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Biological Control of Tomato Damping-off by *Gliocladium virei*

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ABSTRACT

***Gliocladium virens* (IMI No.304061) strongly antagonised *Pythium aphanidermatum*, incitant of tomato damping-off. *G.virens* hyphae ran parallel to, entwined around, penetrated into and killed the host hyphae. Cell-free culture filtrate of *G. virens* was inhibitory to *P. aphanidermatum*. Apron (5-25 µg/ml), Captaf (5-25 µg/ml) and San 506F (50-150 µg/ml) completely inhibited the growth of *P. aphanidermatum in vitro*. Cobox L, Thiram and Captaf (5 g/Kg) seed) gave 64.28, 58.93 and 51.79 per cent control of tomato damping-off in the glass house. *G. virens* was relatively less sensitive to Apron (25 µg/ml), Captaf (150 µg/ml), Cobox L (100 µg/ml) and Fytolan (100 µg/ml). Soil application of inoculum- rich mycelial and/or conidial preparation of *G. virens* on wheat-bran-saw dust (WBSD) media (3:1:3.5 w/w/v) (@ 10 g/2 kg soil) gave 82.77% control of tomato damping-off. Seed coating with conidial suspension (3.78×10^8 /ml) completely protected the seedlings in the glass house. Population of *Pythium* declined in soil amended with *G. virens*. Integration between WBSD preparation of the antagonist and fungicidal seed treatment yielded an additive effect of the two. Seed coating and soil amendment with *G. virens* separately resulted in 128.94, 162.38 and 107.89, 108.92 per cent increase in seedling stand over check in pre and post emergence phases respectively in the field nursery.**

KEY WORDS: Biological control, Antagonist, Seed coating, Damping - off, *Pythium aphanidermatum*, *Gliocladium virens*, Tomato

Damping-off of tomato, caused by *Pythium aphanidermatum* (Edson) Fitz., is the most serious disease appearing in the seedbeds. In most cases, it is responsible for the failure of tomato nursery. Biological control of seedling damping-off can be achieved by soil application of inoculum-rich mycelial and/or conidial preparation and seed coating with conidial suspension of the antagonist. *Gliocladium virens* Miller, Giddens and Fostar, a moniliaceous hyphomycete is a destructive mycoparasite parasitizing the host fungus internally (Tu and Varrataaja, 1981). Gliotoxin, viridin (Webster and Lomas, 1964), gliovirin and viridiol (Howell and Stipanovic, 1983) were reported to be produced by *G.virens*. *G. virens* increased the number of surviving seed lings of cotton in soil infested with *Rhizoctonia solani* (Howell, 1982). Root rot of peas caused by *P. ultimum* was significantly suppressed by *G. virens* and *G. catenulatum* (Teyes and Dirks, 1985).

In the present investigation, we tested the ability of *G. virens* to control tomato damping-off under glass house and field nursery conditions. Effect of integration of *G. virens* and fungicidal seed treatment was also studied.

MATERIALS AND METHODS

P. aphanidermatum was isolated from the hypocotyl of the damped-off tomato seedlings and maintained on potato-dextrose- agar (PDA) medium. Only one isolate of *G. virens* (IMI No.304061) was used.

Flasks with sorghum seeds soaked in 2% glucose solution were autoclaved, seeded with

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P. aphanidermatum and incubated for 7 days at 28 ± 1°C before inoculation (3 g/2 kg soil). Six mm discs each of *P. aphanidermatum* and *G. virens* were placed on sterilized, solidified PDA in Petri plate in such a manner that they lay opposite to each other 4 cm apart. Mycelial fragments were taken out from the zone of interaction of *P. aphanidermatum* and *G. virens* stained with cotton blue and examined. Wheat bran, saw dust and water (WBSD) (3:1:3.5 w/w/v) were mixed thoroughly, put in a polypropylene bag (50 g/bag), sealed on flame and autoclaved (Mukhopadhyay *et al.*, 1986). After cooling to room temperature, the bags were inoculated with four mycelial discs of *G. virens* from 3 day-old culture through four slits made by sterilized blades, sealed with adhesive tape and incubated at 28 ± 1°C for 7 days.

In order to test the effect of culture filtrate, *G. virens* was grown on potato-dextrose-broth (200 g peeled potato, 20 g dextrose, distilled water to make 1000 ml) for five days at 25°C. The culture was then filtered successively through cheese cloth, Whatman No.1 G3, G5 filters to get a cell-free filtrate. The culture filtrate was added to sterilized PDA at 40 ± 1°C in different proportion to achieve final concentration of 5, 10, 15% separately. After solidification, *P. aphanidermatum* mycelial disc was inoculated at the centre.

In vitro study with six fungicides namely, metalaxyl (Apron 35 SD 5, 15, 25 µg/ml), captan (Captaf 75 SD 50, 100, 150 µg/ml), copper oxychloride (Cobox L 150 g/L 25, 50, 100 µg/ml and Fytolan 75 WP 50, 100, 150 µg/ml), oxadixyl (San 506F 50 WP 50, 100, 150 µg/ml) and thiram (Thiram 75 WP 25, 50, 100 µg/ml) was conducted by poisoned food technique separately for *P. aphanidermatum* and *G. virens* (Grover and Moore, 1961).

An experiment was conducted in the glass house with *G. virens*. Sandy loam soil (pH 6.9) was taken in plastic pots (22 cm) at the rate of 2 kg/pot inoculated with pathogen and incubated for 3 days. Thereafter, culture of *G. virens* was added to it (5, 10, 15, 20 g/2 kg soil) and incubated for 7 days. "Pant Bahar", a tomato variety susceptible to damping-off was used. Seeds having 80% germination were sown (30 in each pot) at 3 cm depth. For seed treatment, a concentration of conidia of *G. virens* in water suspension was adjusted to 3.78 x 10^8 per ml. Tomato seeds (120 Nos.) were soaked in conidial suspension (50 ml) and allowed to dry overnight at room temperature before sowing. Soil samples were collected at 0, 10, 20 days after sowing of tomato seeds. The colony forming units (CFU) of *Pythium* population was monitored using soil sprinkling method on *Pythium*-selective medium (Peethambaran, 1975).

Tomato seeds were treated with all the six fungicides (0.5 %) separately. Such seeds were sown in soil inoculated with *P. aphanidermatum* after 7 days of incubation.

Soil moisture was maintained at 80 - 90% throughout the experimental period. Polythene bags were used to create favourable condition for tomato damping-off. Number of healthy seedlings was noted after 21 days of sowing. Per cent disease control was calculated using following formulae:

$$\% \text{ Seedling Mortality} = \frac{\text{Seedling stand in uninoculated soil} - \text{seedling stand in treated soil}}{\text{seedling stand in uninoculated soil}} \times 100$$

$$\% \text{ disease control} = \frac{\%\text{ mortality in control} - \%\text{ mortality in treatment}}{\%\text{ mortality in control}} \times 100$$

A field experiment was carried out at the Horticultural Research Centre, Patthachatta, Pantnagar (U.P.) during July - August, 1988 on a damping-off sick plot. The experiment was conducted in a randomised block design with 3 replicates. Plot size was 0.7 x 1 m and 20 cm raised. *G. virens* was applied either to soil (100 g WBSD preparation / m^2) or with seeds (3.78

x 10^8 conidia / ml). Seeds were treated with fungicides for integrated treatments. After 7 days of straw mulching, seeds were sown in rows (3.5 g in each plot in 7 rows). Seedlings were protected from monsoon rains providing appropriate sheds. Observations on seedlings stand were made twice; 4 days after emergence and a month after sowing. Per cent increase in seedling stand was calculated using the following formula:

$$\% \text{ increase in seedling stand over control} = \frac{\text{Seedling stand in treatment} - \text{seedling stand in control}}{\text{seedling stand in control}} \times 100$$

RESULTS AND DISCUSSION

Initially both *G. virens* and *P. aphanidermatum* grew together in dual culture without showing any zone of demarccation. After the colonies met, growth of *P. aphanidermatum* ceased, and its colony was over grown in 96 h by *G. virens* which covered the entire plate. Attempts to reisolate *P.aphanidermatum* from areas where it had been growing vigourously resulted in recovery of *G. virens* only indicating that *G. virens* was capable of attacking and killing *P. aphanidermatum* in culture.

Upon reaching the host hyphae, *G. virens* hyphae ran parallel to and later coiled around the *P. aphanidermatum* hyphae or produced appressorium or hook- shaped contact branches. Sometimes *G. virens* penetrated the host hyphae and grew intracellularly. Howell and Stipanovic (1983) reported inhibition of *P. ultimum* by *G. virens* without parasitization. Formation of septa in the host hyphae at a point away from zone of contact between the two fungi may probably be a defense mechanism (Mukhopadhyay *et al.*, 1986).

There was a positive correlation between the amount of cell-free culture filtrate and growth inhibition of *P. aphanidermatum*. Inhibition of 12 - 35% was obtained using 5 - 15% culture filtrate (Table 1). *G. virens* produces certain metabolites in culture filtrate which are pernicious to *P. aphanidermatum*. This provides a superfluous chemical weapon to *G. virens* to suppress the pathogen. Gliovirin, a diketopiperazine, was isolated from the culture filtrate of *G. virens* (Howell and Stipanovic, 1983).

All the fungicides tested *viz.*, Apron (5-25 µg/ml), Captaf (5-25 µg/ml), Cobox L (25-100 µg/ml), Fytolan (50-100 µg/ml), San 506F (50-150 µg/ml) and Thiram (25-100 µg/ml) were found to be inhibitory to *P. aphanidermatum*. Apron, San 506F and Captaf were the most effective and completely inhibited the growth of the test fungus, followed by Thiram (81.49-100%), Fytolan (64.81-88.89 %) and Cobox L (44.44-79.63%) (Table 2).

Except San 506F and Thiram, none of the fungicides, namely, Apron (5-25 µg/ml), Captaf (50-150 µg/ml), Cobox L (25-100 µg/ml) and Fytolan (50-150 µg/ml) showed inhibitory effect on *G. virens*. San 506F (50-150 µg/ml), Thiram (150 µg/ml) imposed inhibitory action on *G. virens*. On further incubation, *G. virens* grew and sporulated equally in all the above treatments. Insensitiveness of *G. virens* to considerably high concentrations of metalaxyl opens an avenue for exploitation in the integrated disease management.

Table 1. Effect of different concentrations of culture filtrate of *Gliocladium virens* on the radial growth of *Pythium aphanidermatum*, bioassayed in PDA, incubated at 28 ± 1°C for 24 h

Treatments %	Radial growth (mm*)	Inhibition %
5	79.00	12.22 (20.35)
10	67.00	25.56 (30.36)
15	59.80	35.78 (36.70)
Check	90.00	- -
CD (0.05)	3.29	4.34

* Average of five replications
Figures in the parentheses represent arc sin x $\sqrt{\text{percentage}}$

Table 2. Effect of fungicides on the growth of *Pythium aphanidermatum in vitro* on PDA at 28 ± 1°C

Fungicides	Chemical name	Concentration (µg/ml)	Radial growth (mm)	Inhibition %
Apron 35 SD	Methyl DL-N-(2,6 di-			
	methyl phenyl) - N (2, 5	00.00	100.00	
	methoxy acetyl)	15	00.00	100.00
	alaninate	25	00.00	100.00
Captaf 75 SD	N-trichloro methyl-	5	00.00	100.00
	thio-4-cyclo hexane	15	00.00	100.00
	1,2-di carboximide	25	00.00	100.00
Cobox L	Copper oxychloride	25	50.00	44.44
150 g/L		50	35.00	61.11
		100	18.33	79.63
Fytolan 75 WP	Copper oxychloride	50	31.67	64.81
		100	21.67	75.92
		150	10.00	88.89
San 506F 50 WP	Oxadixyl (2, meth-			
	oxy-N (2 OXO-1, 3 Oxazolidin			
	2yl) acet-2, 6 xylidide) +	50	00.00	100.00
	copper oxychloride in	100	00.00	100.00
	ratio of 10 : 40	150	00.00	100.00
Thiram 75 WP	Tetra methyl	25	16.66	81.49
	thiuram	50	00.00	100.00
	disulphide	100	00.00	100.00
Check		-	90.00	-
CD (0.05)			3.45	3.94

Soil application of inoculum-rich mycelial and/or conidial preparation of *G. virens* on WBSD media (10 g/2 kg soil) recorded the lowest seedling mortality (11.90 %), followed by 5 g (16.67%), 15 g (44.05%) and 20 g (47.62%). Control recorded the maximum (69.05%) in the glass house. Per cent disease control was maximum at the dose of 10 g WBSD preparation per 2 kg pot soil. Coating seeds with conidial suspension (3.78×10^8 /ml) completely eliminated seedling damping-off and was superior to the former (Fig.1A). Seed coating enables the bio-control agent to be introduced to specific courts of infection where seedlings are most susceptible. *G. virens* as seed coating allows its use in small quantities and methods involving application in larger quantities may not be economically feasible. The ability of *G. virens* to be effective as seed coating is probably associated with its ability to grow and sporulate on seeds and therefore

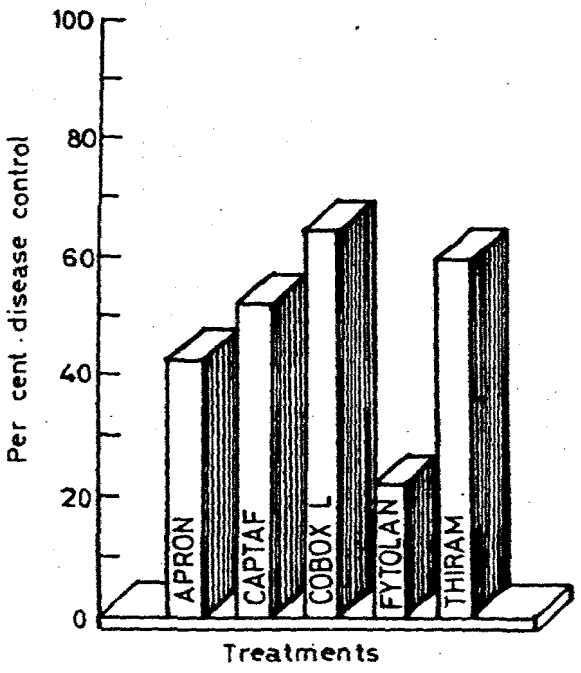

Fig. 1A. Effect of soil amendment and seed coating with *G. virens* on tomato damping-off

Table 3. Colony forming unit (CFU) of *Pythium aphanidermatum* in pot soil amended with various doses of WBSD preparations of *G.virens* at different time intervals

G. virens rate in g per 2 kg soil	CFU per g soil 10 days after sowing		
	0	10	20
5	184.50	151.25	162.50
10	190.00	116.50	142.00
15	219.50	102.00	131.50
20	223.00	87.50	107.00
Seed coating*	209.00	96.00	118.50
Check	239.50	197.50	198.00
CD (0.05)	66.6	11.2	4.2

* Concentration of conidia 3.78×10^8 / ml.

becomes established in the rhizosphere. Colonization of rhizosphere as in *Gliocladium* (Chao *et el.*, 1986) and mycoparasitism as in *Trichoderma* (Lifshitz *et el.*, 1986) were not observed in the present case.

In the antagonist amended soil, population of *Pythium* was on the wane with time and with increased *G. virens* application rates. Colony forming units (CFU) /g was lowest in 20 g *G. virens* mass culture in both the soil samples 10 days (87.5 x 10) and 20 days (107.0 x 10) after sowing. *Pythium* population was minimum (96.0 x 10 CFU /g) at 10 days after sowing of *G. virens* spore-coated seeds (Table 3). The decline in the population of *Pythium* in soil treated with *G. virens* may be largely attributed to the antagonistic action of *G. virens*.

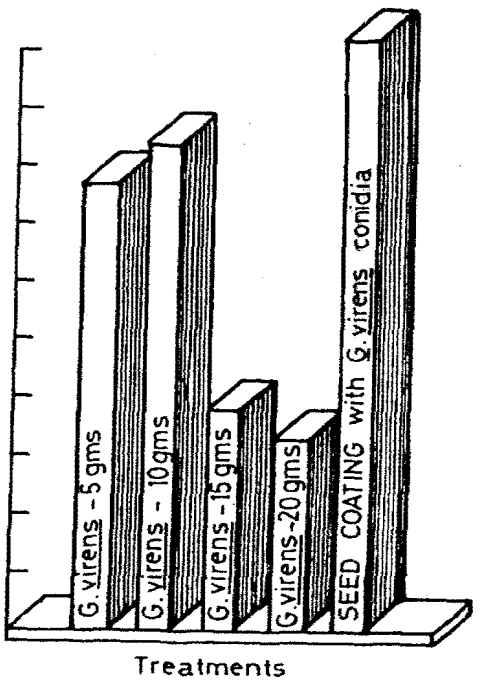

Fig. 1B. Effect of fungicidal seed treatment on tomato damping-off in glass house

Cobox L gave highest control of tomato damping-off (64.28%), followed by Thiram (58.93%), Captaf (51.79%), Apron (42.85 %) and Fytolan (21.43 %) in the glass house (Fig. 1B). San 506F was not effective against tomato damping-off.

Combination of *G. virens* and Thiram produced highest disease control (100 %) followed by *G. virens* + captaf (88.75%), *G.virens* + Apron (82.49%), *G.virens* + Cobox L (71.25 %) and *G. virens* + Fytolan' (56.24%). This juxtaposition of fungicides and *G. virens* improved the biocontrol efficacy. The result of integration was additive. Fungicides might have weakened the pathogen and made it vulnerable, thus allowing *G.virens* to become more virulent on a weak pathogen (Upadhyay and Mukhopadhyay, 1986). Sensitivity of *G. virens* to the fungicides was previously tested in order to integrate the former with the latter. The fungicides may take care of the pathogen in the rhizosphere and spermosphere whereas *G. virens* does so beyond the rhizosphere (Mukherjee *et al.*, 1989). This probably gave an additive effect.

In the field experiment, the pathogens were so aggressive that they formed a white cottony

Table 4. Effect of biological, fungicidal and integrated control on the pre and post emergence damping-off of tomato under field condition

Treatments	Pre-emergence damping-off		Post-emergence damping-off	
	No. of seedlings/plot* (0.7 sq.m)	% increase in seedling stand over check	No. of seedlings/plot* (0.7 sq.m)	% increase in seedling stand over check
Seed coating with conidial suspension of *G.virens* $	87.00	128.94	107.66	162.58
G.virens on WBSD media @ 100 g/sq.m	79.33	107.89	85.66	108.62
Thiram	158.33	315.78	134.00	226.82
G. virens + Thiram	137.33	260.52	118.33	188.60
Cobox L	68.00	78.94	67.00	63.41
G. virens + Cobox L	174.00	357.89	112.33	173.17
Check	38.00	-	41.00	-
CD (0.05)	45.23	-	58.46	-

* Average of three replications
$ Concentration of conidia 3.78×10^8 per ml

mycelial mat on the soil. In the pre- emergence phase, combination of Cobox L + *G. virens* increased seedling stand over check by 357.89 % followed by seed treatment with Thiram alone (315.78 %), *G. virens* + Thiram (260.52 %) and Cobox L (78.94 %). In the post emergence phase, Thiram alone gave 226.82 % increase in stand over check followed by *G. virens* + Thiram (188.60 %), *G. virens* + Cobox L (173.17 %) and Cobox L (63.14%). Seed coating and soil amendment with *G. virens* separately resulted in 128.94, 162.38 and 107.89, 108.92 per cent increase in stand respectively in the pre and post emergence phases (Table 4). Post-emergence phase was more severe than pre-emergence phase. Thiram alone proved superior to combination of *G. virens* + Thiram. Thiram at the dose applied may inhibit *G. virens* which is supported by *in vitro* observation. Only biological control either seed coating or soil amendment with *G. virens* could give the seedlings total protection after emergence.

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