and other pseudomonads

To screen antagonistic *Pseudomonas* against foot rot of black pepper a new technique was developed by Anith and co-workers (2002, 2003). An assay was performed on the basis of interaction of the pathogen, antagonist and the host plant, which better resembled the field conditions. Suppression of lesion formation on the excised shoots on artificial inoculation with the pathogen in the presence of the antagonist could be correlated with their biological control potential. In their study, the isolate that gave greater suppression of the lesion development in the assay, *Pseudomonas fluorescens* PN-026R, showed the highest degree of biocontrol efficiency in the *in vivo* test. The strain PN026R has been commercially developed into a talc based formulated product and the Kerala Agricultural University has been recommending the same for the management of foot rot disease of black pepper.

Paul et al. (2005b) found that the strains of fluorescent pseudomonads isolated from black pepper roots showed varying degrees of inhibition of *P. capsici* (36.3%–70.0% by non-volatile metabolites and 2% to 23% by volatile-metabolites). The isolate IISR-51, caused maximum inhibition of *P. capsici* by producing non-volatile and volatile metabolites. Six strains of *P. fluorescens*, which had been found efficient in root-rot suppression in black pepper caused by *P. capsici* were tested for their ability in rejuvenating *Phytophthora* infected black pepper cuttings (Paul et al., 2005c). These strains significantly increased the total root biomass of black pepper plants by 30–135%. Paul and Sarma (2005) reported that strains of *P. fluorescens* increased the levels of defence enzymes viz., peroxidase (PO), catalase, phenylalanine ammonia lyase (PAL) and poly phenol oxidase (PPO) in leaves of black pepper indicating the systemic protection offered to the plant againsts the pathogen. They also reported a relatively higher level of lignification (30–100% over control) in the bacterized roots compared to the untreated plants which also resulted in significant root rot suppression in black pepper. The volatile and non-volatile metabolites produced by the strains of

Pseudomonas spp. inhibited the different stages in the lifecycle of *P. capsici* in terms of mycelial growth, sporangial formation, release of zoospores, and germination of zoospores (Paul and Sarma, 2006).

Fluorescent pseudomonads with the capability to produce biosurfactants have also been isolated from the black pepper rhizosphere in Vietnam and their potential to manage *P. capsici* induced foot rot disease has also been evaluated (Tran et al., 2008). *Burkholderia (Pseudomonas)* sp capable of producing hydrolytic enzymes and bioactive substances was effective in managing foot rot disease and promoting growth of black pepper in Malaysia (Sopheareth et al., 2013; Khairulmazmi and Tijjani, 2017).

Endophytic *Pseudomonas* strains showing disease suppression have also been reported in black pepper. Three black pepper associated endophytic bacteria identified as *P. aeruginosa* IISRBP 35, *P. putida* IISRBP 25 and *B. megaterium* IISRBP 17 were found promising for suppression of *P. capsici*. This work provides the first evidence of black pepper associated endophytic bacteria against *P. capsici* (Aravind et al., 2009).

4.2. Bacillus spp.

Bacillus spp. have been used against different disease-causing species of *Phytophthora* in many hosts. When used against *Phytophthora capsici* attacking black pepper plants, reduction in disease severity with plant growth promotion have been observed (Akgul and Mirik, 2008). Majority of the endophytes with antagonistic property against foot rot pathogen associated with *Piper* spp. have been reported as *Bacillus* spp. (Kollakkodan et al., 2017) which is dealt in detail separately in this review.

4.3. Other bacterial bioagents

Among the other recognized bacterial biological agents, *Streptomyces*, is considered as quite unique group with the remarkable potential to produce an array of bioactive secondary metabolite compounds. Actinomycetes obtained from black pepper rhizosphere and vermicompost showed antagonistic activity against *P. capsici* infecting black pepper. A consortium of *Streptomyces* spp. is found to be more effective than individual isolates (Bhai et al., 2016). An *in vitro* and *in planta* assay was carried out to evaluate the foot rot disease suppressing ability of three actinomycetes isolated from black pepper rhizosphere namely, IISRBPAct1, IISRBPAct25 and IISRBPAct42 by Thampi and Bhai (2017). Even though all the three isolates inhibited the growth of pathogen in both the assays, *Streptomyces* spp. IISRBPAct25 significantly reduced the disease incidence (80.73%). Recently, Anusree et al. (2019) proved *Streptomyces* spp. as efficient agents for the extraction of anti-oomycete and antifungal moieties. Crude culture filtrate of *Streptomyces* spp. isolated from black pepper rhizosphere have been shown to inhibit mycelial growth, whereas diluted culture filtrate inhibited the sporangia formation of *P. capsici*. High resolution UPLC- (ESI)-QToF-MS analysis of the extracts could detect the presence of a variety of anti-oomycete compounds such as brevianamide F, enniatin B, harzianopyridone 1, harzianopyridone 2, isonitric acid E etc. Taking into account the biocontrol ability of *Streptomyces* spp. against the foot rot pathogen *P. capsici*, more research attention needs to be paid to develop them as promising biocontrol agents in the field.

Currently, intensive research is being undertaken to explore a vast number of novel characteristics of *Burkholderia* to fight against foot rot disease. First evidence on the biocontrol potential of *Burkholderia cepacia* against foot rot pathogen was given by Dinesh et al. (2014). Treatment of black pepper plants with *Burkholderia cepacia* (BRB 21) isolated from the rhizosphere of black pepper variety Panniyur-1 significantly reduced the foot rot incidence of plants when challenge inoculated with *P. capsici,* and improved the plant growth under greenhouse conditions. *B. cepacia* (BRB 21) inoculated plants recorded lowest foot rot incidence (32.77%) and this was found on par with chemical treatment (metalaxyl-mancozeb @ 1.25 g L^{-1}).

It has been documented that *Serratia marcescens* secrete cell wall lysing enzymes like chitinase and β-1,3-glucanases. Cellulose fraction of cell wall of *P. capsici* could be lysed by β-1,3-glucanases and β-1,4-glucanases produced by *Serratia marcescens* (El-Tarabily et al., 2000). Of seventy-four PGPR isolates obtained from different varieties of black pepper, three isolates namely, *Burkholderia cepacia*-BRB21, *Pseudomonas aeruginosa*-BRB28 and *Serratia marcescens*-BRB49 showed more than 70% inhibition of *P. capsici* in dual culture plate assay (Dinesh et al., 2014). *Serratia marcescens*-BRB49 inhibited the pathogen with an inhibition percent of 74.4. However, the study reported the poor performance of *S. marcescens* (BRB49) in the greenhouse biocontrol assay.

4.4. Endophytic bacteria

Despite several reports of abundant occurrence of endophytic bacteria in black pepper and their related species, experimentation on their influence on suppression of foot rot disease is limited. Different bacterial strains belonging to various genera have been isolated, characterized and *in vitro* antagonism against *P. capsici* found out by many workers. However, field studies on foot rot disease suppression in black pepper are very limited. Philip et al. (2000) reported the occurrence of bacterial endophytic colonization in black pepper and other *Piper* spp. Kulkarni et al. (2007) have reported the presence of four different endophytic bacteria in tissue cultured plantlets of black pepper. Both the above reports described endophytic bacteria as contaminants in tissue cultured system and therefore the aim was not to characterize and use them for biocontrol activity. Aravind et al. (2009) isolated 74 endophytic bacterial isolates from different varieties of black pepper. All the isolates were screened for biological control properties against the foot rot pathogen. Three isolates, *Pseudomonas aeruginosa* IISRBP 35, *P. putida* IISRBP 25 and *Bacillus megaterium* IISRBP 17 were effective in *Phytophthora* suppression in a multilevel screening procedure and they recorded more than 70% disease suppression in greenhouse trials. The isolate *P. putida* IISRBP 25 was able to induce expression of defense genes in black pepper (Agisha et al., 2017). It also produced several volatile organic compounds like 2, 5-dimethyl pyrazine; 2-methyl pyrazine; dimethyl trisulphide; 2-ethyl 5-methyl pyrazine; and 2-ethyl 3, 6-dimethyl pyrazine which inhibited the growth of *P. capsici* and many other fungal phytopathogens (Agisha et al., 2019).

In a study by Ngo et al. (2020), 352 endophytic bacterial strains were isolated from 90 root samples from 30 black pepper farms in three provinces in the Central Highlands of Vietnam. They were screened for antifungal activity and six of them were selected for greenhouse experiments. They were identified as isolates belonging to *Bacillus siamensis*, *B. velezensis*, and *B. methylotrophicus*. Antifungal enzyme activity of the potent isolates showed that they produced high amount of chitinase and protease enzymes. Bacterized seedlings were used for foot rot disease suppression assay in greenhouse conditions and seedlings treated with these bacterial isolates had the lowest rate of root disease (8.45–11.21%) and lower fatal rate (11.11–15.55%) compared to the control group (24.81% and 24.44%). Further to this, these isolates were used by Nguyen et al. (2021) to make different biological formulations and they were tried under field conditions. A formulation containing the strains *Bacillus velezensis* KN12, *Bacillus amyloliquefaciens* DL1, *Bacillus velezensis* DS29, *Bacillus subtilis* BH15, *Bacillus subtilis* V1.21 and *Bacillus cereus* CS30 showed effect against *Phytophthora* and *Fusarium* induced diseases of black pepper. The population density of *P. capsici* was reduced drastically in the root region of black pepper plants under field conditions.

Wild relatives of black pepper also possess endophytic bacteria abundantly. *Piper colubrinum* Link. (Piperaceae), a woody shrub, native to South America is resistant to infection by *P. capsici* and *Radopholus similis*, the causal agent of foot rot and slow decline disease respectively (Ravindran and Remashree, 1998). Zachariah et al. (2005) observed that black pepper grafted on to *P. colubrinum* rootstocks had lesser collar infection caused by *P. capsici*. Kollakkodan et al. (2017) isolated culturable endophytic bacterial communities associated with *Piper nigrum* and *Piper colubrinum*. A total of 26 and 21 isolates were obtained from different parts of *P. colubrinum* and *P. nigrum* respectively. *Bacillus* spp. were found to be predominant among the endophytes. *In vitro* dual culture plate screening for pathogen growth suppression resulted in selection of 12 antagonistic bacterial isolates. Disease suppression on the foliage of plants bacterized with *Rhizobium radiobacter* PCRE10 and *Bacillus velezensis* PCSE10 was more than 70% compared to that in the control plants in a greenhouse trial (Kollakkodan et al., 2021).

Developing a biocontrol system with efficient bacterial endophytic colonizers will be of great practical significance, as bacterization of cut end of the runner shoots, the planting material of black pepper, with a stable bacterial formulation before planting will ensure colonization of the applied bacteria in the internal tissues of the planting material.

5. Formulated bioagents for foot rot management

Usually carrier-based formulations, especially with talc, are used in black pepper plantations, which are applied along with organic manures to the basins of the vines. Currently, many successful products are available in the market with either organic or inorganic carrier materials. A coffee husk-based *Trichoderma* formulation has been reported to be very effective in the control of *Phytophthora* foot rot of black pepper (Sawant and Sawant, 1996). This formulation is widely being used in foot rot affected pepper plantations in south India. A drastic decrease in the population density of *P. capsici* was noticed when it is present in the substrate alongside with *T. harzianum* (Ahmed et al., 1999). Later, in tune with this study, Noveriza and Quimio (2004) reported that formulations in the form of substrate and pellet could reduce disease intensity about 66.7% and 36%, respectively.

Shobha et al. (2019) tested the efficacy of talc-based bio-formulation of strains of *B. subtilis*, *P. fluorescens, T. asperellum* and *T. viride* individually against *P. capsici*. Talc-based bio-formulation of *Bacillus subtilis* conspicuously reduced the foot rot incidence by 20%, whereas *P. fluorescens* showed a reduction of 31% disease incidence compared to untreated control plants (68%). Predominantly, talc-based formulations are used in black pepper plantation for drenching the basins and spraying the foliage. Liquid formulations are preferred by the farmers, but the cost of production of the same restricts the producers supply them to the users at a reasonable price.

6. Field level biocontrol of foot rot using formulated microbial products

For the management of foot rot of black pepper, *Trichoderma asperellum*, *Pseudomonas fluorescence* and native Arbuscular Mycorrhizal Fungi are recommended for application at the time of planting, both in the nursery and in the main field by the Kerala Agricultural University (KAU, 2016). *Trichoderma* is recommended for application around the base of the vine @ 50 g/vine (this quantity is recommended for a substrate containing *Trichoderma* @ 10^{10} cfu/g) with the onset of monsoon (May–June). A second application of *Trichoderma* is to be given during August–September. Commercial formulation of *Trichoderma* @ 1–2 kg is applied to 100 kg of cow dung-neem cake mixture (9:1) and incubated at 7–8 days before application in the field.

Foot rot disease in the plantations can be managed by the application of *Pseudomonas fluorescens* strain PN026, (commercially distributed by Kerala Agricultural University) at the rate of 10 g talc-based formulation mixed with 2 kg of well decomposed farmyard manure or compost in the basin of pepper vine in the field. A 1–2% aqueous suspension of talc-based formulation is recommended for soil drenching and spraying. Drenching the nursery plants immediately after planting followed by one to two sprays depending on the extent of disease occurrence is found to be effective. For managing the disease in the main field, drenching the base of the vines, and spraying the plants with talc-based formulation of *P. fluorescens* at the rate of 10 g/litre during the onset of monsoon is recommended and a second spray may be given, if necessary, during the mid-monsoon period.

7. Conclusion and future perspectives

Foot rot disease incidence in black pepper could effectively be managed with rhizospheric microbial antagonists. However, developing bioagents for biocontrol of diseases of spice crops pose many challenges. One of the reasons is the perennial nature of the crops. Black pepper, which takes three to four years for establishment and regular bearing is the best example for such a scenario. Biological control of foot rot disease in black pepper with microbial agents is also faced with lots of bottlenecks at different stages of their development. They start from the isolation of bioagents, screening them for biological efficiency, and end with the field-use efficacy. Methods to assess the efficacy of the bioagents in young plants, that may ultimately reflect on the final outcome in the fully bearing plants need to be developed. Miniaturized system that uses "bush pepper" would help the researchers to understand the response of the crop with regard to application of bioagents in a shorter period of time.

Development of bioagents having multiple beneficial traits is yet another area with challenging opportunities in plant health management of black pepper. Rhizospheric antagonistic bacteria and fungi use various mechanisms to combat *Phytophthora* infection in black pepper (Tables 1 and 2; Fig. 3). Understanding the specific mechanisms for suppression by the bioagents will help improve the strains through molecular and biotechnological tools. Biological agents possessing multiple disease suppressiveness, with improved colonization capability at different sites of application such as soil, rhizosphere, internal tissues, leaf surface, fruits etc. also assume importance. Another approach could be use of consortium or mixtures of biological agents. However, development of stable compatible mixtures is always a difficult task. User-friendly biological formulations with longer shelf life having high population count, for multiple mode of application are to be developed for commercial use in black pepper plantations.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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