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Efficacy of Bio - Agents and *Lantana camara* against Damping - Off (*Pythium aphanidermatum*) of Chilli (*Capsicum annuum* L.)

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A B S T R A C T

In chilli (*Capsicum annuum* L.) several diseases are caused from fungal, bacterial and viral origin. Among the fungal diseases, damping off caused by species of *Pythium* is very common in the nursery which causes about 90 per cent mortality in nurseries and fields. Present experiment was carried out in *in-vitro* and *in-situ* conditions at Department of Plant Pathology, SHUATS in Completely Randomized Design with eight treatments and three replications each. The dominant pathogen, which causes damping-off of chilli, was isolated and identified as *Pythium aphanidermatum.* Bio-control agents *Trichoderma harzianum* and *Pseudomonas fluorescens* were isolated from different crops of healthy rhizosphere soils in different geographical regions. The *in-vitro* studies revealed that *Lanatana camara* (20 ml) carbendazim fungicide (8ppm) showed the highest mycelial growth inhibition (100 %) over the control and both antagonists were compatible with each other. Under nursery conditions, maximum germination percentage was recorded in carbendazim seed treatment (80 %), *Lantana camara* (100 ml/kg) soil treatment (76.00 %), [*Trichoderma harzianum*+ *Pseudomonas fluorescens*] +*Lantana camara* [seed] + soil treatment (70.66 %), *Trichoderma harzianum* + *Pseudomonas fluorescens* seed treatment (68.00 %), *Trichoderma harzianum* seed treatment (64.00%), *Pseudomonas fluorescens* seed treatment (60.00 %) when compared to the T_0 - relative control (treated control) (13.33 %) and T_1 - absolute control (untreated control) (78.66 %).Maximum root length was recorded in treatment carbendazim (4.29 cm) followed by T_5 - *Trichoderma harzianum*+ *Pseudomonas fluorescens* (3.80 cm). Maximum shoot length was recorded in treatment T_7 – carbendazim (4.52 cm) followed by T_5 - *Trichoderma harzianum*+ *Pseudomonas fluorescens* (4.1 cm).

Introduction

Among the fungal diseases, damping off caused by *Pythium* species is essentially soil borne disease fungi, which causes seedling rot and damping off in many crops including a chilli and tomato. In nursery plot, disease show infection in patches and within two to

four days the entire lot of seedling may be destroyed (Ghutukade *et al.,* 2013). Damping-off incited by *Pythium aphanidermatum* (Edson) Fitz. caused more than 60% mortality of seedlings in both nurseries and field grown crops (Muthukumar *et al.,* 2010). *Pythium aphanidermatum*, a member of class oomycetes, is an unspecialized parasite that has a wide host range. It affects the plant both in pre and post emergence stage in nursery beds (Jeyaseelan *et al.,* 2012).

Biological control agents colonize rhizosphere and provide protection against various soil borne plant pathogens (Kloepper *et al.,* 1989). Fungi in the genus *Trichoderma* are of increasing interest as bioprotectants.

Another important group of biocontrol agents are rhizobacteria. Among them *Pseudomonas florescens* is capable of suppressing wide range of plant pathogens (Raghunandan *et al.,* 2013). Utilization of plant extracts which are natural source of antimicrobial substances, considered as safe and degraded by natural microorganism.

Materials and Methods

Isolation of the pathogen

Isolation of *Pythium* species was carried out from infested nursery soil by using French bean/bottle guard as bait. Fruits were treated with carbendazim (500ppm) + streptocycline (10 0ppm) solution for 24 hours and then it was transferred to infested soil.

The entire french bean / bottle guard fruits were covered with white fluffy mycelial growth within 24 hours and it was aseptically transferred to potato dextrose agar plates.

Thus, the pathogen was isolated within five days without any other fungal and bacterial contamination (Patel *et al.,* 2014).

Identification of the pathogen

Identification of *Pythium aphanidermatum* was based on standard keys suggested by (Plaats-Niterink, and Van der, A. J. 1981; Butler, 1907 and Dick, 1990). Slides were prepared from the culture and stained with cotton blue according to Parija and Prabhakar, (1995) and examined under the light microscope.

Mass multiplication of *Pythium aphanidermatum*

The mass culture of *Pythium aphanidermatum* was prepared on sorghum grain medium using the method of Khan *et al.,* (2004). Sorghum grain and water were mixed in ratio of 1:1.25 (w/v) and boiled up to two whistles in pressure cooker.

Then 200 gm of such mixture was filled in 500 ml Erlenmeyer flasks. The sorghum grains were sterilized in an autoclave at 15 lbs for 20 minutes. After that, the flasks were inoculated with 5 mm mycelial discs of *Pythium aphanidermatum* and incubated for 7 days at 28$\pm$1°C. The grains turn whitish due to mycelial growth of the test fungus. For soil application in nursery experiment, the grains colonized by *Pythium aphanidermatum* was mixed in soil as such @ 10 g/kg sterilized soil.

Isolation of *Trichoderma harzianum* and *Pseudomonas fluorescens*

Trichoderma harzianum was isolated by serial dilution technique using TSM agar plate. The obtained strains were purified on TSM agar plates using sub-culture technique.

Pseudomonas fluorescens was isolated by serial dilution method using King's B agar. The fluorescent strains were purified on King's B agar plates using single spore technique.

Purification and identification of *Trichoderma* sp.

Trichoderma sp. was purified by single spore culture (Tuite, 1969b). Identification and maintenance of *Trichoderma* sp. was based on colony characters (Gams and Bisset, 1998).

Purification and Identification of *Pseudomonas fluorescens*

The green fluorescent colonies under UV light were picked up, purified by repeated streaking on the same medium and checked for their fluorescens (Sandheep *et al.,* 2013). For the identification of efficient antagonist rhizobacteria biochemical and functional tests were done and identified according to Bergey's manual of systematic bacteriology.

In-vitro evaluation of fungicide, botanical and bio-agents

Poison Food Technique

The extract was prepared from *Lantana camara* leaves which were antifungal in nature. Fresh leaves were grinded with the help of pestle and mortar by taking (1:1 w/v) one gram of extract was added in 1 ml distilled water and filtered through muslin cloth and 100% plant extract solution was prepared. The extracts were poured in the flasks plugged with cotton and heated at 100°C for 10 minutes to avoid contamination.

Appropriate concentration (20%) of plant extract was incorporated to potato dextrose medium agar for inoculation of the test pathogen in sterilized petridishes. The isolated pathogen was grown on potato dextrose agar medium was placed at the center of petridishes containing defined concentration of the poisoned medium and incubated at 27±2°C for 6 days. Radial growth (cm) of fungus was measured after inoculation till 6 days at an interval of 24 hrs. per cent growth inhibition of the test pathogen over control was worked out (Vincent, 1927) as follows,

$$I = \frac{C-T}{C} \times 100$$

Where, C = Radial growth in control, T= Radial growth in treatment.

Dual culture Technique

The antagonistic activity of *Trichoderma* sp. and *Pseudomonas fluorescens* against *Pythium aphanidermatum* was studied in dual culture method (Falck, 1907). So the antagonist was evaluated by dual culture technique. The pathogen was inoculated on one side of the petri plate filled with 20 ml of PDA and antagonist was inoculated at exactly opposite side of the same plate by leaving 3-4 cm gap.

For this, actively growing five days old culture was used. In case of bacterial antagonist evaluation, bacterial antagonist was streaked in the plates and fungal discs were placed at one corner of the plates. After a period of incubation, when the growth of the pathogen was measured at 48, 72, 96 and 120 hrs. Percent inhibition over check was worked out according to the equation given by (Vincent, 1927).

PIRG=R1-R2/R1 X 100

Where, R1 = Radius of *Pythium aphanidermatum* colony in dual control plate;

R2 = Radius of *Pythium aphanidermatum* colony in dual culture plate

Testing of compatibility between fungal and bacterial bio-control agents

The method described by Nikam *et al.,* (2007) was used for *in - vitro* testing. Bacterial antagonist was streaked with the help of sterilized inoculating needle at one end of the

PDA Petri plate at a distance of 5 mm from the periphery of petriplate. The bacterial isolate was allowed to grow for 24 hr at 26±2°C. A 5 mm diameter plug from a 5- day-old culture of *Trichoderma harzianum* was placed in the opposite direction of the plate (approximately four cm apart). After 3 days incubation at 26±2°C the zone of inhibition, if any, was measured.

In- situ evaluation of fungicide, botanical and bio-agents

The *in-situ* experiment was conducted in greenhouse which is located in the research plot of the department of Plant Pathology, Sam Higginbotton University of Agriculture, Technology and Sciences, during the *Rabi* (February) season of 2017-18. Experiment was laid - out in completely randomized design with three replications in green house.

Details of Experiment

Experimental design : CRD

Number of replications : 3

Number of treatments : 8

Total number of trays : 24

Tray size : 23 x 15cm

Season : *Rabi*

Selected crop : Chilli

Variety : G-4

Seed rate : 1kg/ha

Nursery

The present experiment was conducted to test the efficacy of *Trichoderma harzianum*, *Pseudomonas fluorescens*, *Lantana camara* alone and combined applications against damping-off of chilli. The normal surface soil was collected randomly from the field and it was thoroughly mixed, sieved through screen. Then the soil was sterilized at 15 pound pressure per inch for 1 hrs consequently for two times with an interval of 24 hrs. The well sterilized plastic containers (disinfected with 5 % solution of copper sulphate) with drainage hole of 1 cm diameter at the bottom were filled with sterilized amended soil with 1kg of each. The sterilized soil was mixed with pathogen inoculum (@10gm kg^{-1} soil prior to one week of sowing).

The soil inoculated with pathogen was covered and not disturbed in order to facilitate the growth of the pathogen. Trays were irrigated regularly. Seed and soil treatments were given and chilli seeds were sown in these trays (@ 25 seeds per tray). Non-treated seeds sown in the infested soil served as positive control. Non-treated seeds sown in non - infested soil served as negative control.

Seed treatment

Seeds were smeared with bio - agents one day before sowing. Bio-agent culture from 1 Petri plate was used for smearing 48 seeds. Treated seeds were sown in the infested soil in plastic containers. For the combined application of bio-control agents the same was followed (Zagade *et al.*, 2012).

Soil Treatment

The 20% aqueous extract of *Lantana camara* was applied (alone and in combination with biocontrol agents) as soil drenching (100 ml/kg soil) (Gholve *et al.*, 2014).

Observations to be recorded

Germination percentage at 25 DAS

Damping - off incidence at 15 DAS

Shoot length and root length at 45 DAS

Disease incidence will be calculated by using the following formula

Disease incidence (%)

$= \frac{X1}{X2} X\ 100$ Where,

X_1 = No. of infected plants/leaves

X_2 = Total no. of plants/leaves

Results and Discussion

In vitro evaluation

Compatibility between *Trichoderma harzianum* and *pseudomonas fluorescens*

Absence of inhibition zone between the two biocontrol agents indicated that these were compatible with each other.

Efficacy of selected treatments on the mycelial growth of *Pythium aphanidermatum* by dual culture technique and poisoned food technique

The two isolates *Trichoderma harzianum*, *Pseudomonas fluorescens* and one botanical extract *Lantana camara* and fungicide carbendazim were screened against *Pythium aphanidermatum* by dual culture and poison food test for their antagonistic and fungicide ability.

Maximum inhibition growth was recorded in both T_7 - treated check (Carbendazim) (100 %) and T_4 - *Lantana camara* - (100 %), followed by T_5 - *Trichoderma harzianum* + *Psuedomonas fluorescens* (81.11 %), T_2 *Trichoderma harzianum* (76.66 %), T_3 - *Psuedomonas fluorescens* (72.22 %) as compared to the untreated control. At 7th DAI the percent mycelial inhibition of the all the treatments were significant over the control, however treatments (T_4 and T_7) are statistically non significant to each other.

In situ evaluation

Germination percentage of chilli seedlings at 25 DAS

Results revealed that maximum germination percentage was recorded in T_7 - Carbendazim [seed treatment] (80.00 %) followed by T_4 - *Lantana camara* [soil treatment] (76.00 %), T_6 - *Trichoderma harzianum* + *Pseudomonas fluorescens* + *Lantana camara* [seed + soil treatment] (70.66 %), T_5 - *Trichoderma harzianum* + *Pseudomonas fluorescens* [seed treatment] (68.00 %), T_2 - *Trichoderma harzianum* [seed treatment] (64.00 %), T_3 - *Pseudomonas fluorescens* [seed treatment] (60.00 %) when compared to the T_0 - relative control (treated control) (13.33 %) and T_1 - absolute control (untreated control) (78.66 %).

Efficacy of treatments on the disease incidence of *Pythium aphanidermatum* in chilli at 15 days after sowing

Results revealed that minimum disease incidence was recorded in T_7 - Carbendazim seed treatment (20.00 %) followed by T_4 - *Lantana camara* [soil treatment] (24.00 %), T_6 - *Trichoderma harzianum* + *Pseudomonas fluorescens* + *Lantana camara* [seed + soil treatment] (29.33%), T_5 - *Trichoderma harzianum*+ *Pseudomonas fluorescens* [seed treatment] (33.33 %), T_2 - *Trichoderma harzianum* [seed treatment] (36.00 %), T_3 - *Pseudomonas fluorescens* [seed treatment] (41.33 %) when compared to the T_0 - relative control (treated control) (86.66 %) and T_1 - absolute control (untreated control) (21.33%).

Table.1 Efficacy of selected treatments on the mycelial growth of *Pythium aphanidermatum* by dual culture technique and poisoned food technique

Tr no.	Treatments	Mycelial growth of the pathogen (mm)	Per cent growth inhibition
T_0	Relative control	90	0
T_2	*Trichoderma harzianum*	21	76.66
T_3	*Pseudomonas fluorescens*	25	72.22
T_4	*Lantana camara*	0	100
T_5	*Trichoderma harzianum* + *Pseudomonas fluorescens*	17	81.11
T_7	Carbendazim (check)	0	100
F –test		S	S
S.Ed. (±)		0.17	-
C.D. (at 0.05%)		0.428	-

Table.2 Germination percentage of chilli seedlings at 25 DAS

Tr no.	Treatments	Germination percentage
T_0	Relative control	13.33
T_1	Absolute control	78.66
T_2	*Trichoderma harzianum*	64
T_3	*Pseudomonas fluorescens*	60
T_4	*Lantana camara*	76
T_5	*Trichoderma harzianum* + *Pseudomonas fluorescens*	68
T_6	[*Trichoderma harzianum* + *Pseudomonas fluorescens*] + *Lantana camara*	70.66
T_7	Carbendazim (check)	80
F –test		S
S.Ed. (±)		1.03
C.D. (at 0.05%)		2.205

Table.3 Efficacy of treatments on the disease incidence of *Pythium aphanidermatum* in chilli at 15 days after sowing

Tr no.	Treatments	Disease Incidence
T_0	Relative control	86.66
T_1	Absolute control	21.33
T_2	*Trichoderma harzianum*	36.00
T_3	*Pseudomonas fluorescens*	41.33
T_4	*Lantana camara*	24.00
T_5	*Trichoderma harzianum* + *Pseudomonas fluorescens*	33.33
T_6	[*Trichoderma harzianum* + *Pseudomonas fluorescens*] + *Lantana camara*	29.33
T_7	Carbendazim (check)	20.33
F –test		S
S.Ed. (±)		1.02
C.D. (at 0.05%)		2.170

Table.4 Root length of chilli seedlings 45 days after sowing

Tr no.	Treatments	Root length (cm)
T_0	Relative control	2.26
T_1	Absolute control	3.80
T_2	*Trichoderma harzianum*	3.56
T_3	*Pseudomonas fluorescens*	3.20
T_4	*Lantana camara*	3.11
T_5	*Trichoderma harzianum* + *Pseudomonas fluorescens*	3.80
T_6	[*Trichoderma harzianum* + *Pseudomonas fluorescens*] + *Lantana camara*	3.75
T_7	Carbendazim (check)	4.29
F –test		S
S.Ed. (±)		0.10
C.D. (at 0.05%)		0.18

Table.5 Shoot length of chilli seedlings 45 days after sowing

Tr no.	Treatments	Shoot length (cm)
T_0	Relative control	2.10
T_1	Absolute control	4.31
T_2	*Trichoderma harzianum*	3.46
T_3	*Pseudomonas fluorescens*	3.26
T_4	*Lantana camara*	2.98
T_5	*Trichoderma harzianum* + *Pseudomonas fluorescens*	4.10
T_6	[*Trichoderma harzianum* + *Pseudomonas fluorescens*] + *Lantana camara*	3.90
T_7	Carbendazim (check)	4.52
F –test		S
S.Ed. (±)		1.02
C.D. (at 0.05%)		0.116

Root length and shoot length of chilli seedlings 45 days after sowing

Maximum root length was recorded in treatment in *Trichoderma harzianum*+ *Pseudomonas fluorescens* (3.80 cm) as compared to the treated control carbendazim (4.29 cm) and maximum shoot length was recorded in treatment *Trichoderma harzianum* + *Pseudomonas fluorescens* (4.1 cm) as to the treated control carbendazim (4.52 cm).

Based on the result it was observed that *Lantana camara* (soil application) proved to be most effective treatment under *in-vitro* and nursery conditions when compared to the carbendazim. The present study indicated that application of biocontrol agents singly and in combination with *Lantana camara* was found to be effective in controlling the chilli damping-off disease.

Hence, there is a need to search for an environmentally safe and economically viable strategy for the control of diseases and to reduce the dependence on the synthetic agrochemicals. In addition, bio- control agents are generally not effective if once the plants have been infected by *Pythium* and thus should not be considered curative control treatments. However, the present research findings are limited to one crop season under Allahabad agro-climatic condition as such more trials are required in future to validate the findings.

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