

Original Research Article

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Efficacy of Fungicides and Bioagents against Damping off in Chilli caused by *Pythium aphanidermatum*

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Pythium damping-off of Chilli (*Capsicum annuum* L.) is very common problem in fields and greenhouses, whereas the organism kills newly emerged seedlings. In present investigation repeated isolations from rotten seeds as well as toppled seedlings of Chilli cv. Pusa Jwala have yielded the cultures of *P. aphanidermatum*. The damping off mainly being soil borne, the primary infections are needed to be controlled with suitable fungicides. The different fungicides and bioagents were evaluated as seed dressers and soil drenching. The result revealed that there was significant difference in per cent disease incidence at 15, 30 and 45 day after sowing. Seed dressing of metalaxyl reduced the damping off disease significantly as compared to all other treatments and at par with thiram. Among bioagents, *Trichoderma harzianum* was proved effective in controlling the disease. In per cent mortality it was found that metalaxyl reduced the plant mortality significantly over control followed by thiram. Among the bioagents, *Trichoderma harzianum* was superior over all other treatments followed by *T. viride*.

Introduction

Chilli (*Capsicum annuum* L.) is an important commercial vegetable crop in India and belongs to Solanaceae family. It is also called as nature's wonder, hot pepper, and cayenne pepper. Chilli is the fourth most important vegetable crops in the world and first in Asia. It suffers to various foliar, soil, seed borne and transmissible diseases. The important diseases reported are Anthracnose (*Colletotrichum capsici*), Cercospora leaf spot (*Cercospora capsici*), damping-off and root rot (*Rhizoctonia solani*, *Pythium* sp., and

Fusarium sp.), Fusarium wilt (*Fusarium oxysporum* f.sp. *capsici*), gray mould (*Botrytis cinerea*), powdery mildew (*Leveillula taurica*) etc. (Vidhyasekaran and Thiagarajan 1981; Meon and Nick, 1988; pandey *et al.*, 2012; Ahila and Prakasham, 2014). Among these diseases, damping off due to *Pythium* spp. is of considerable importance.

Most common fungi causing damping off disease are *Pythium aphanidermatum*, *Pythium butleri*, *Pythium debaryanum* and *Pythium ultimum*. Damping off is responsible

for poor germination and stand of seedling in nursery bed. Chilli crop suffer from two phases of damping off, one is pre-emergence and another is post-emergence. In pre-emergence damping off, the young seedlings are killed before they reach the surface of soil. The post-emergence damping off is very conspicuous.

Infection usually occurs at or below ground level and the infected tissue appears water soaked and soft. As the disease advances the stem become constricted at the base and plant collapse. Seedlings that are apparently healthy one day may have collapsed by the following morning (Singh1985). The aim of the present study was to evaluate the efficacy of seed treatments with different fungicides and bioagents either alone or combination against damping-off disease of Chilli plants.

Materials and Methods

The field experiment was conducted at Plant Pathology Section, College of Agriculture, Nagpur during 2015-16 to find out the effect of different treatment for the management of damping off in Chilli. For this investigation Randomized Block Design was used with three replications and ten treatments were taken.

Seed dressers

Evaluation of fungicides and bioagents as seed dressers

The damping off mainly being soil borne, the primary infections are needed to be controlled with suitable fungicides. For testing different fungicides in the sterilized soil the experiment was planned in RBD with 3 replications and 10 treatments. The relative control without seed treatment with inoculation by sterilized soil was maintained. The absolute control without seed treatment, soil inoculation and

stagnation was also maintained. For evaluation of different biocontrol agents as seed dressers, seeds of Chilli variety Pusa Jwala were procured. Experiment was conducted in RBD with 3 replications and 10 treatments.

Seeds were treated with following bioagents, one day before sowing. Treated seeds were sown in the sterilized soil in plastic tray. Observations on per cent germination were recorded after 10th days of sowing.

Evaluation of fungicides and biocontrol agents by soil drenching

For evaluation of fungicides and biocontrol agents by soil drenching, an experiment was planned in *Kharif* 2015-16 on variety Pusa Jwala. The experiment was planned in R.B.D. with 10 treatments and 3 replications.

The formulation required in gram per liter was calculated by using the following formula,

$$M_f = \frac{C \times V}{A. I. (\%)}$$

Where,

M_f = Mass of formulation required in gram

C = Concentration of the A.I. desired.

V = Volume of the solution/medium to be prepared.

A.I. (%) = Percentage of Active Ingredient in formulation.

Evaluation of fungicides and bioagents *in vitro*

Efficacy of (Five) fungicides and (Three) bioagents were evaluated by employing poisoned food technique and dual culture technique respectively.

In vitro efficacy of fungicides against *Pythium aphanidermatum* by poisoned food technique

Efficacies of five fungicides were evaluated *in vitro* against *Pythium aphanidermatum* by poisoned food technique. Based on active ingredient, the requisite quantity of each fungicide was calculated and mixed thoroughly with autoclaved and cooled Potato Dextrose Agar medium (PDA) in conical flask to obtain desired concentrations. Plain PDA medium without fungicides served as untreated control. Fungicide amended PDA medium was then poured aseptically in petriplates (90 mm diameter) and allowed to solidify at room temperature. After solidification of the medium, all the plates were inoculated aseptically with 4 mm culture disc of the test fungus obtained from a week old actively growing pure culture of *Pythium aphanidermatum*.

The disc was placed on PDA in the centre of the petriplates and the plates were incubated at 27 ± 1°C. Each treatment was replicated thrice. Observations on radial mycelial growth/colony diameter were recorded on 3rd, 5th and 7th days after inoculation. Per cent mycelial growth inhibition of the test pathogen with the test fungicides over untreated control was calculated by applying the formula given below based on the average of colony diameter. The data of mycelial growth was also subjected to statistical analysis and conclusions were drawn (Gomez and Gomez, 1984).

$$\text{Percent inhibition (I)} = \frac{C - T}{C} \times 100$$

Where,

C = Growth (mm) of test fungus in untreated control plates

T = Growth (mm) of test fungus in treated plates

In vitro evaluation of bioagents by dual culture technique

Antagonistic activity of *Trichoderma viride*, *Trichoderma harzianum* and *Pseudomonas fluorescens* on growth of *Pythium aphanidermatum* was studied by dual culture technique on PDA plates. Discs of 4 mm of *Pythium aphanidermatum* were placed at center of respective PDA plates. Then bacterial antagonists were streaked on side of fungal pathogens leaving 3 cm distances between them. In case of fungal antagonist each plate was inoculated with 4 mm mycelial disc of fungal pathogens and *Trichoderma viride* were placed at side by side on medium in each plate approximately at a distance of 4 cm away from each other. Similarly one set of each fungus without any bioagents culture served as control.

The inoculated plates were incubated at 28±2°C for 3 days in case of *Pythium aphanidermatum*. Observations regarding antagonistic effect of all these bioagents against test pathogens were recorded at 3rd, 5th and 7th days after inoculation. After 3rd and 7th days of incubation as mentioned earlier colony diameter of test fungus in each plate was measured and mean diameter and per cent inhibition in each case was worked out. The growth inhibition of each fungal pathogen was calculated by using the formula given below by Anand *et al.*, 2010 as follows,

$$\text{Per cent inhibition (I)} = \frac{C-T}{C} \times 100$$

Where,

C= Colony diameter in control. T = Colony growth in treatment.

Results and Discussion

Effect of seed dresser on seed germination

The data from Table 1 indicated that the highest seed germination was recorded with metalaxyl (87.22 per cent) at par with thiram (81.36 per cent). Among fungicides, the lowest per cent seed germination was recorded in carbendazim (68.24 per cent). Among the bioagents, the highest seed germination was recorded by *Trichoderma harzianum* 69.21 per cent at par with *Trichoderma viride* (56.18 per cent). This was followed by *Pseudomonas fluorescens* (50.33 per cent).

The present results are in argument with the findings of Zagade *et al.*, (2012) who reported metalaxyl alone or in combination with *T. hamatum* has synergistic effect for reducing the seed rot and improving the germination and was used against Oomycetes fungi like *Pythium* @ 3g/ kg seed. And thiram, captan, carbendazim were at par and were inferior to metalaxyl + *T. hamatum* and metalaxyl alone. Muthukumar *et al.*, (2010) reported significant increase in the germination, shoot length and root length of Chilli plants. Chakraborty *et al.*, (2005) recorded the maximum seed germination (86%) and minimum pre and post damping off in brinjal. Similar studies were given by Deshmukh *et al.*, (2012), Ghutukade *et al.*, (2013), Manoranjitham *et al.*, (2000), Bohra *et al.*, (2006), Korekar *et al.*, (1992), Thakur and Tripathi (2015), Zalte *et al.*, (2013) and Kaiser *et al.*, (1983).

Effect of soil drenching on seed germination

The data from Table 2 revealed that the metalaxyl was most effective in increasing the seed germination by 79.35 per cent over control and at par with thiram (74.23 per

cent), followed by captan (68.51 per cent), chlorothalonil (61.19 per cent) and carbendazim (57.44 per cent). The lowest per cent seed germination was recorded in control (41.12 per cent). Among the bioagents, the highest seed germination was recorded with *Trichoderma harzianum* (54.21 per cent) at par with *Trichoderma viride* (49.64 per cent) and *Pseudomonas fluorescens* (46.33 per cent).

The above results were also in agreement with the findings of Rathaiah (1982), Kuruchev and Padmavathi (1998), Chakraborty *et al.*, (2005), Ganeshan and Sinha (2002) and Manoranjitham *et al.*, (2001) (Table 3 and 4).

Disease incidence of pre emergence damping off

It is evident from the Table 5 that significant reduction in pre emergence damping off of chilli was recorded in metalaxyl (31.92 per cent), which was significantly superior to all other treatments. The treatment thiram (49.72 per cent) was at par with captan (55.51 per cent) and chlorothalonil (59.60 per cent). Higher pre emergence damping off disease was recorded in inoculated control (72.11 per cent). Among the bioagents, *Trichoderma harzianum* recorded less disease incidence (53.58 per cent) and at par with *Trichoderma viride* (57.26 per cent) and *Pseudomonas fluorescens* (67.38 per cent).

The present results are similar with the finding of Zagade *et al.*, (2012) who concluded that Metalaxyl + *T. hamatum* seed treatment was significantly superior over rest of the fungicides tested. Metalaxyl alone ranked 2nd. Metalaxyl alone was also significantly superior over rest of the fungicide tested. Cyamoxanil + *T. hamatum* was at par with metalaxyl alone (on basis of percentage value) reported by Thakur and Tripathi (2015), Deshmukh *et al.*, (2012) and Muthukumar *et al.*, (2011).

Disease incidence of post emergence damping off

It is evident from Table 6 that there was significant difference in incidence of damping off disease due to various treatments at 15, 30 and 45 days over control. At 15 DAS the effect of metalaxyl @ 0.2 % was found in minimising disease incidence (10.81 per cent) and at par with thiram @ 0.3 % treatment (15.38 per cent) and captan @ 0.3 % treatment (16.00 per cent). This was followed by chlorothalonil (18.18 per cent) and carbendazim (23.80 per cent). Among the bioagents, *Trichoderma harzianum* (25.00 per cent) was significantly superior followed by *Trichoderma viride* and *Pseudomonas fluorescens* (30.43, 35.00 per cent respectively).

Result of this experiment was similar with the results recorded by Muthukumar *et al.*, (2011), Muthukumar *et al.*, (2010), who reported minimum pre and post emergence damping off of (9.10 and 12.33 per cent respectively), which was on par with metalaxyl treatment. Saha *et al.*, (2008), Usharani and Satheesh (2007), reported that thiram recorded lowest disease incidence. Sharma and Sain (2005), Jain *et al.*, (2014), Thakur and Tripathi (2015), Manoranjitham and Prakasam (1999) reported that *T. harzianum* and *T. viride* effectively reduced the pre and post emergence damping off. At 30 and 45 DAS metalaxyl treatments recorded minimum percent disease incidence (13.51% and 18.71%) and at par with thiram (19.23% and 23.07%), captan (20.00% and 28.00%) and chlorothalonil (22.72% and 31.18%) followed by carbendazim (33.33% and 38.09%). Among the bioagents, *Trichoderma harzianum* recorded less disease incidence (37.50 % and 41.66 %) and at par with *Trichoderma viride* (43.47% and 47.82%) and *Pseudomonas fluorescens* (45.00% and 56.09% respectively).

The present results are similar with findings of Sawant and Mukhopadhyay (1990) who reported seed treatment with 1000 ppm metalaxyl gave complete protection against damping off for entire period. Salman and Abuamsha (2012) reported that the metalaxyl-M treatment applied alone or in combination with *P. fluorescens*, significantly protected tomato seedling against damping off. Ghutukade *et al.*, (2013) reported that metalaxyl showed maximum reduction in damping off (4.44 per cent) in nursery. Saha *et al.*, (2008) found that thiram was proved superior to captan and loss recorded was (13.7 per cent) and (18.35 per cent) respectively. Deshmukh *et al.*, (2012) and Mehetre and kale (2011) recorded *T. harzianum* seed treatment, with corresponding significantly highest reduction in post emergence followed by *Trichoderma viride* and *Pseudomonas fluorescens* respectively. Dar *et al.*, (2012) showed damping off of papaya caused by *Pythium debaryanum* was reduced by soil and seed application of talc based formulation of *P. fluorescens*. The antagonistics and chemical metalaxyl increased the seed germination, shoot length, root length, fresh and dry weight of papaya seedlings.

Mortality in soil drenching

The data regarding the mortality presented in Table 7 indicated significant difference due to various treatments over control. Among the treatments, it was found that metalaxyl (10.36 per cent) reduced the plant mortality significantly over control. Followed by thiram (17.87 per cent), captan (23.85 per cent), chlorothalonil (25.54 per cent) and carbendazim (31.82 per cent). All these treatments were statistically at par with each other. Among the bioagents, *Trichoderma harzianum* (35.27per cent) is superior to all other treatments followed by *T. viride* and *P. fluorescens* (37.27per cent and 48.50 per cent respectively).

The present results are similar with the findings of Zagade *et al.*, (2012) who reported that drenching with either metalaxyl or cymoxanil reduced the plant mortality significantly over control and rest of the fungicides tested. Metalaxyl and cymoxanil were at par in reducing the percent mortality. Jahagirdar (2012) found that the drenching with metalaxyl-M + mancozeb reduced minimum disease incidence. Rathaiah (1982) reported that the soil drenching with Ridomil, reduced the incidence of rhizome rot as compared to control. Deshpande (1985) concluded that only metalaxyl could protect

the seedling from post emergence mortality followed by captan and thiram. Manoranjitham *et al.*, (2000) reported soil application of *T. viride* and *P. fluorescens* effectively checked the pre and post emergence damping off of tomato. Hegde and Kulkarni (2001) revealed that captan, thiram, *T. harzianum* and *P. fluorescens* reduced the damping off of seedling to the maximum extent, thus can minimize the secondary spread of the inoculum. Kanse *et al.*, (2011) recorded that *T. harzianum* was superior followed by *P. fluorescens* and *T. viride*.

Table.1 Seed dressers

Treatment No.	Treatments details	Doses
T ₁	MetalaxyI 35% SD	0.2%
T ₂	Captan 75% WP	0.3%
T ₃	Thiram 75% WP	0.3%
T ₄	Carbendazim 50% WP	0.1%
T ₅	Chlorothalonil 78% WP	0.3%
T ₆	<i>Trichoderma viride</i> (10 ⁷ cfu)	4g/kg
T ₇	<i>Trichoderma harzianum</i> (10 ⁷ cfu)	4g/kg
T ₈	<i>Pseudomonas fluorescens</i> (10 ⁸ cfu)	4g/kg
T ₉	Uninoculated control	-
T ₁₀	Inoculated control	-

Table.2 Soil drenching

Treatment No.	Treatments details	Rate of application (g/kg)
T ₁	MetalaxyI 35% SD	2
T ₂	Captan 75% WP	3
T ₃	Thiram 75% WP	3
T ₄	Carbendazim 50% WP	1
T ₅	Chlorothalonil 78% WP	3
T ₆	<i>Trichoderma viride</i>	1×10 ⁷ cfu/ml
T ₇	<i>Trichoderma harzianum</i>	1×10 ⁷ cfu/ml
T ₈	<i>Pseudomonas fluorescens</i>	1×10 ⁸ cfu/ml
T ₉	Uninoculated Control	-
T ₁₀	Inoculated Control	-

Table.3 Effect of seed treatment with fungicides and bioagents on per cent seed germination

Treatment No.	Treatments Details	Seed germination (%)
T ₁	Metalaxyl	87.22**
T ₂	Captan	76.40
T ₃	Thiram	81.36*
T ₄	Carbendazim	68.24 least
T ₅	Chlorothalonil	72.04
T ₆	<i>Trichoderma viride</i> (10 ⁷ cfu)	56.18*
T ₇	<i>Trichoderma harzianum</i> (10 ⁷ cfu)	69.21**
T ₈	<i>Pseudomonas fluorescens</i> (10 ⁸ cfu)	50.33
T ₉	Uninoculated Control	91.18
T ₁₀	Inoculated Control	44.37
	'F' Test	Sig.
	SE ± (m)	3.87
	CD (P=0.05)	15.77

Table.4 Effect of soil drenching with fungicides and bioagents on per cent seed germination

Treatment No.	Treatments Details	Seed germination (%)
T ₁	Metalaxyl	79.35 **
T ₂	Captan	68.51
T ₃	Thiram	74.23*
T ₄	Carbendazim	57.44
T ₅	Chlorothalonil	61.19
T ₆	<i>Trichoderma viride</i> (10 ⁷ cfu)	49.64
T ₇	<i>Trichoderma harzianum</i> (10 ⁷ cfu)	54.21**
T ₈	<i>Pseudomonas fluorescens</i> (10 ⁸ cfu)	46.33*
T ₉	Uninoculated Control	83.05
T ₁₀	Inoculated Control	41.12 lowest
	'F' Test	Sig.
	SE ± (m)	3.43
	CD (P=0.05)	10.26

Table.5 Effect of sees treatment with fungicides and bioagents on pre emergence damping off

Treatment No.	Treatments Details	% Incidence
T ₁	Metalaxyl	31.92 (34.40)*
T ₂	Captan	55.16 (47.96)*
T ₃	Thiram	49.72 (44.84)*
T ₄	Carbendazim	68.79 (56.04)*
T ₅	Chlorothalonil	59.60 (50.53)*
T ₆	<i>Trichoderma viride</i> (10 ⁷ cfu)	57.26 (49.17)*
T ₇	<i>Trichoderma harzianum</i> (10 ⁷ cfu)	53.58 (47.05)*
T ₈	<i>Pseudomonas fluorescens</i> (10 ⁸ cfu)	67.38 (55.17)*
T ₉	Uninoculated Control	02.86 (09.74)*
T ₁₀	Inoculated Control	72.11 (58.12)*
	'F' Test	Sig.
	SE ± (m)	2.96
	CD (P=0.05)	8.86

*(Figures in parentheses are Arc sine transformed values)

Table.6 Effect of fungicides and bioagents on post emergence damping off

Treatment No.	Treatments details	Percent disease incidence		
		15DAS	30DAS	45DAS
T ₁	Metalaxyl	10.81 (19.20)*	13.51 (21.57)*	18.71 (25.63)*
T ₂	Captan	16.00 (23.58)*	20.00 (26.57)*	28.00 (31.95)*
T ₃	Thiram	15.38 (23.09)*	19.23 (26.01)*	23.07 (28.71)*
T ₄	Carbendazim	23.80 (29.20)*	33.33 (35.26)*	38.09 (38.11)*
T ₅	Chlorothalonil	18.18 (25.24)*	22.72 (28.47)*	31.18 (33.94)*
T ₆	<i>Trichoderma viride</i> (10 ⁷ cfu)	30.43 (33.48)*	43.47 (41.25)*	47.82 (43.75)*
T ₇	<i>Trichoderma harzianum</i> (10 ⁷ cfu)	25.00 (30.00)*	37.50 (37.76)*	41.66 (40.20)*
T ₈	<i>Pseudomonas fluorescens</i> (10 ⁸ cfu)	35.00 (36.27)*	45.00 (42.13)*	56.09 (48.50)*
T ₉	Uninoculated Control	01.40 (06.80)*	02.68 (09.42)*	04.22 (11.85)*
T ₁₀	Inoculated Control	63.93 (53.09)*	69.42 (56.43)*	70.40 (57.04)*
	'F' Test	Sig.	Sig.	Sig.
	SE ± (m)	1.64	1.98	2.19
	CD (P=0.05)	4.92	5.95	6.56

*(figures in parentheses are Arc sine transformed values)

Table.7 Effect of soil drenching with fungicides and bioagents on per cent mortality in damping off

Treatment No.	Treatments details	Per cent Mortality	
		20 DAS	30 DAS
T ₁	Metalaxyl	07.23 (15.60)*	10.36 (18.78)*
T ₂	Captan	20.67 (27.04)*	23.85 (29.23)*
T ₃	Thiram	16.38 (23.87)*	17.87 (25.01)*
T ₄	Carbendazim	27.84 (31.85)*	31.82 (34.34)*
T ₅	Chlorothalonil	23.72 (29.85)*	25.54 (30.36)*
T ₆	<i>Trichoderma viride</i> (10 ⁷ cfu)	36.44 (37.13)*	37.27 (37.63)*
T ₇	<i>Trichoderma harzianum</i> (10 ⁷ cfu)	31.12 (33.91)*	35.27 (36.43)*
T ₈	<i>Pseudomonas fluorescens</i> (10 ⁸ cfu)	46.08 (42.75)*	48.50 (44.14)*
T ₉	Uninoculated Control	02.19 (08.51)*	04.62 (12.41)*
T ₁₀	Inoculated Control	73.41 (58.96)*	76.54 (61.03)*
	'F' Test	Sig.	Sig.
	SE ± (m)	1.93	2.05
	CD (P=0.05)	5.78	6.16

*(Figures in parentheses are Arc sine transformed values)

Table.8 *In vitro* effect of fungicides and bioagents on radial mycelial growth at 7th DAI

Treatment No.	Treatment details	Conc. %	Mean colony diameter (mm)	Per cent growth inhibition
T ₁	Metalaxyl	0.2	00	100 (90.00)*
T ₂	Captan	0.3	16.32	79.43 (63.02)*
T ₃	Thiram	0.3	11.22	85.66 (67.74)*
T ₄	Carbendazim	0.1	22.17	72.66 (58.47)*
T ₅	Chlorothalonil	0.3	18.64	76.24 (60.82)*
T ₆	<i>Trichoderma viride</i>	-	30.43	61.65 (51.73)*
T ₇	<i>Trichoderma harzianum</i>	-	26.12	67.65 (55.33)*
T ₈	<i>Pseudomonas fluorescens</i>	-	32.29	59.23 (50.31)*
T ₉	Control		79.35	
	'F' Test		Sig.	
	SE ± (m)		1.98	
	CD (P=0.01)		8.16	

*(Figures in parentheses are Arc sine transformed values)

Evaluation of fungicides and bioagents on *in vitro*

Observation on average colony diameter at 7th day after inoculation and per cent growth inhibition was recorded. The fungicides and bioagents showed their potentiality to check the mycelial growth of pathogen *Pythium* spp.

The data presented in Table 1, fungicides viz., Metalaxyl, Captan, Thiram, Carbendazim, Chlorothalonil, and biocontrol agents like *Trichoderma viride*, *Trichoderma harzianum* and *Pseudomonas fluorescens* were evaluated at recommended concentration in the laboratory for their efficacy against *Pythium aphanidermatum* by applying poisoned food technique and dual culture technique.

Fungicides and bioagents significantly inhibited the growth of *Pythium aphanidermatum*. Fungicides like metalaxyl was found significantly superior to arrest the growth of pathogen with (100 per cent) inhibition followed by thiram which was recorded (85.66 per cent) growth inhibition over control (Table 8). Lowest per cent growth inhibition was observed in carbendazim (72.66 per cent) as compared to control. The above results correlated with the results recorded by Yadav and Joshi (2012), Gupta *et al.*, (2012), Gholve *et al.*, (2014) and Zalte *et al.*, (2013). Among the bioagents *Trichoderma harzianum* recorded (67.65 per cent) growth inhibition as compared to *Trichoderma viride* (61.65 per cent) and *Pseudomonas fluorescens* (59.23 per cent). The present results are similar with the findings of Muthukumar *et al.*, (2010), Ramamoorthy *et al.*, (2002), Muthukumar (2011), Gulhane *et al.*, (2005) and Sushir *et al.*, (2006).

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