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# EFFECT OF BIOCONTROL AGENTS AND FUNGICIDES ON DAMPING OFF DISEASE OF TOMATO

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**ABSTRACT**
Inhibition effect of the potential biocontrol agent's *viz.*, *Pseudomonas fluorescens* and *Bacillus subtilis* was studid *in vitro* and under green house condition against *Pythium aphanidermatum*. After *in vitro* screening of *P. fluorescens* and *B. subtilis,* the most effective isolate from each bioagent was selected for further studies. Amongst these, *P. fluorescens* (35.37%) was found more effective than *B. subtilis* (32.84%) when tested *in vitro*. In green house condition seed treatment with *P. fluorescens* @ 10g + metalaxyl @ 2g/kg seed had most significant effect to manage pre (13.36%) and post emergence damping off (21.12%). *Pythium* population was in decreasing order in all treatments except in control. Seed treatment with *P. fluorescens* @ 10g + metalaxyl @ 2g/kg seed was found effective to reduce pathogen population from $9.33 \times 10^2$ to $3.33 \times 10^2$cfu/g soil at 30 DAS. Under green house experiment, similar treatment gave maximum seeding emergence (76.55%), shoot length (6.14 cm), root length (7.35 cm) and vigour index (1032.65). This is because of growth promotion activity in *P. fluorescens* and *B. subtilis*. It may be concluded that *P. fluorescens* and *B. subtilis* are effective to manage disease as well as improve seedling health.

## INTRODUCTION

Tomato (*Lycopersicon esculentum*) is one of the most important vegetable crops in many countries and has a worldwide economic and nutritive importance (Meraj-ul-Haque and Nandkar, 2012). Damping off disease caused by *Pythium* spp. can cause severe losses in Tomato crop in India. The most common species of *Pythium* causing damping off disease are *P. aphanidermatum*, *P. debaryanum* and *P. ultimum*. Among these *P. aphanidermatum* is one of the most important pathogen to cause damping off in Tomato in nurseries and is a major constraint in the production tomato (Manoranjitham *et al.,* 2001). *Pythium* spp. is essentially soil borne (Berkley, 1925; Horsfall, 1930) and poses a genetic problem in disease management. The most common means to check the disease in nurseries is by using fungicides but frequent and indiscriminate use of fungicides often leads to environmental pollution and also there is a great possibility of development of resistance within a very little span of time. Hence alternative management stratergies are most desirable. Several organisms have been successfully used as biocontrol agents such as *P. fluorescens* (Chen *et al.,* 2000; Boominathan and Sivakumar, 2012) and *B. subtilis* (Christy *et al.*, 2012). Integration of seed treatment fungicides with these bioagents could gave the way of complete solution for management of the disease. The chemical fight and physical control of this fungal pathogen are very difficult to realize and in view of its broad host range, crop rotation also not completely control the pathogen. Moreover, use of chemicals to control the disease is also criticized throughout the world due to its detrimental effects on environment, as they are harmful for human and animal health as well as soil. Besides this, *P. aphanidermatum* became resistant to the common fungicides used against it. Eventually, it has forced scientist to find out the best alternatives to control this notorious pathogen. Hence here in this review paper the different control measures as well as use of plant extracts used against *Pythium* by researchers and many workers is summarized in brief (Tahira Parveen and Kanika Sharma, 2015). *Pseudomonas fluorescens* and *B. subtilis* are common inhabitants. Previous reports have demonstrated that the control efficiencies of *Pseudomonas* spp. and *Bacillus* spp. Were directly related to the ability of these microbes to colonize roots (Ji *et al.,* 2008). Biological control is an efficient and environmentally friendly way to prevent amping off disease. Many microbial species such as *Pseudomonas fluorescens* (Nagrajkumar, 2004) and *Bacillus subtilis* (Asaka and Shoda, 1996) has shown to effectively. *Pythim* spp. are the are the most important phytopathogen responsible for damping off disease in tomato. Thus, the objective of this research was to evaluate the efficacy of the bioagents in disease suppression and growth promotion of tomato seedlings.

## MATERIALS AND METHODS

*Pythium aphanidermatum* was isolated from infected tomato seedlings. Pathogenicity of isolate was assessed on tomato seedlings under greenhouse condition (22-26ºC and 60-70% RH). Tomato seeds were disinfected with *2%* sodium hypochlorite solution for 15 min, rinsed with sterile distilled water and sown in pots containing steam sterilized soil. All pots were maintained in a greenhouse and plants were

watered twice a week. Mass multiplied culture of *Pythium* was incorporated @ 250 g / kg soil to make soil sick. Control pots were without inoculums of pathogen. After 10 days damping off disease affected plants were counted. *P. fluorescens* (Kings *et al.,* 1954) and *B. subtilis* were isolated from rhizosphere of vegetable crops.

**Antagonistic activity**

The antagonistic effect of *P. fluorescens* and *B. subtilis* were tested *in vitro* to see their ability to produce antifungal substance against *P. aphanidermatum* using dual culture assay on PDA. *P. fluorescens* and *B. subtilis* were spotted at the edge of the plates at equal distance. After 24 hr. of incubation at 27ºC, a single 5 mm diameter mycelia disc of pathogen was placed in the centre of the plate. Five replications were maintained foreach biocontrol agent. As a control, a disc of *P. aphanidermatum* was placed at the centre of PDA. Plates were incubated for one week at 27ºC and reduction in fungal growth was measured. Relative growth inhibition was calculated and statistically analized(Weller and Cook 1986; Oktay and Kemal, 2010).

**Production of volatile antibiotics**

Two hundred $\mu$ l of bacterial suspension ($1 \times 10^{7}$cfu/ml, was placed at the centre of a Petri-plate containing NA medium and a 5-mm disk of 7 days old pure culture of *P. aphanidermatum* was placed at the centre of another petriplate containing PDA media. Then both half plates were and placed face to face preventing any Physical contact between the pathogen and bacterial suspension. Plates were then sealed to isolate the atmosphere inside with parafilm to prevent loss of any volatiles (Zakira Naureen *et al*., 2009). In the control plates bacterial suspension was replaced with sterile water. Plates were incubated at 27-29ºC, for 48h and the percentage of inhibition zone were measured. Experiments were repeated twice with four replicates for each treatment. (Zakira Naureen *et al.,* 2009; Fiddaman and Rossall, 1993).

**Greenhouse experiments**

Two isolates including *P. fluorescens, B. subtilis* and also fungicides for seed treatment were selected for green house study. Tomato seeds were soaked in a *P. fluorescens*/*B. subtilis* methyl cellulose suspension for 30 min and dried under laminar airflow. Control treatment consists of non treated dry seeds. Seeds were sown in sterilized soil infected with *Pythium aphanidermatum* at conc. of $10^{5}$cfu / g soil. A complete randomized design with 3 replications and 25 seeds in each pot were used.

**Assessment of disease incidence**

Measurement of growth factor was performed 30 days after seed sowing. Pre and post emergence damping off was recorded. The disease incidence was measured. Also, shoot length, root length and vigour index was calculated (Paulitz and Baker, 1987).

## RESULTS AND DISCUSSION

The most virulent isolate was selected on the basis of pathogenicity test by using water culture and pot culture experiments.

In dual culture assay, *P. fluorescens* (35.37%) and *B. subtilis* (32.84%) were isolated from the rhizosphere of tomato showing substantial inhibition zone against *P. aphanidermatum in vitro* (Table 1). Boominathan and Sivakumar (2012) evaluated the efficacy of seven Rhizobacterial isolates for their ability to inhibit the growth of *Pythium aphanidermatum*, the causal agent of turmeric rhizome rot. *In vitro* studies revealed that *P. fluorescence* (PF25) and *B. megaterium* (BM29) showed the highest

**Table 1: Effect of *P. fluorescens* and *B. subtilis* on growth of *P. aphanidermatum***

| Sr.No | Bio-control agent | Growth inhibition (%) | Volatile antibiotics (% inhibition) |
|---|---|---|---|
| 1 | *P. fluorescens* | 35.37 | 12.10 |
| 2 | *B. subtilis* | 32.84 | 9.60 |
| 3 | Control | - | - |

**Table 2: Effect of different treatments on the population of *P. aphanidermatum* and pre and post damping off of tomato**

| Tr. No. | Treatment detail | Cfu/g soil $\times 10^{2}$ 0 Day | 10 Day | 20 Day | 30 Day | Pre damping-off | Post damping-off |
|---|---|---|---|---|---|---|---|
| $T_1$ | Seed treatment with *Pseudomonas fluorescens*@ 10 g/kg seed | 9.00 | 5.65 | 4.15 | 2.85 | 25.11(30.07)* | 38.86(38.59)* |
| $T_2$ | *Bacillus subtilis*@ 20 g/kg seed. | 9.66 | 6.32 | 4.92 | 3.70 | 30.00(33.21)* | 43.37(41.09)* |
| $T_3$ | *Pseudomonas fluorescens*@ 10 g/kg seed + *Bacillus subtilis* @ 10 g/kg seed | 9.33 | 6.00 | 4.70 | 3.53 | 21.83*(27.90) | 36.60*(37.23) |
| $T_4$ | Metalaxyl @ 2 g/kg | 9.00 | 5.67 | 4.05 | 3.91 | 19.38(26.06)* | 27.84(31.88)* |
| $T_5$ | *Pseudomonas fluorescens* @ 10 g/kg + Metalaxyl@ 2 g/kg seed | 9.33 | 6.00 | 4.50 | 3.33 | 13.36(21.39)* | 21.12(27.35)* |
| $T_6$ | *Bacillus subtilis* @ 10 g/kg + Metalaxyl@ 2 g/kg seed | 8.66 | 5.34 | 3.73 | 2.53 | 18.06(25.10)* | 26.77(31.18)* |
| $T_7$ | Thiram@ 3g/kg seed | 9.66 | 6.34 | 4.82 | 4.66 | 30.89(33.77)* | 43.93(41.50)* |
| $T_8$ | Control with *Pythium aphanidermatum* | 8.66 | 10.00 | 11.29 | 12.76 | 34.4(35.91)* | 71.57(57.67)* |
| C.D. (p = 0.01) | | | 1.46 | 0.17 | 0.27 | 4.66 | 5.03 |

*Values in the parenthesis are arc sine transformed

**Table 3: Effect of different treatments on growth promoting characters of tomato**

| Tr. No. | Treatment detail | Pcrcont seedling emergence | Shoot length(cm) | Root length(cm) | Vigour index |
|---|---|---|---|---|---|
| $T_1$ | Seed treatment with *Pseudomonas fluorescens* @ 10 g/kg seed | 61.84 (51.83)* | 5.53 | 6.51 | 744.55 |
| $T_2$ | *Bacillus subtilis* @ 20 g/kg seed. | 60.00(50.77) | 4.96 | 5.83 | 647.40 |
| $T_3$ | *Pseudomonas fluorescens*@ 10 g/kg seed + *Bacillus subtilis* @ 10 g/kg seed | 64.88(53.67) | 6.10 | 7.20 | 862.90 |
| $T_4$ | Metalaxyl @ 2 g/kg | 70.60(57.17) | 5.60 | 6.26 | 837.31 |
| $T_5$ | *Pseudomonas fluorescens* @ 10 g/kg + Metalaxyl @ 2 g/kg seed | 76.55(61.00) | 6.14 | 7.35 | 1032.65 |
| $T_6$ | *Bacillus subtilis* @ 10 g/kg + Metalaxyl @ 2 g/kg seed | 71.90(57.35) | 5.79 | 7.23 | 959.14 |
| $T_7$ | Thiram @ 3g/kg seed | 57.34(50.77) | 3.13 | 5.98 | 674.89 |
| $T_8$ | Control with *Pythium aphanidermatum* | 40.20(39.35) | 4.11 | 3.78 | 277.78 |
| $T_9$ | Control without *Pythium aphanidermatum* | 90.00(71.57) | 4.00 | 4.34 | 760.50 |
| C.D.(p = 0.01) | | | 4.38 | 1.33 | 0.59 |

* Values in the parenthesis are arc sine transformed

inhibition of mycelial growth (72.4%; 76.2%) of *P. aphanidermatum*. Christy *et al*. (2012) studied in vitro efficacy of two species of *Trichoderma* and nine species of *Bacillus* against tomato damping off caused by *Pythium aphanidermatum* by dual culture technique. Out of nine species of Bacillus, *B. Polymyxa* produced highest (5.2 mm) zone of inhibition.

Results indicated that both bacterial isolates caused mycelial reduction of the pathogen. The highest percent inhibition of pathogen was observed by *P. fluorescens* (12.1%) followed by *B. subtilis* (9.6%). Both isolates were found to produce volatile compounds but do not have sufficient effect on pathogen (Table 1). Production of secondary metabolites such as antibiotics and emergence of volatile compounds by antagonist bacteria caused mycelial inhibition of *P. aphanidermatum* (Weller and Cook, 1988; Meenu Saraf *et al*., 2014). Biocontrol agents may express different mechanism against pathogen during their antagonistic activity. It is weakening or destroying the pathogen by parasitizing the pathogen directly, by producing antimicrobial compounds, by competing for space and nutrition, by producing enzymes that attack the cell components of the pathogen. Production of antibiotic compound and inhibition of other microbes is the most important mechanism expressed by the antagonistic bacteria (Koche *et al*., 2013). In this study the antagonistic study expressed by the *P. fluorescens* and *B. subtilis* in dual culture method might be due to one or combination of above mechanisms. However results are mostly depend on the ability of producing antimicrobial compounds and degradative enzymes by the tested antagonistic bacteria. It has been already reported that *P. fluorescens* has ability to produce high level of chitinase, â-l 3-glucanace, cellulase, fungitoxic metabolites and siderophores (*Jayraj et al*., 2007) also *Bacillus spp*. have ability to produce bactericin, gramicidin, S. polymyxin, tyrotricidin, bacilysin, chlotelaine, iturin A, mycobacilin, bailomycin, mycosubtilin, fungistatin and subsporin(Trevor *et al*., 2004; Meenu Saraf *et al*., 2014). Similar results were reported by some other researchers (Intana *et al*., 2008).

**Efficiecy of antagonistic bacteria and seed treatment fungicides under greenhouse condition**

Efficiency of *P. fluorescens* and *B. subtilis* were tested in pot culture under *Pythium* sick soil. Three days old growth of *Pythium* was incorporated in the proportion of 1:9 in sterilized soil. Seeds of tomato were sown after application of different treatments. Seed treatments with antagonist recorded highest seed emergence and minimum disease incidence of damping off in sick soil. Among the different treatments most significant treatment was seed treatment with *Pseudomonas fluorescens* @ 10 g + Metalaxyl @ 2 g /kg seed i.e. 76.55% followed by the *Bacillus subtilis* @ 10g + Metalaxyl @ 2 g / kg seed *i.e.* 71.90% (Table 2). The lowest percent seed emergence was registered in control with *Pythium* 40.20% (Muthukumar *et al*., 2010).

Minimum pre and post damping off disease incidence was recorded in seed treatment with *Pseudomonas fluorescens* @ 10 g + Metalaxyl @ 2 g / kg seed *i.e.* 13.36% and was significantly superior to all other treatments. Seed treatments with *B. subtilis* @ 10 g + Metalaxyl @ 2 g / kg seed recorded 18.06 per cent pre emergence damping off (Table 2). Maximum pre emergence damping off disease was recorded in control 34.4% (Table 2). Similarly, minimum post emergence damping off was recorded in *P. fluorescens* @ 10 g + Metalaxyl % 2 g / kg seed *i.e.* 21.12% and was significantly superior to Tl, T2, T3, T7 and T8 but at par with T4 and T6 (Table 2). Maximum post emergence damping off disease was recorded in control i.e. 71.57% (Table 2). Combined seed treatment with *P. fluorescens* and the fungicides resulted in a significant improvement in disease control. This is in accordance with findings of Salman and Abuamsha, 2012.

Seed treatment with *P. fluorescens* @ 10 g + Metalazxyl @ 2 g/kg seed reduced the population of *P. aphanidermatum* from 9.33 X $10^2$ to 3.33x $10^2$cfu/ g soil at 30 days after sowing. This was at par with *B. subtilis* @ 10 g + Metalaxyl @ 2 g / kg seed in reducing the pathogen population (Table 2). The reduction in population of *P. aphanidermatum* in the soil might be the reason for lesser incidence of pre emergence and post emergence damping off of tomato in seed treatment with fungicides and antagonist bacteria. This clearly indicated that in soil the antagonist competes with the pathogen and brings down the inoculums of the pathogen there by reducing the disease incidence.

**Effect of treatments on plant growth promotion**

The data regarding the effect of antagonist on growth of tomato

seedling present in Table 3 indicates that seed treatment with *P. fluorescens* @ 10 g + Metalaxyl @ 2 g / kg seed recorded maximum percent increase in shoot length (6.14 cm) root length (7.35 cm ). This was closely followed by *B. subtilis* @ 10 g + Metalaxyl @ 2 g / kg seed (Table 3.) Increase in root and shoot length due to antagonist in combination with fungicidal seed treatment is in accordance with the findings of Ramanathan (1989) and Emayavaramban (1994) who worked on damping off management in Chilly. Research has also shown that many micro organisms from rhizosphere such as PGPR can have positive influence on plant growth and plant health (Zakira Naureen *et al.,* 2009). The role of *P. fluorescens* in promoting root and shoot growth of different crops have also been demonstrated in past (Singh *et al*., 2013). This indicates that bacterial isolates could be used as a plant growth promoter and improvement in plant health (Zakira Naureen *et al*., 2009, Naureen *et al*., 2005). Further research needed to see role of environmental factors in antibiotic synthesis-modulation by *P. fluorescens* and *B. subtilis* in order to improve efficacy of bacterial strain in supporting the *P. aphanidermatum* under field condition

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