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Biological control agents in the management of Bell pepper nursery diseases: A review

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Abstract

Bellpepper (*Capsicum annum* L.) is the most popular and highly remunerative vegetable crop of India. It is consumed as fresh vegetable or condiment and is grown in cooler parts of India. This crop falls prey to various phytopathogens causing various diseases during different growth stages of crop *viz.* damping off, Phytophthora blight, root rot and fruit rot, Fusarium wilt, frog eye leaf spot, Verticillium root rot, Rhizoctonia root rot, powdery mildew, anthracnose etc. Damping off disease caused by different fungi like *Pythium*, *Phytophthora*, *Fusarium*, *Rhizoctonia*, *Sclerotium*, *Phomopsis*, *Colletotrichum*, etc. in the nursery is the most destructive malady resulting in plant death as seed decay or germination failure, pre and post emergence damping off, vascular wilt and root rot. At present, these soil borne pathogens are managed by application of various fungicides. However, non-judicious use of chemical fungicides and their persistence in soil is leading to phytotoxicity, environmental degradation and posing threat to human health, besides, development of resistance to agrochemicals by these pathogens is of major concern. Hence, an eco-friendly and sustainable strategy is required for managing these soil borne pathogens. Use of biocontrol agents in plant disease management is an emerging approach to counteract the effect of these soil borne pathogens.

Keywords: Biocontrol, pathogens, damping off, soil borne diseases

Introduction

Bell pepper (*Capsicum annum* L.), commonly known as sweet pepper, pepper or capsicum is an important annual herbaceous vegetable crop grown in India. It belongs to nightshade family and thought to be originated in Mexico and Central America (Andrews 1984) [5]. It is one of the most popular and highly remunerative vegetable crops grown for fresh fruits throughout world as it is utilized in variety of ways for home consumptions, catering and industries round the year (Obidiebube *et al.*, 2012) [48]. This crop is widely consumed as fresh vegetable or condiment and used for pharmaceutical and cosmetic purposes (Bosland and Votava, 2003) [11]. Bell peppers are 94 per cent water, 5 per cent carbohydrates, negligible fat and protein. As per the USDA nutrient database, these are rich source of vitamin C, containing 80.4 mg (134%) of the Reference Daily Intake (RDI) in a 100 gram reference amount. Red bell peppers have more vitamin C content than green bell peppers. Their vitamin B6 content is moderate 0.2 mg (11% RDI), with no other micronutrients present in significant amounts.

Bell pepper is grown in India, China, Italy, Mexico, Spain, Romania, Bulgaria, USA, Hungary, Hong Kong and in other European, Central and South American countries. China is the world's largest producer of bell and chilli peppers followed by Mexico, Turkey, Indonesia and the United States of America. India ranks 5th in bellpepper production. India is one of the largest producer of capsicum in world accounting 3,26,000 metric tonnes of production (Anonymus, 2018) [6] grown in an area of 24,000 hectare. Himachal Pradesh, Uttar Pradesh, Jammu and Kashmir, Arunachal Pradesh, Nilgiri hills of Tamil Nadu and Karnataka are primary capsicum growing regions. Some areas produce fruit round the year in green houses including Pune, Bangalore and other parts of Karnataka. Delicious taste and pleasant flavor makes bell pepper a high value crop (Singh *et al.*, 1993; Sreedhara *et al.*, 2013) [67,73]. Bell pepper consumption in India is increasing nowadays and can be attributed to urban consumers. However, its productivity in India is very low compared to western countries. Like other vegetable crops, bellpepper production is also hampered by various abiotic and biotic stresses resulting in poor yield of the crop (Ochoa-Alejo and Ramirez-Malagon, 2001) [49]. Occurrence of several calamitous diseases in bellpepper like damping off, Phytophthora blight, root rot and fruit rot, Fusarium wilt, frog eye leaf spot, Verticillium root rot, Rhizoctonia root rot, powdery mildew, anthracnose etc. (Bose *et al.*, 2002; Gupta and Paul, 2002; Chadha, 2003; Gupta and Thind, 2006) [10, 28, 16, 27] can be witnessed which ruin the crop and quality to the worst level.

Among various devastating diseases, damping off disease caused by different fungi like *Pythium*, *Phytophthora*, *Fusarium*, *Rhizoctonia*, *Sclerotium*, *Phomopsis*, *Colletotrichum*, etc. (Singh, 1987) ^[68] in the nursery is the most destructive malady affecting almost all the commercial cultivars. Damping-off caused by *Pythium aphanidermatum* (Edson) Fitz. in nurseries is a major constraint in capsicum production causing 62 per cent mortality of seedlings (Ramamoorthy *et al.*, 2002) ^[57]. Wilting of capsicum seedlings due to *Fusarium oxysporum* f. sp. *capsici* in nurseries have a great impact on crop yield (Sahi and Khalid, 2007; Abada and Ahmed, 2014) ^[62, 1]. Similarly, *Rhizoctonia solani* also cause several types of damage at nursery stages such as seed decay, pre- and post- emergence damping-off, wire stem and root rot (Mannai *et al.*, 2018) ^[40].

The available literature has been reviewed under the following heads.

1. Symptomatology
2. Causal organism
3. Predisposing factors
4. Perpetuation of pathogens
5. Review of research work on disease management

1. Symptomatology

***Pythium* sp.:** Damping off caused by *Pythium* occurs in poorly drained soils. Symptoms include collapse and death of young seedlings due to rotting of stem at the soil level. Seedlings die in the seed bed. Damping off is a very common disease in nursery beds showing irregular patches. It is responsible for 90% of plant death either as pre or post emergence damping off (Sowmini, 1961) ^[72].

Pre emergence symptom: It causes seeds and young seedlings to rot before they emerge from the growing medium and results in patchy growth. Seeds become soft, rotten and fail to germinate (Whipps and Lumsden, 1991) ^[78]. This fungus may cause root rot of seedlings or kill the seedlings before they emerge (Roberts, 2005) ^[60].

Post emergence symptom: It occurs when seedlings decay, wilt and die after emergence. Newly emerged seedlings are killed by causing a water soaked, soft brown lesion at the stem base, near soil line, that pinches off the stem causing the seedling to topple over. In this case, stems of germinating seeds are affected with characteristic water-soaked lesions formed at or below the soil line (Cram, 2003; Landis, 2013) ^[18, 38]. Seedling stems become thin and tough (commonly known as “wirestem”), which often leads to reduced seedling vigour. Overall, the symptoms on the stem of the seedlings include water-soaked, sunken lesion at or slightly below the ground level and some time also below ground line (i.e., on the roots), causing the plant to fall over (Wright, 1944; Filer and Peterson, 1975) ^[79, 25]. Surviving plants are stunted and affected areas often show uneven growth.

Rhizoctonia solani often cause serious disease in nurseries during April-May when warm humid conditions prevail. Fungus infects both leaves and stems. It can infect seed, preventing germination. Symptoms on young seedlings are similar to those caused by *Pythium* species. Grey sunken spots and mycelial threads appear on the leaves. On the stem, the infection occurs as dark brown lesions which spread both upwards and downwards. New flushes subtending the points of infections gradually droop and dry up. *Rhizoctonia solani* attacks the seedling at or near the soil surface causing necrosis of the cortical tissues of the stem at the base that results in fall of the seedlings (Dube, 2018) ^[22].

Fusarium oxysporum causes wilting of seedlings in nurseries.

Premature loss of cotyledons, developing leaves and basal stem necrosis are the major symptoms (Dube, 2018) ^[22].

Basal wilt caused by *Sclerotium rolfsii* during June to September is devastating disease of nursery. On the leaves, white mycelium is seen at the advancing edges of the lesion. Mycelium later girdles the stem resulting in drooping of leaves beyond the point of infection and in advanced stages, the roots dry up. Small whitish to cream coloured grain like sclerotial bodies appear on the mature lesions.

Phytophthora species viz. *Phytophthora capsici* are usually associated with root rot of established plants but also cause damping off diseases. It enters the roots and causes root rot similar to *Pythium*. Symptoms on the roots appear as rotting of the entire system. Infections are also noticed on leaves, stems and roots of seedlings in the nursery. Dark spots with fimbriate margins appear on the leaves, which spread rapidly resulting in defoliation. Infections on the stem are seen as black lesions which results in blight like symptoms.

2. Causal organism

Pythium

Damping off caused by *Pythium* sp. is very common problem in open fields and greenhouses, where the organism kills newly emerged seedlings (Jarvis, 1992) ^[32]. In capsicum crop two species of *Pythium* have been reported. i.e. *Pythium ultimum* (Trow. Fitz.) and *Pythium aphanidermatum* (Edson). The genus *Pythium* (Domain: Eukarya; Kingdom: Chromista/Stramenopila; Phylum: Oomycota; Class: Oomycetes; Subclass: Peronosporomycetidae; Order: Pythiales and Family: Pythiaceae) is cosmopolitan in its distribution. The most of species are soil inhabitants and are found in aquatic habitats. The pathogen infects large range of hosts (Owen-Going *et al.*, 2002) ^[50] including economically important crops like wheat, mustard, tomato, brinjal, chilli, capsicum, sugarbeet, papaya, tobacco, forest pine trees and grasses (Van der and Niterink, 1981) ^[76], causing notable economic losses to these crops.

Fusarium oxysporum

Fusarium oxysporum (Domain: Eukarya; Kingdom: Fungi; Phylum: Ascomycota; Sub-phylum: Sordariomycotina; Class: Sordariomycetes; Order: Hypocreales; Family: Nectriaceae and Teleomorph: *Nectria*) was reported as the causal agent of damping off and root rot of pepper seedlings (Perez-Hernandez *et al.*, 2014) ^[53]. Symptomatic plants exhibited damping off, necrosis of the primary and secondary roots and sometimes necrotic streaks on the stem. *Fusarium oxysporum*, causes vascular wilt of many other crops, was found in association with wilt disease of *Capsicum annum* (Kraft and Papavizas, 1983; Kucuk and Kivanc, 2003; Siddiqui and Akhtar, 2007) ^[36, 37, 66].

Rhizoctonia solani

Rhizoctonia solani Kuhn. (Domain: Eukarya; Kingdom: Fungi; Phylum: Basidiomycota; Sub-phylum: Agaricomycotina; Class: Agaricomycetes; Order: Cantharellales; Family: Ceratobasidiaceae and Teleomorph: *Thanatephorus*) is an anamorphic soil borne pathogen causing damping off disease and produces a sclerotia. The most common symptoms of *Rhizoctonia* is “damping off” or germination failure of infected seeds. It may invade the seed before it has germinated to cause this pre-emergence damping off or it can kill very young seedlings soon after they emerge from the soil. Seeds that do germinate before being killed by the fungus have reddish-brown lesions and cankers on stems

and roots. It grows vigorously as a saprobe in soil decomposing cellulose. *R. solani* has an extensive host range and causes different diseases on important crop plants in family Asteraceae, Brassicaceae, Fabaceae, Poaceae and Solanaceae (Carling *et al.*, 2002; Ceresini *et al.*, 2007; Mikhail *et al.*, 2010) [14, 15, 43].

Phytophthora capsici

Phytophthora capsici (Domain: Eukarya; Kingdom: Chromista/ Stramenopila; Phylum: Oomycota; Class: Oomycetes; Subclass: Peronosporomycetidae; Order: Peronosporales and Family: Peronosporaceae) is also responsible for causing damping off in nursery of bellpepper (Akgul and Mirik, 2008) [3].

Sclerotium rolfsii

Sclerotium rolfsii (Domain: Eukarya; Kingdom: Fungi; Phylum: Basidiomycota; Class: Agaricomycetes; Order: Atheliales; Family: Atheliaceae and Teleomorph: *Athelia*) also known as mustard seed fungus is a basidiomycete that typically exists as mycelium and sclerotia. The pathogen has a very large host range, affecting over 500 plant species (including tomato, onion, snapbean and pea). The fungus attacks the host crown and stem tissues at the soil line by producing a number of compounds such as oxalic acid, in addition to enzymes that are pectinolytic and cellulolytic (Agrios, 2005) [2].

3. Predisposing factors

- Seedlings are susceptible to infection particularly during first 3 weeks.
- Factors that favour disease development, are pathogen infested soils, high soil moisture (due to over watering or poor drainage), inadequate light, overcrowding, poor ventilation and excess of nitrogen in the soil.
- Nematodes may enhance infection by making wounds that facilitate infection.
- The various damping off fungi have different environmental requirements *Pythium* and *Phytophthora* cause damping off in cool and wet soils while *Rhizoctonia*, *Sclerotium* and *Fusarium* prefer warm and dry soil.

4. Perpetuation of pathogens

Pythium, *Phytophthora*, *Rhizoctonia*, *Fusarium* and *Sclerotium* are known to be important soil borne pathogens causing diseases in nursery as well as mature plants. Among these, *Pythium* sp. is mainly found to be associated with pre and post emergence damping off resulting in collapse of seedlings. *Pythium* and *Phytophthora* are soil inhabitants and form thick-walled oospores for perennation over a longer period in the soil and host debris. *Fusarium* survives in the soil through its resting structure termed as chlamydospores, whereas, *Rhizoctonia* and *Sclerotium* perennates as dormant mycelium and sclerotia (Dube, 2018) [22].

5. Review of research work on disease management:

At present, these soil borne pathogens are managed by application of fungicides with promising results but indiscriminate and non-judicious use of fungicide and their residues are leading to environmental pollution and posing threat to human health, besides, development of fungicide resistance by *Pythium* spp. discourages its use for disease control (Punja and Yip, 2003; Cuthbertson *et al.*, 2010) [55, 19]. Other control measures like host resistance has not yet

become a viable measure as no resistant variety has yet been developed and released against these soil borne pathogens causing damping off at seedling stage (Koike *et al.*, 2000) [35]. Hence, such situations have prompted researchers to look out for alternate strategies for managing the disease. "Food Security" and "Sustainability" is the need of the hour. Nowadays, to increase the agricultural production without deteriorating the natural resources like soil and water is a big challenge. It has become necessary to develop an alternative and sustainable strategy for these chemical fungicides so as to reduce the pollution in the environment as well as to maintain the soil fertility and protect the beneficial organisms. Environmental and consumer concerns have focused interest on development of biological control agents as an environment friendly and sustainable strategy to counteract the effect of these phytopathogens (Dunne *et al.*, 1998) [23], an alternative to the chemical fungicides (Ravikumar, 1998; Biju, 2000) [58, 9], thus gaining importance in modern agriculture.

Suppression of diseases by bio-control agents are result of sustainable balance in interactions among the plant, bio-control agent and the microbial community on and around the plant (Handelsman and Stabb, 1996) [29]. It has been found that phylogenetically diverse microorganisms can suppress the growth of several phytopathogens (Cook, 2000) [17]. Species of *Trichoderma* and various other microflora like *Bacillus subtilis*, *Pseudomonas fluorescens* and *Streptomyces* sp. have been extensively tested and used as biocontrol agent against a wide range of plant pathogens viz., *Pythium*, *Rhizoctonia*, *Fusarium*, *Sclerotium*, *Sclerotinia*, *Alternaria* and *Macrophomina* sp. etc. (Mishra, 2010; Amin *et al.*, 2010; Muthukumar *et al.*, 2010; Muthukumar *et al.*, 2011; Meenakshisundaram *et al.*, 2013) [44, 4, 46, 45, 42].

Among different biocontrol agents, *Trichoderma* is an excellent versatile microbe, characteristically distinguished as green mould fungus and a common saprophyte in soil. It is a free living, filamentous fungi, reproduces asexually and multiplies rapidly on different substrates. It acts as a mycoparasite and capable of producing different antibiotics, antifungal compounds and enzymes, hereby making it ecologically successful. Growth inhibition of phytopathogens by *Trichoderma* metabolites has been well researched (Dennis and Webster, 1971) [21]. Similarly, various fungi and bacteria viz. *Bacillus*, *Pseudomonas*, *Streptomyces*, *Penicillium* and *Aspergillus* are also prolific producers of secondary metabolites that parasitize and suppress the growth of phytopathogens.

Various mechanisms of these rhizospheric biocontrol agents like competition for nutrients and ecological niche, mycoparasitism or hyperparasitism, antibiosis, production of lytic enzymes and siderophores are responsible for suppression of phytopathogens (Nakkeeran *et al.*, 2006) [47]. Restricted colonization of pathogen also depends on nature, quality and amount of inhibitory substances released by the antagonists (Dennis and Webster, 1971; Skidmore and Dickinson, 1976) [21, 69]. For example, hyphal coiling was observed when *Trichoderma harzianum* interacted with *Rhizoctonia solani*. Formation of knob like haustoria inside the hyphae of pathogen resulting in shrinkage and coagulation of cytoplasm of pathogen was also seen during microscopic studies. Only cell wall was clearly visible without cytoplasm (Seema and Devaki, 2012) [64]. Similar malformation in hyphal growth of the pathogen *Rhizoctonia solani* and *Fusarium oxysporum* due to penetration and release of toxic metabolites from *Trichoderma harzianum* and *Trichoderma*

viride was found. A clear lytic area due to antibiotics produced by bacteria *Bacillus subtilis* was also observed (Mahmoud, 2015) [39].

Successful management of damping off caused by *Pythium* species in various crops by application of *Trichoderma* has been previously reported (Jayaraj *et al.*, 2006) [33]. Bioefficacy of fungal antagonists reported that *Trichoderma viride* and *Trichoderma harzianum* significantly inhibited the growth of *Pythium aphanidermatum* responsible for causing damping off disease in chilli (Manoranjitham *et al.*, 1999) [41]. Similarly, Muthukumar *et al.* (2011) [45] obtained eight isolates of fungal antagonist *Trichoderma* sp. from rhizosphere by serial dilution technique on *Trichoderma* selective media and were evaluated *in vitro* for management of damping off of chilli caused by *Pythium aphanidermatum* and found that TVC 3 recorded maximum growth inhibition of pathogen as compared to control. Antibiotic potentiality of various species of *Trichoderma* has been reported for suppressing the growth of *Pythium debaryanum*, causing damping off disease in Chilli (Gomathi *et al.* 2011) [26]. Likewise, *Trichoderma viride* 1433 and *T. harzianum* inhibited the radial growth of *Pythium aphanidermatum*, respectively (Mishra, 2010; Patel *et al.*, 2014) [44, 52].

Similar evidences showed that *Trichoderma* isolate TR 55 obtained from tomato rhizosphere was found to be the most effective isolate against damping off pathogens *Pythium* spp. and *Rhizoctonia solani* Kuhn of tomato followed by other isolates like TR 66, TR 122 and TR 136 (Biam *et al.*, 2019) [8]. Similarly, isolate Tr16, Tr11 and Tr08 of *Trichoderma harzianum* exhibited inhibition of *Fusarium solani* causing Fusarium wilt in chilli (Wani *et al.*, 2014) [77]. Similar evidences of colony reduction of *Fusarium oxysporum* f. sp. *capsici* by different *Trichoderma* species were reported by Sahi and Khalid, (2007) [62]. ANR 1 isolate of *Trichoderma harzianum* inhibited the mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici*, causal organism of wilt in tomato when compared to all other native isolates (Sundaramoorthy and Balabhaskar, 2013) [74].

Biological control agents also stimulate growth of host plant along with suppression of plant pathogens. *Trichoderma harzianum* produces a butenolide metabolite called harzianolide that both stimulates plant growth and induces defence mechanisms (Cai *et al.*, 2013) [12]. Similarly, some bacteria also produces volatiles like 2,3-butanediol, acetoin, aldehydes and ketones which may also play important role in promoting plant growth. Several biocontrol strains are known to produce multiple antibiotics which can suppress one or more pathogens. Pseudomonads are known to produce the antibiotic 2,4-diacetylphloroglucinol (DAPG) may also induce host defenses (Iavicoli *et al.*, 2003) [30].

Bacterial antagonists like *Pseudomonas fluorescens* inhibited growth of *Pythium aphanidermatum*, causation of chilli damping off (Tekale *et al.*, 2019) [75]. Similarly, EBS 20, an endophytic isolate of *Pseudomonas fluorescens* produced the largest inhibition zone and the least mycelial growth of *Pythium aphanidermatum*, causal agent of damping off of chilli under *in vitro* conditions (Muthukumar *et al.*, 2010) [46]. Prasad *et al.* (2017) [54] reported that *Pseudomonas fluorescens* isolate 3 have shown inhibition of radial growth of *Pythium debaryanum*, causal agent of damping off under *in vitro* conditions. Likewise, *Pseudomonas fluorescens* as well as *Bacillus subtilis* significantly inhibited the growth of pathogen *Pythium aphanidermatum* and *Fusarium oxysporum* f. sp. *lycopersici* (Manoranjitham *et al.*, 1999; Basco *et al.*, 2017) [41, 7]. *Pseudomonas fluorescens* isolate Ps1 and *Bacillus*

sp. isolate B1 were found most effective in inhibiting the mycelial growth of pathogens viz. *Rhizoctonia solani*, *Sclerotium rolfsii*, *Fusarium solani*, *F. oxysporum*, *F. pallidoroseum* and *Pythium* sp. under *in vitro* conditions (Dar *et al.*, 2015) [20]. Similar evidences of inhibition of *Phytophthora capsici* by biosurfactant producing *Pseudomonas* isolates were reported by (Ozyilmaz and Benglioglu, 2013) [51]. Similarly, *Pseudomonas aeruginosa* isolate Tr20 inhibited radial growth of pathogen *Fusarium oxysporum* f. sp. *lycopersici* and *A. solani*, respectively (Sachdev and Singh, 2018) [61].

In the same way, *Bacillus subtilis* reduced the radial growth of *Pythium* sp. (Patel *et al.*, 2014) [52]. *Bacillus thuraciensis*, *B. circulans*, *B. polymyxa* and *B. sphaericus* have antagonistic effect on *Pythium aphanidermatum*, inducing damping off in tomato (Jeyseelan *et al.*, 2012) [34]. Abada and Ahmed, (2014) [1] found that *Bacillus thuringiensis* inhibited the growth of *Fusarium oxysporum* f. sp. *capsici*, causal agent of wilt in sweet pepper followed by *B. pumillus* and *B. subtilis* under *in vitro* and field conditions. Mannai *et al.* (2018) [40] reported significant reduction in pre and post emergence damping off of sweet pepper caused by *Rhizoctonia solani* on treatment with *Trichoderma harzianum*, *T. viride*, *Gliocladium virens*, *Bacillus pumilus* and *Bacillus putida*. *Bacillus subtilis* isolate 16 showed the highest per cent inhibition of *Rhizoctonia solani*, causal agent of root rot of chilli followed by BS isolate 30 and the least inhibition of mycelial growth of (12.85%) was observed in case of BS17 compared to check isolate with (38.55%) inhibition of mycelial growth (Rajkumar *et al.*, 2018) [56]. Similarly, when pepper seedlings were inoculated with phosphate solubilising strains of *Bacillus megaterium*, suppression of pathogen *Phytophthora capsici* was observed with an increased yield of the crop (Akgul and Mirik, 2008) [3].

Application of *Trichoderma harzianum* Tr20 and *Trichoderma pseudokoningii* alone and in combination with *Pseudomonas fluorescens* P28 were found effective in suppressing *Rhizoctonia solani* and *Fusarium oxysporum* infecting tomato under greenhouse and field conditions (Rini *et al.*, 2005) [59]. BCAs utilizes same energy sources, nutrients and ecological niches as pathogens and prevents its establishment in environment (Sneh, 1998; Ellis *et al.*, 1999; Card *et al.*, 2009) [71, 24, 13]. BCAs are able to produce iron sequestering siderophores and thus depriving the fungi of iron (Jan *et al.*, 2011; Santoyo *et al.*, 2012) [31, 63].

Hence, use of biocontrol agents in plant disease management is helpful in maintaining the sustainability and its commercialization will open new vistas for entrepreneurs.

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