

Review Article

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Damping Off in Chilli and Its Biological Management-A Review

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ABSTRACT

Chilli (*Capsicum annuum* L.) is one of the most important vegetable crop grown in India for both unripe (green) and ripe (red) fruit. Chilli crop is attacked by several diseases viz. damping-off, wilt, anthracnose, die back, root rot, bacterial wilt etc. Among the fungal diseases, damping-off caused by *Pythium aphanidermatum* (Edson) Fitz. in nurseries is a major constraint in chilli production causing 62% mortality of seedlings and is responsible for 90 per cent of plant death either as pre or post-emergence damping off in the nursery fields and is very common problem in fields and greenhouse, where the organism kills newly emerged seedlings. Traditionally, this disease is controlled by the application of synthetic fungicides like azoxystrobin (strobilurin), fosetyl-aluminum (alkyl phosphonate), mefenoxam and metalaxyl (acylanilide), etridiazole (triazazole), and propamocarb (carbamate) but phytotoxicity by these fungicide residues are major problems. For this reason, a number of restrictions are imposed in the licensing, registering and using of each chemical. Development of fungicide resistance by *Pythium* spp. further discourages its use for disease control. Other control measures like host resistance has not yet become a viable measure. No resistant variety has yet been developed and released against these soil borne pathogens causing damping-off at seedling stage. Hence, such situations have prompted researchers to look out for alternate strategies for managing the disease. One of the key elements of such sustainable agriculture is the application of biological controlling strategies, for plant protection. Biological control has been known since 1874, when Roberts showed the suppressive activity of *Penicillium glaucum* against bacteria and regarded this phenomenon as antagonism. The soil has an enormous untapped potential of antagonistic microbes such as *Trichoderma* spp., *Bacillus* spp., and the fluorescent *pseudomonads*, which show antagonistic effects against soil borne plant pathogenic organisms such as *Pythium* spp.

Keywords

Bacillus, Damping off, *Penicillium glaucum*, *Pythium*, *Trichoderma*

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Introduction

Chilli (*Capsicum annuum* L.) belongs to Solanaceae family and is a native of tropical

America. It is grown throughout the world for its green and red ripe fruits as it is a lucrative crop and forms an indispensable adjuvant almost in every house. India is one of the

major chilli growing countries, producing 1.446 million tonnes of ripe dry chillies with an area of 0.869 million hectares (Anonymous, 2013) and has been the second largest exporter of chilli in the international market, exporting products ranging from dried form of chilli to chilli powder and oleoresins across 90 countries (Muthukumar *et al.*, 2010). India also leads in the context of maximum area covered under chilli cultivation. Chilli fruit is used fresh, cooked, pickled and canned in sauces and as powder for hot spices (Parey *et al.*, 2013). Nadkarni (1927) has reported many medicinal value of chilli. Its paste is externally used as rubefacient and as local stimulant for the tonsils in tonsillitis. It is irritant internally and produces gastroenteritis. It is used with many ingredients for local remedies.

Chilli crop is attacked by several diseases of biotic and abiotic nature leading to great loss to cultivators. Damping off disease is one of them that causes heavy losses to the growers. It is responsible for 90 per cent of plant death either as pre or post-emergence damping off in the nurseries and fields (Sowmini, 1961). These diseases of vegetables and field crops are considered an important limiting factor in successful cultivation of crop plants throughout the world.

Etiology

Causal organism

Most *Pythium* species cause seed rot and damping off of many crops including chilli and tomato (Shah and Burns, 1996). The genus *Pythium* (kingdom: Straminopila; phylum: Oomycota; class: Oomycetes; subclass: Peronosporomycetidae; order: Pythiales and family: Pythiaceae) is a complex genus containing over 200 described species that occupy a variety of terrestrial and aquatic ecological habitats (Dick, 2001),

perhaps the most economically important members of this genus are plant pathogens (Hendrix and Campbell, 1973), many of which have a broad host range and cause losses by both pre- and post-emergence damping off (Erwin and Ribeiro, 1996), as well as reduction in plant growth and yield due to root rot (Plaats-Niterink, 1981). Seeds attacked by this fungus usually fail to germinate whereas seedlings can be damaged in two ways: the roots may rot causing the seedling to wilt and die quickly, or the seedling may be attacked on the stem at the ground line, causing the seedling to collapse.

Pythium aphanidermatum (Edson) Fitz. is causal organism causing damping off of disease in chillies. *P. aphanidermatum* is cosmopolitan in distribution and one of the most common plant parasitic pathogen of a number of other crop plants in warmer parts of the world. *P. aphanidermatum* is also known to cause infection on a wide range of plant species, belonging to different families viz., Amaranthaceae, Amaryllidaceae, Araceae, Basellaceae, Bromeliaceae, Cactaceae, Chenopodiaceae, Compositae, Coniferae, Convolvulaceae, Cruciferae, Cucurbitaceae, Euphorbiaceae, Gramineae, Leguminosae, Linaceae, Malvaceae, Moraceae, Passifloraceae, Rosaceae, Solanaceae, Umbelliferae, Violaceae, Vitaceae, Zingiberaceae (Waterhouse and Waterston, 1964).

Symptomology

Damping off occurs in two stages, i.e. the pre-emergence and the post-emergence phase. In the pre-emergence phase the seedlings are killed just before they reach the soil surface. The young radical and the plumule are killed and there is complete rotting of the seedlings. The post-emergence phase is characterized by the infection of the young, juvenile tissues of the collar at the ground level. The infected

tissues become soft and water soaked. The seedlings topple over or collapse (Agrios, 2005).

Predisposition

The disease is soil borne in nature and practice of monoculture together make the crop prone to disease. The cultivars grown are not resistant to the pathogen which also helps to spread the disease. *P.aphanidermatum* is an aggressive pathogen at high temperature (Gold and Stanghellini, 1985).

Perpetuation of pathogen

Many *Pythium* species are known to be important soil borne plant pathogens (Hendrix and Campbell, 1973). These species are most commonly identified as causal agents of pre- and post-emergence damping-off, leading to poor stands and low crop vigor (Agrios, 2005). Some *Pythium* species can infect mature plants, causing significant damage to yields, such as the species that cause carrot cavity spot (Suffert and Guibert, 2007). *Pythium* species survive in the soil via oospores or sporangia (Hendrix and Campbell, 1973; Plaats-Niterink, 1981). The oospore, a sexual spore, is the primary survival structure of many *Pythium* species because of the thick-wall enables the spore to survive in the absence of a host, even in the presence of unfavorable soil conditions (Dick, 1990). The sporangia and hyphal swellings of *Pythium*, on the other hand, serve as the asexual reproductive structures for many of species (Hendrix and Campbell, 1973; Plaats-Niterink, 1981).

Management

Cultural methods

Cultural management practices are the primary strategies used to address disease

problems (Sullivan, 2004). Site selection, such as choosing land without history of soil borne diseases, and planting in fields with well-drained soil, is important for preventing damping-off (Koike *et al.*, 2000). Abawi and Widmer (2000) reviewed the impact of some cultural practices on soil health management practices in controlling soil borne pathogens and concluded that the cultural practices such as cover cropping and green manure incorporation, use of composts, crop rotation, and tillage practices improve soil compaction, increase drainage, and increase soil temperature, all of which have an impact on the physical soil characteristics as well as increasing diversity of the soil biota and ultimately reducing the pathogen inoculum.

The use of resistant cultivars can be the most important weapon for damping off disease control. However desirable cultivars with resistance to important soil borne diseases are not always available (Koike *et al.*, 2000).

Chemical management

Management of soil borne pathogens has been attempted by many workers/researchers by manipulation of cultural practices and chemicals. However, according to Haware and Kannaiyan (1992) the management of seed borne and soil borne diseases has always been problematic. Satija and Hooda (1987) evaluated fungicides belonging to different chemical groups for their efficacy in controlling damping-off tomato and chili caused by *Fusarium solani* and *Pythium aphanidermatum*. As seed treatment, copper oxychloride was found best fungicide in controlling damping-off of the both the tests plants caused by *F. solani* whereas for the control of damping-off due to *P. aphanidermatum* MEMC was found the best fungicide. However, for controlling damping-off caused by both the fungi MEMC and captan were very promising on tomato and

captafol on chill. Bucherau *et al.*, (1981) obtained good control of pre-emergence damping off with Ridomil (metalaxyl) caused by *P. ultimum* in alfalfa. Efficacy of Ridomil (metalaxyl) has been confirmed as seed dresser against *P. ultimum* in case of carrot, chick pea, cotton, cucumber, pea and sugar beet significantly superior in reducing the seed rot percentage (Kaiser and Hanan, 1983; Rama, 1980). Ayub *et al.*, (1998) reported that Ridomil (metalaxyl) at all concentrations effectively controlled the damping off disease in tomato. Abdel Rahim and Abu Surrieh (1991) tested 9 fungicides such as Bavistin, Benlate, Topsin M, Miltox special, Dithane M-45, Dithane S-60, Ridomil MZ, Rovral and Captan 50 against damping off of cucumber and found Ridomil MZ best among them in preventing the disease. Hickman and Michailides (1998) reported that fallow field soil, solarized soil, methyl bromide treated soil and metalaxyl soil drench treatments were all effective against damping off caused by *P. aphanidermatum* of cucumber.

Although fungicides have shown promising results in controlling the damping-off disease, phytotoxicity and fungicide residues are major problems leading to environmental pollution and human health hazards. For this reason, a number of restrictions are imposed in the licensing, registering and using of each chemical (Cuthbertson *et al.*, 2010). Hence use of environmental friendly bio-control agents can more effectively control the soil borne phyto-pathogens (Nam *et al.*, 1988 and Saleem *et al.*, 2000).

Biological management

Biological control of soil borne plant pathogens has received tremendous attention on global level on account of the growing public concern about health hazardous and pollutant effects of the pesticides (Ooijkass *et al.*, 1998). Plant ecosystem bestowed with a

number of microorganisms, acts as competitors or antagonists to pathogens and act as viable and potent alternative to plant conventional chemicals for the management of plant diseases (Jeyarajan and Angappan, 1998). In sustainable agriculture, biological control assumes major importance especially, where soil borne diseases are serious (Harman, 2006). Various biocontrol agents have been tried against different species of *Pythium* and got considerable results.

Growth inhibition of the phytopathogens by the *Trichoderma* metabolites has been well researched (Dennis and Webster, 1971). Successful management of damping-off caused by *Pythium* species in various crops by application of *Trichoderma* has been previously reported (Jayaraj *et al.*, 2006). Gomathi *et al.*, (2011) evaluated the antibiotic ability of local isolates of *Trichoderma spp.* against damping off disease. *Trichoderma spp.* showed maximum activity in controlling the disease with influence on soil physico-chemical parameters. Hanson (2003) evaluated various isolates of *Trichoderma virens* and found almost all isolates effective against damping off of sugarbeet seedlings caused by *Rhizoctonia solani* in the greenhouse and also found peroxidase activity significantly higher in roots of plants treated with biocontrol effective strain. Subash *et al.*, (2013) evaluated *T. harzianum* against damping off disease caused by *Fusarium oxysporium*, *Rhizoctonia solani* and *Alternaria alternata* and found *T. harzianum* efficient not only to inhibit the growth of all the above pathogens but also to enhance the growth of the plants.

One of the mechanisms observed to be adopted by *Trichoderma* species to parasitize pathogenic organisms is by competition. *Trichoderma* suppress the growth of these phytopathogenic fungus through the over growth. On this basis the twelve isolates of

Trichoderma were evaluated by Arunachalam & Sharma (2012). All the *T. harzianum* isolates were found to be more profuse than *T. viride* isolates with isolate Th3 having the maximum effect in inhibiting the growth of *P. aphanidermatum* (86.4%).

In addition, several studies have reported the use of bacteria and actinomycetes (El-Tarabily *et al.*, 2009). Bacterial strains of *Pseudomonas fluorescens* and other species are the most commonly reported fluorescent pseudomonads used to suppress damping-off (Martin and Loper, 1999). Carisse *et al.*, (2003) reported efficient control of *Pythium* damping off using *Bacillus marinus*, *P. fluorescens* and *P. aeruginosa*. *Pseudomonas* antagonists were found to be superior to *Bacillus* antagonists in the control of damping-off of cucumber and sugar beet (Georgakopoulos *et al.*, 2002). Other studies reported successful use of pseudomonads as biocontrol agents in greenhouses (Rankin and Paulitz, 1994) and fields (Weller and Cook, 1986). In addition, *Pseudomonas* species were used successfully to control several diseases in crops other than cucumber (Amein *et al.*, 2008). *Pseudomonas fluorescens* (Elad and Chet, 1987) and native isolates have been successfully used for the biocontrol of damping-off disease. Ardakani *et al.*, (2010) found *P. fluorescens* strains more effective than the common seed dressing fungicides (Carboxin/Thiram) for controlling damping off of cotton under greenhouse conditions. Several other studies reported antagonistic activity among *Pseudomonas* species including *P. fluorescens* and *P. corrugate* against *Pythium* species (Hultberg *et al.*, 2000). *Pseudomonas* spp. in combination with other biocontrol agents or applied singly have been tested for their biocontrol potential against many diseases as well as for the damping off of chilli (Jain *et al.*, 2012; Muthukumar *et al.*, 2010). Kumar *et al.*, (2010) observed increase in yield parameters

viz., shoot length, fruit number and biomass production in chilli with *Pseudomonas fluorescens* combined with other antagonists.

The mycoparasitic potential of *Pseudomonas* spp. is well documented by earlier workers (Anitha and Tripathi, 2001). Fluorescent pseudomonads not only colonize the root system but they also produce a variety of antagonistic secondary metabolites that include siderophores, antibiotics and volatile compounds such as cyanide (Fravel, 1988; Kloepper *et al.*, 1980). Several cell wall degrading enzymes such as chitinase and β -1,3-glucanase are involved in this process and they are capable of degrading chitin and β -1,3-glucan respectively, the major components of fungal cell walls (Bhowmik *et al.*, 2002).

Bacillus has shown promising results for the biological control of various plant pathogens as well as growth promoters of some crops (Podile and Laxmi, 1998). Kumaresan *et al.*, (2005) reported significant decrease in the damping off disease caused by *P. aphanidermatum* by treatment with the *B. subtilis* with increase in the shoot length and root length respectively. *Bacillus subtilis* Cohn has been successfully used for control of diseases caused by *Pythium*, *Rhizoctonia*, *Gaeumannomyces*, *Sclerotinia*, *Fusarium* and others (Schmiedeknecht *et al.*, 1998; Ryder *et al.*, 1999; Bacon *et al.*, 2001).

The antagonistic activities of *B. subtilis* are mainly attributed to the production of antibiotic substances, most of which are dipeptides or cyclic peptides (Loeffler *et al.*, 1990). The bacterium is also capable of producing certain volatile extra-cellular metabolites that have antifungal activity (Podile and Prakash, 1996). *B. subtilis* is also a well-known producer of hydrolytic enzymes, including cellulase, chitinase and β -1,3-glucanase (Marten *et al.*, 2000), which

causes lysis of fungal cell walls and membranes. In addition to direct antagonistic activity against several soil borne fungal pathogens, *B. subtilis* known to promote plant growth and yield (Krebs *et al.*, 1998). There is also emerging evidence indicating that the bacterium could be involved in induction of systemic acquired resistance in plants (Podile and Laxmi, 1998; Ghonim, 1999; Collins and Jacobson, 2003).

It has been reported that application of a single strain of a biological control agent is less effective than chemical protection and its effect may not be consistent (Weller, 1998). This low and unstable efficacy or variability of plant protection of biological control agents may be due to the complexity of the ecosystem (Guetsky *et al.*, 2002). Possible approaches of enhancement of the efficacy of biological control agent against soil borne pathogens has been reviewed and it has been identified that one way to improve the biological control ability may be the use of mixtures or combinations of biocontrol agents (Spadaro and Gullino, 2005). Pierson and Weller (1994) reported that combining different *Pseudomonas fluorescens* strains increased the control of wheat take when compared to other strains used singly. Raupach and Kloepper (1998) showed that the use of combined applications of bacterial biocontrol agents increased plant protection against the cucumber pathogens significantly. Apart from the bacterial combinations, some reports indicated the combination of bacterial bio-agent with *Trichoderma* spp. showed increased plant protection than they showed when used single. Brewer and Larkin (2005) reported that combination of *Bacillus subtilis* and *Trichoderma virens* enhanced resistance against *R. solani* on potato than these biocontrol agents when used singly. A positive probably synergistic interaction between *Trichoderma* spp. strains and bacterial strains has been reported for

combined applications in controlling plant pathogens (Fogliano *et al.*, 2002). Sudharani *et al.*, (2014) reported a positive interaction between PGPR's and fungal bio-agents with increased seedling vigour and root and shoot biomass. The combination, *T. harzianum* (TR20) + *P. fluorescens* (P28), was found most effective in reducing disease incidence (66.7%) than the control (Rini and Sulochana, 2006). Highest per plant yield was also recorded in the treatment combination TR20+P28.

Chilli (*Capsicum annum* L.) both unripe (green) and ripe (red) fruit, is one of the most important vegetable crop grown in India as an indispensable condiment, digestive stimulant and a colouring agent. Besides pungency due to Capsaicin and vitamins such as vitamin A and C, the green fruit is also known to contain vitamin P (rutin) in appreciable proportion.

Chilli crop is attacked by several diseases viz. damping-off, wilt, anthracnose, die back, root rot, bacterial wilt etc. Among the fungal diseases, damping-off caused by *Pythium aphanidermatum* (Edson) Fitz. in nurseries is a major constraint in chilli production and is responsible for 90 per cent of plant death either as pre or post-emergence damping off.

Traditionally, this disease is controlled by the application of synthetic fungicides belonging to different classes of chemicals including azoxystrobin (strobilurin), fosetyl-aluminum (alkyl phosphonate), mephenoxam and metalaxyl (acylanilide), etridiazole (triazazole), and propamocarb (carbamate).

Although these fungicides have shown promising results in controlling the damping-off disease but phytotoxicity by fungicide residues are major problems leading to environmental pollution and human health hazards such as adverse genetic effects, causing morphological and physiological

abnormalities in test animals. Development of fungicide resistance by *Pythium* spp. further discourages its use for disease control.

Other control measures as host resistance have not yet become a viable measure.

Hence, such situations have prompted researchers to look out for alternate strategies for managing the disease.

One of the key elements of such sustainable agriculture is the application of biological controlling strategies, for plant protection.

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