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Research Article

Evaluation of gliotoxin phytotoxicity and gliotoxin producing *Trichoderma virens* for the suppression of damping off of tomato

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ABSTRACT: Gliotoxin is a potent antibiotic showing antifungal activity against various phytopathogenic fungi. It is produced by Q strains of *Trichoderma virens* and gliotoxin non-producing strains of *T. virens* are designated as P strains. There is no detailed study on effect of gliotoxin on suppression of damping off of tomato caused by *Pythium aphanidermatum* and its phytotoxicity effect on tomato plants. Thus, the present study was carried out to assess the effect of gliotoxin on inhibition of mycelial growth of *P. aphanidermatum,* its phytotoxicity effect on tomato and its role on the suppression of damping off of tomato. Culture filtrates of Q strains of *T. virens* containing gliotoxin highly inhibited the mycelial growth of *P. aphanidermatum* compared to that of P strains of *T. virens*. Purified gliotoxin but not bis-thiomethyl gliotoxin effectively inhibited the mycelial growth of *P. aphanidermatum.* Tomato seeds treated with purified gliotoxin did not inhibit the germination of seeds, its root and shoot length even at higher concentration that is at 1000 ppm (fivefold inhibitory concentration against *P. aphanidermatum*). Foliar spray of gliotoxin on tomato plants did not show any phytotoxic effect at lower concentration but showed scorching effect at higher concentration. Seed treatment with gliotoxin producing Q strains of *T. virens* showed greater suppression of damping-off tomato compared to P strains of *T. virens*. This study clearly showed that gliotoxin producing *T. virens* could be used in suppression of damping-off disease incidence in tomato.

KEY WORDS: Bio-control, damping-off, gliotoxin, *Trichoderma virens*

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INTRODUCTION

Damping-off disease caused by *Pythium* spp. in vegetable crops is economically very important worldwide (Whipps and Lumsden, 1991). Rapid germination of sporangia of *Pythium* in response to root exudates followed by immediate infection make the management strategies of the pathogen very difficult (Nelson, 1987; Osburn *et al*., 1989; Whipps and Lumsden, 1991). Though fungicides are effective in controlling the damping-off disease, development of fungicide resistance in *Pythium* strains, fungicide residues and phytotoxicity is major concerns leading to environmental pollution and human health hazards. Since the pathogen is soil-borne and survives in soil as oospore for several years, biological control is one of the alternative strategies to chemical control due to its eco-friendly nature and environmentally safe approach.

Soil has enormous untapped potential fungal and bacterial antagonistic microbes such as *Trichoderma* spp. and Plant Growth Promoting Rhizobacteria (PGPR) showing antagonistic effects against various soil-borne phytopathogenic fungi. Various *Trichoderma* spp. are used for management of soil-borne diseases (Mukherjee *et al*., 2013). Among the various *Trichoderma* spp. *T. virens* is one of the

beneficial soil saprophytes and act as a biocontrol agent for suppression of various soil-borne plant pathogens. Though *T. virens* inhibits the phytopathogenic fungi through various mechanisms such as competition, mycoparasitism, antibiosis and induced systemic resistance, antibiosis by production of gliotoxin is one of the important traits by which *T. virens* inhibits the soil-borne phytopathogenic fungi and bacteria. Among the various species of *Trichoderma*, only *T. virens* produces gliotoxin. All strains of *T. virens* do not produce gliotoxin. Based on their ability to produce gliotoxin, *T. virens* strains are classified into two groups namely P and Q group. The strains belonging to P group do not produce gliotoxin and Q group produce gliotoxin (Dennis and Webster, 1971; Webster and Lomas, 1964; Atanasova *et al*., 2013; Bulgari, *et al*., 2020; Howell, 1999; Park *et al*., 1992).

Gliotoxin is a disulfide bond containing antimicrobial compound and belongs to an epidithiopiperazines (ETPs) group (Gardiner and Howlett, 2005; Dolan *et al*., 2015). It has broad-spectrum antifungal and antibacterial activity on various phytopathogenic fungi and bacteria. Both purified gliotoxin and gliotoxin producing *T. virens* are inhibitory against several phytopathogenic fungi such as *Rhizoctonia solani, Botrytis cinerea, Colletotrichum* spp., *Pythium ultimum, Fusarium* spp. etc (Wright, 1956; Harris and Lumsden, 1997; Highley, 1997; Howell, 1999). *T. virens* mutant strain lacking gliotoxin production was less effective in suppression of damping off disease in cotton and zinnia plants compared to its isogenic wild-type strain (Wilhite *et al*., 1994; Vargas *et al*., 2014). *T. virens* strain G-20, which produces the highest amount of gliotoxin, was the first microbial agent commercially developed and marketed as a bio-pesticide in the name of SoilGard® (Certis, USA) (Lumsden *et al*., 1992). In addition to disease suppression, *T. virens* improves seed germination and enhances the plant growth and yield (Contreras-Cornejo *et al*., 2009; Fiorentino *et al*., 2018; Halifu *et al*., 2019).

Earlier from our lab, we isolated several P and Q strains of *T. virens* from rhizosphere soil of various crop plants and evaluated their gliotoxin production ability in various culture media (Oviya, 2019). Since there is no detailed study on the efficacy of gliotoxin producing *T. virens* in comparison with gliotoxin non-producing strains on the suppression of tomato damping off disease, the present study was carried out to assess the *in vitro* effect of gliotoxin on inhibition of *P. aphanidermatum* causing damping off disease in tomato and phytotoxicity of purified gliotoxin on tomato seed germination, its root and shoot growth and phytotoxicity effect on leaves. Finally, the efficacy of gliotoxin producing *T. virens* was compared with that of gliotoxin non-producing *T. virens* on the management of damping-off of tomato.

MATERIALS AND METHODS

Fungal culture and growth media

Pythium aphanidermatum was isolated from the infected tomato plant and its coenocytic mycelial growth pattern, vesicle formation on the sporangia was observed under light microscope. It was confirmed as *P. aphanidermatum* by ITS sequence analysis. Several gliotoxin producing *T. virens* (Q strains) and gliotoxin non- producing *T. virens* (P strains) were isolated using gliotoxin amended medium as described by Park *et al.* (1992) and Howell (1999). They were identified as *T. virens* by ITS sequence analysis and based on *Gliocladium* type of conidiophore. They were grouped into Q strain and P strain based on gliotoxin production (Oviya, 2019).

Analysis of gliotoxin production by *T. virens*

Six isolates of *T. virens* (three gliotoxin producing Q strains *viz.,* 15-2, 16-1 and 16-2 and three gliotoxin non-producing P strains *viz*., 1A, 2A and 3A) were used in this study. They were tested for gliotoxin production by Thin Layer Chromatography (TLC) to confirm as Q strains (gliotoxin producer) and P strains (gliotoxin non-producers). One hundred microlitre conidial suspensions (containing 1×10^7 conidia/ml) of *T. virens* was inoculated in 50 ml of Weindling medium and incubated for four days in a shaker at 30°C; 150 rpm. One millilitre of the culture filtrate was aliquoted in the Eppendorf tube and extracted with half the volume of chloroform. The chloroform extracts were separated; dried completely; then re-dissolved in 50 µl of methanol and spotted on TLC silica gel 60 F_{254} plate (Merck, USA) for analytical purpose. The extracts were resolved using chloroform: acetone (70:30 v/v) solvent mixture. The presence of gliotoxin on TLC was visualized under UV light at 254 nm and again confirmed by spraying the TLC plates with 2% silver nitrate dissolved in 50% aqueous acetone. The presence of gliotoxin produced by *T. virens* was confirmed by comparing the retention time of the standard gliotoxin (purity ≥ 98%) purchased from Sigma, USA.

Assay of *In vitro* antifungal activity of culture filtrate of *T. virens*

In vitro antifungal activity of culture filtrates of *T. virens* were tested against *P. aphanidermatum*. Three Q strains of *T. virens viz.,* 15-2, 16-1, and 16-2 and three P strains of *T. virens viz.,* 1A, 2A and 3A were inoculated in Weindling medium and grown for four days. PDA medium was incorporated with culture filtrates of *T. virens* at 1 and 5 % levels separately. PDA medium without culture filtrate served as control. Actively growing culture discs of *P. aphanidermatum* was inoculated on the medium and incubated at 25°C. Mycelial growth on the culture filtrate amended PDA medium and control PDA medium was measured. Per cent growth inhibition by the culture filtrate of *T. virens* was calculated using the formula

I= (C-T)/C × 100.

Where,
C – Mycelial growth in control plate,
T – Mycelial growth in culture filtrates of *T. virens* amended PDA plates
I – Per cent inhibition of mycelial growth

Assay of *In vitro* antifungal activity of gliotoxin and bis-thiomethyl gliotoxin

Gliotoxin is produced by *T. virens* at the early logarithmic growth phase. Gliotoxin is converted into bis-thiomethyl gliotoxin during late stationary growth phase (Premalatha, 2020). Large scale production of gliotoxin and bis-thiomethyl gliotoxin was carried out using preparative TLC. Antifungal effect of gliotoxin and bis-thiomethyl gliotoxin was tested by poisoned food technique. Required volume of gliotoxin (for making 25, 50, 100 and 150 ppm levels in 5 ml of PDA medium) was aliquoted in the Eppendorf tube from the stock gliotoxin solution and dried. Then dried gliotoxin solution was redissolved uniformly in 50 µl of methanol. A sterilized PDA medium was amended (poisoned) with gliotoxin at 25, 50, 100 and 150 ppm levels. Similarly, sterilized PDA medium was incorporated with bis-thiomethyl gliotoxin at similar concentration levels used for antifungal assay of gliotoxin. PDA medium without addition of gliotoxin or bis-thiomethyl gliotoxin served as control. PDA medium amended with 50 µl of methanol also served as control. Actively growing culture discs of *P. aphanidermatum* was inoculated at the centre of the media plate and incubated at 25ºC until the growth of the mycelium covered in any one of the treatments. Per cent growth inhibition by the gliotoxin or bis-thiomethyl gliotoxin was calculated using the formula

I= (C-T) / C × 100.

Where,
C – Mycelial growth in control plate,
T – Mycelial growth in gliotoxin or bis-thiomethyl gliotoxin amended PDA plates
I – Per cent inhibition of mycelial growth

Phytotoxic effects of gliotoxin on tomato seed germination

Gliotoxin was prepared in 1% methanol. To assess the phytotoxic effects of gliotoxin, tomato seeds were treated for overnight with different concentrations of purified gliotoxin *viz.,* 100, 250, 500, 750 and 1000 ppm and next day seeds were placed in germination paper by roll towel method (ISTA, 1993). The seeds soaked with 1% methanol served as a control. The biometric observations *viz.,* germination per cent, root length and shoot length were recorded. The vigour index was calculated using the following formula:

Vigour index = per cent germination × seedling length (shoot length + root length) (Abdul-Baki and Anderson, 1973).

Phytotoxic effects of gliotoxin on tomato plants

The phytotoxicity effect of purified gliotoxin (prepared in 1% methanol) on tomato plants was tested by spray method under glass house conditions. The purified gliotoxin at different concentrations *viz.,* 100, 250, 500, 750 and 1000 ppm were sprayed on tomato leaves and incubated for a week. The tomato leaves sprayed with 1 % methanol served as a control. After spraying with gliotoxin, the plants were observed on daily basis for phytotoxicity such as yellowing, scorching etc.

Management of damping off disease by seed treatment of gliotoxin producing and gliotoxin non-producing strains of *T. virens*

Pots were filled with sterilized soil that was made sick by inoculating *P. aphanidermatum* (5% w/w) mass-multiplied in sand maize medium. Conidial suspension of *T. virens* was prepared in sterile water containing 0.5% Carboxy Methyl Cellulose (CMC) as sticking agent. Tomato seeds were treated with conidial suspension ($1x10^5$conidia/ml) of various gliotoxin producing *T. virens* Q strains *viz.,* 15-2 16-1, and 16-2 and gliotoxin non-producing *T. virens* P strains *viz.,* 1A, 2A and 3A. Fifty seeds per pot were sown for each treatment with three replications. Tomato seeds were densely sown in order to create conducive nature for damping off infection. Pathogen-inoculated treatment served as positive control. Pathogen un-inoculated treatment served as negative control. Seeds treated with metalaxyl (4g/kg of seeds) served as chemical control. Disease incidence and plant height were recorded at 15 days after sowing. All treatments were replicated thrice. The experiment was repeated twice.

The details of the treatments are as follows:

S. No	Tr. No	List of treatments
1	T_1	*T. virens* isolate 15-2
2	T_2	*T. virens* isolate 16-1
3	T_3	*T. virens* isolate 16-2
4	T_4	*T. virens* isolate 1A
5	T_5	*T. virens* isolate 2A
6	T_6	*T. virens* isolate 3A
7	T_7	Metalaxyl
8	T_8	Pathogen inoculated control
9	T_9	Pathogen uninoculated control

RESULTS AND DISCUSSION

Analysis of gliotoxin production by *T. virens*

In this study, three gliotoxin producing *T. virens* Q strains *viz*., 15-2, 16-1 and 16-2 and gliotoxin non-producing *T. virens* P strains *viz*., 1A, 2A and 3A were used and all these strains appeared greenish color and showed cultural variability (Fig. 1A). Upon TLC analysis for gliotoxin production, all the Q strains of *T. virens viz*., 15-2, 16-1 and 16-2 produced gliotoxin as revealed from the blue band upon exposure of the TLC plate to UV light (Fig. 1B upper panel) and also brown band upon spraying the TLC plate with silver nitrate (Fig. 1B lower panel). The Rf value was found to be as 0.65 for gliotoxin. Among the several *Trichoderma* spp. only *T. virens* produced gliotoxin (Webster and Lomas, 1964). Howell *et al*. (1993) characterized several *T. virens* and grouped them into P and Q strains based on gliotoxin production. Gliotoxin producing Q strains could be selectively isolated on the PDA medium amended with gliotoxin which prevent the growth of other fungi (Howell, 1999; Park *et al*., 1992).

Antifungal activity of culture filtrates of *T. virens* against *P. aphanidermatum*

Antifungal activity of culture filtrate of *T. virens* against fungal pathogen indicates the production of potent antimicrobial compound. Thus, antifungal effect of culture filtrates of *T. virens* strains was tested by poisoned food technique by incorporating PDA medium with culture filtrates at 1% and 5% levels. Culture filtrates of gliotoxin producing strains, when incorporated at 1% level, showed upto 57 to 60% inhibition of mycelia growth of *P. aphanidermatum* and complete inhibition was observed at 5% level of culture filtrate amendment. Whereas, culture filtrates of gliotoxin non-producing strains showed 23 to 41% inhibition of mycelia growth at 1% of culture filtrate amendment and 53 to 64% of mycelia growth inhibition was observed at 5% level of culture filtrate amendment. This study revealed that the culture filtrates of gliotoxin producing *T. virens* strains effectively inhibited the mycelial growth of *P. aphanidermatum* compared to gliotoxin non-producing strains of *T. virens* (Fig. 2; Table 1). Earlier studies also indicated that culture filtrates of *T. virens* were highly effective against the mycelial growth of *Rhizoctonia solani* (Weindling, 1934). Similarly, culture filtrates containing gliotoxins were very highly inhibitory to

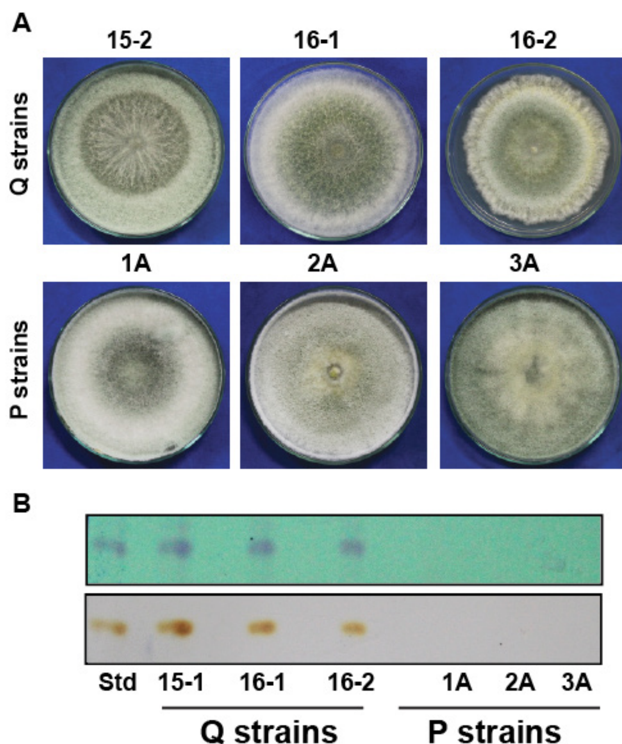

Fig. 1. Analysis of gliotoxin production by *T. virens*. Cultural variability of six isolates of *T. virens* on PDA medium B. Analysis of gliotoxin production by Thin Layer Chromatography

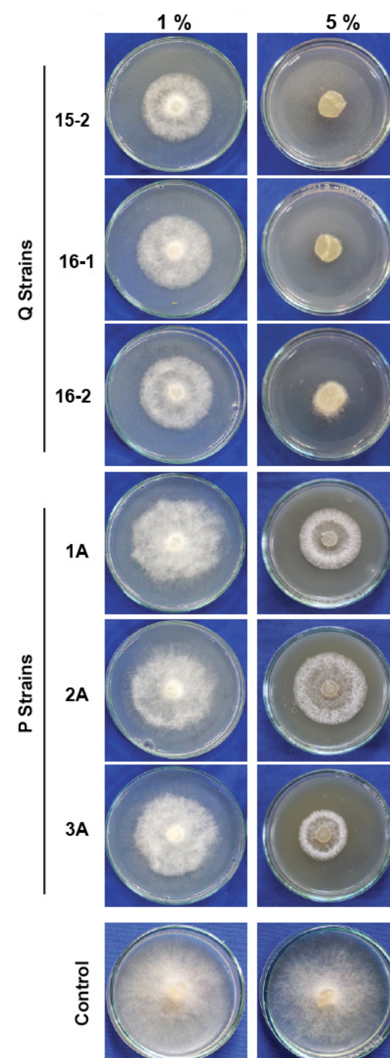

Fig. 2. Antifungal activity of culture filtrates of *T. virens* on the mycelial growth of *P. aphanidermatum*. Inhibitory effect of 1% and 5% culture filtrates of P and Q strains of *T. virens* was tested against the mycelial growth of *P. aphanidermatum*.

Table 1. Antifungal activity of culture filtrates of *T. virens* on the mycelial growth of *P. aphanidermatum*

S. No	*T. virens* isolates	Mycelial growth (cm)*		Growth inhibition (%)	
		1 %	5 %	1 %	5 %
1	Q strain 15-2	3.70[f]	0.50[e]	58.89	94.44
2	Q strain 16-1	3.90[e]	0.00[f]	56.67	100.00
3	Q strain 16-2	3.60[f]	0.00[f]	60.00	100.00
4	P strain 1A	5.30[d]	3.95[c]	41.11	56.11
5	P strain 2A	5.80[c]	4.23[b]	35.56	53.00
6	P strain 3A	6.90[b]	3.25[d]	23.33	63.89
7	Control	9.00[a]	9.00[a]	0.00	0.00
	CD value (0.05%)	0.18	0.13		

The treatment means are compared using Duncan Multiple Range Test (DMRT).
In each row, mean values followed by a common letter are not significantly different (p=0.05)
*Values are means of three replications

Colletotrichum lini, Fusarium caeruleum, Botrytis alli (Brian and Hemming, 1945). Culture filtrates of wild type *T. virens* inhibited *R. solani* by 70% whereas that from the isogenic mutant deficient in gliotoxin production inhibited by 25-30% (Howell and Stipanovic, 1995). Thus, the present study clearly showed that gliotoxin present in the culture filtrates is highly inhibitory to *P. aphanidermatum.*

Antifungal activity of purified gliotoxin and bis-thiomethyl gliotoxin against *P. aphanidermatum*

The antifungal activity of purified gliotoxin and bis-thiomethyl gliotoxin on mycelia growth of *P. aphanidermatum* was tested by poisoned food technique by amending PDA medium with gliotoxin or bis-thiomethyl gliotoxin and *P. aphanidermatum* was inoculated at centre of the medium. The gliotoxin completely inhibited the mycelial growth of *P. aphanidermatum* at 150 ppm followed by 64% mycelial growth inhibition at 100 ppm. The minimum inhibitory effect (6%) was noticed at 25 ppm concentration levels. In contrast, no inhibitory effect was observed even at 150 ppm concentration level of bis-thiomethyl gliotoxin amendment in PDA medium (Fig. 3; Table 2). This study clearly revealed that the gliotoxin had antifungal property whereas modified gliotoxin did not. Similar to the present study, purified gliotoxin was shown to be strongly inhibitory against the sporangial germination and growth of *P. ultimum,* mycelial growth of *R. solani* and sclerotial germination and growth of *Sclerotium rolfsii* (Lumsden *et al*., 1992).

Assessment of phytotoxic effects of gliotoxin on tomato plants

To test whether gliotoxin inhibit germination of tomato seeds or not, tomato seeds were treated with gliotoxin at 100, 250, 500, 750 and 1000 ppm levels and placed in germination paper in roll towel method for assessing phytotoxicity effect. Seeds treated with gliotoxin even at maximum level of 1000 ppm germinated normally and shoot length and root length also appeared normal as in control treatment without gliotoxin treatment. There is no much difference on the vigour index of gliotoxin treated seeds and the control seeds without gliotoxin treatment (Fig. 4; Table 3).

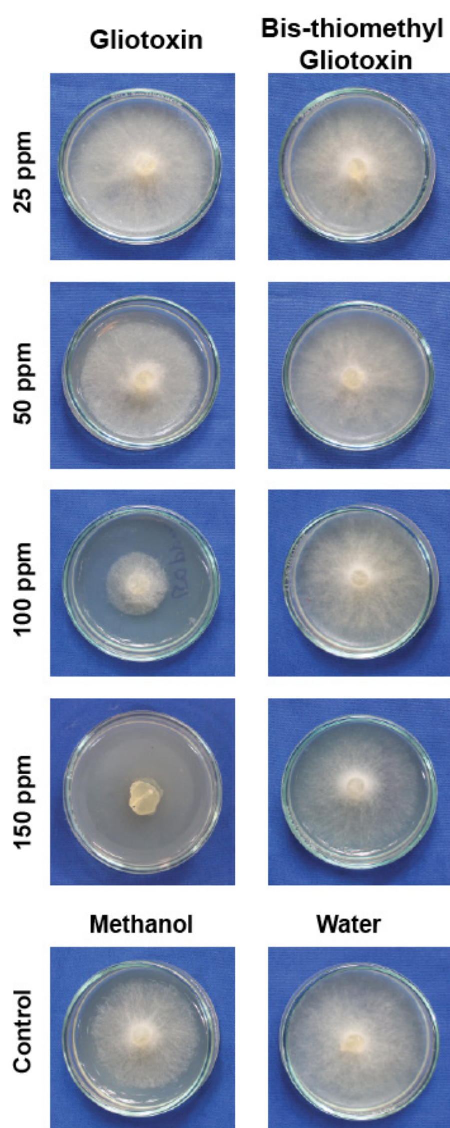

Fig. 3. Antifungal activity of gliotoxin and bis-thiomethyl gliotoxin on the mycelial growth of *P. aphanidermatum*. Inhibitory effect of gliotoxin and bis-thiomethyl gliotoxin at different concentration levels was tested against the mycelial growth of *P. aphanidermatum*.

Thirty days old tomato plants were sprayed with purified gliotoxin at different concentration levels *viz*., 100, 250, 500, 750 and 1000 ppm and observed for phytotoxicity effect. There is no phytotoxic effect of gliotoxin when the leaves were sprayed upto to 500 ppm concentration levels. However, scorching effect was noticed when the leaves were sprayed

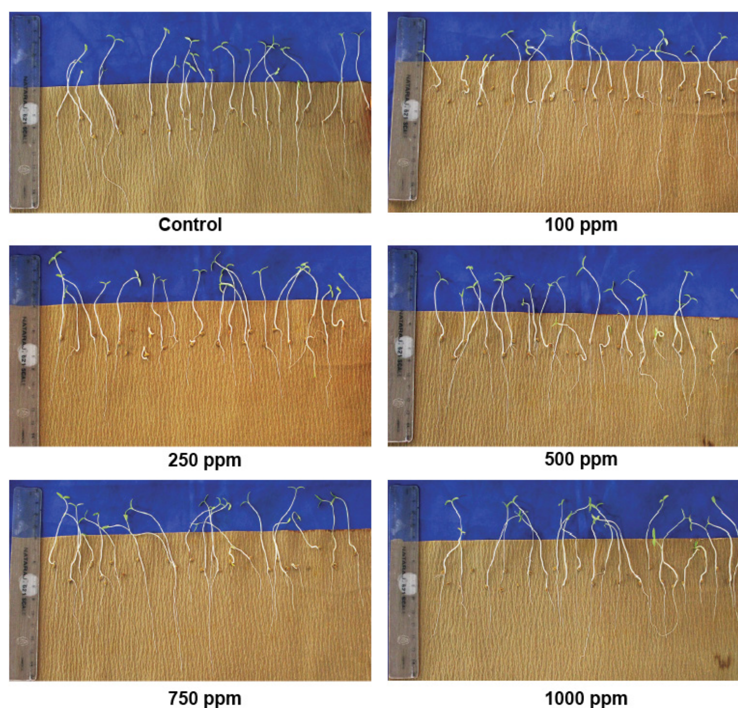

Fig. 4. Assessment of phytotoxic effects of purified gliotoxin on tomato plants by roll towel method Seeds were treated with different concentration of gliotoxin.

Table 2. Antifungal activity of gliotoxin and bis-thiomethyl gliotoxin on the mycelial growth of *P. aphanidermatum*

S. No	Gliotoxin concentration (ppm)	Mycelial growth (cm)*	Growth inhibition (%)	Bis-thiomethyl gliotoxin concentration (ppm)	Mycelial growth (cm)*	Growth inhibition (%)
1	25	3.76^{b}	6.00	25	4.00^{a}	0.00
2	50	3.29^{c}	17.60	50	4.00^{a}	0.00
3	100	1.44^{e}	64.00	100	3.92^{a}	2.00
4	150	0.00^{f}	100.00	150	3.79^{b}	5.00
5	Methanol	2.60^{d}	35.00	Methanol	2.60^{c}	35.00
6	Control	4.00^{a}	0.00	Control	4.00^{a}	0.00
	CD value (0.05%)	0.13			0.09	

The treatment means are compared using Duncan Multiple Range Test (DMRT).
In each row, Mean values followed by a common letter are not significantly different (p=0.05)
*Values are means of three replications

Table 3. Assessment of phytotoxic effects of purified gliotoxin on tomato plants by roll towel method

S. No	Seed treatment with purified gliotoxin (ppm)	Germination (%)	Shoot length (cm)*	Root length (cm)*	Vigour index
1	Control (Water alone)	92	7.33	5.39	1171.16^{a}
2	100	89	7.12	4.93	1076.23^{bc}
3	250	88	7.14	4.76	1047.26^{c}
4	500	87	7.25	5.04	1068.94^{bc}
5	750	87	7.27	5.15	1080.08^{bc}
6	1000	88	7.34	5.26	1107.63^{b}
	CD value (0.05%)				49.52

The treatment means are compared using Duncan Multiple Range Test (DMRT).
In each row, Mean values followed by a common letter are not significantly different (p=0.05)
*Values are means of three replications

Table 4. Effects of gliotoxin producing and non-producing *T. virens* on the management of damping off of tomato

S. No	Tr. No	Treatments	TRAIL – I		TRAIL - II	
			Disease incidence (%)*	Plant height (cm)*	Disease incidence (%)*	Plant height (cm)*
1	T_1	*T. virens* isolate 15-2	25.00 (29.99)[e]	11.86[bc]	23.33 (28.88)[c]	11.89[abc]
2	T_2	*T. virens* isolate16-1	18.33 (25.35)[f]	12.38[a]	20.00 (26.56)[d]	12.35[a]
3	T_3	*T. virens* isolate 16-2	26.67 (31.09)[e]	12.12[ab]	25.00 (29.99)[c]	12.25[ab]
4	T_4	*T. virens* isolate 1A	30.00 (33.20)[d]	11.39[cd]	31.67 (34.25)[b]	11.75[bc]
5	T_5	*T. virens* isolate 2A	33.33 (35.26)[c]	11.43[cd]	35.00 (36.25)[b]	11.58[c]
6	T_6	*T. virens* isolate 3A	36.67 (37.27)[b]	11.01[d]	33.33 (35.26)[b]	11.47[c]
7	T_7	Metalaxyl	35.00 (36.28)[bc]	11.35[d]	31.66 (34.24)[b]	11.65[c]
8	T_8	Pathogen inoculated control	83.33 (65.95)[a]	5.22[f]	91.67 (73.49)[a]	4.00[e]
9	T_9	Pathogen uninoculated control	8.33 (16.78)[g]	10.40[e]	5.00 (12.92)[e]	10.42[d]
		CD value (0.05)	1.78	0.49	2.29	0.55

The treatment means are compared using Duncan Multiple Range Test (DMRT).
In each row, Mean values followed by a common letter are not significantly different (p=0.05)
*Values are means of three replications
Figures in the parentheses are arc sine transformed values
Disease incidence = (No. of seeds sown - no. of seeds germinated) + (No. of germinated seedlings - No. of infected seedlings)/No. of seeds sown*100

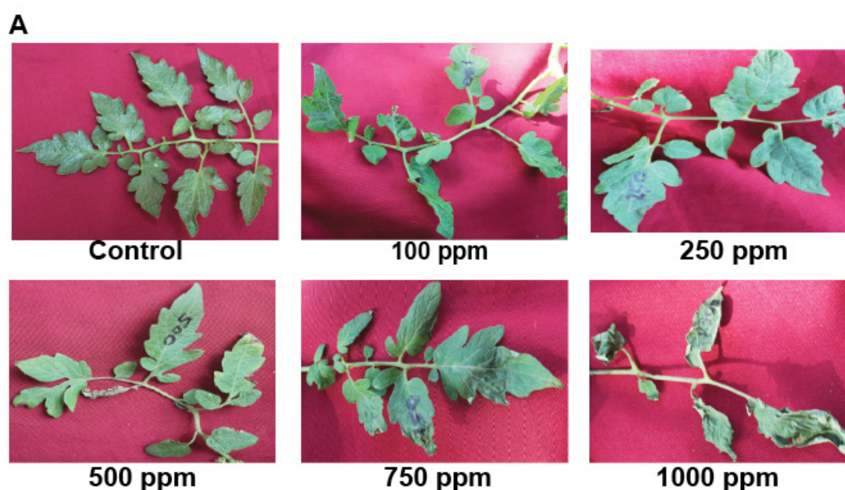

Fig. 5. Assessment of phytotoxic effects of purified gliotoxin on tomato leaves. Tomato leaves were sprayed with different concentration of gliotoxin and phytotoxicity effect was recorded.

at higher concentration *viz*., 750 and 1000 ppm. This study indicated that spraying of gliotoxin at higher concentrations showed phytotoxicity on leaves (Fig. 5). Earlier studies indicated that gliotoxin showed phytotoxic effect in some crops and not in others. Inhibition of seed germination and reduction of root growth was noticed when mustard seeds were germinated on the agar medium containing gliotoxin (Wright, 1956). Hagaruchi *et al.* (1996) studied the effect of gliotoxin on growth of plant cells and tissues, and reported that gliotoxin treated seedling showed slight inhibition on the shoot growth and root growth. However, Brian and Hemming (1945) reported that seed (wheat, barley and oats) dressing with gliotoxin did not affect germination and did not show any phytotoxic effect. In experimental trials, no phytotoxic effects of gliotoxin-producing *T. virens* on plants have been noticed (Howell, 2006). This study shows that gliotoxin and gliotoxin producing *T. virens* do not show drastic phytotoxic effect.

Effects of gliotoxin producing and gliotoxin non-producing *T. virens* on the management of damping off pathogen of tomato

Since purified gliotoxin and culture filtrate of Q strains of *T. virens* effectively inhibited the mycelial growth of *P. aphanidermatum* under *in vitro* condition, the efficacy of Q strains of *T. virens* on the management of damping off of tomato was assessed.

Seed treatment with Q strains of *T. virens* recorded 18-27% damping off disease incidence compared to P strains of *T.*

virens which showed 30-37% damping off disease incidence. In addition, treatment of seeds with gliotoxin producing Q strains of *T. virens* resulted in higher plant growth compared to P strains of *T. virens*. Among the six strains of *T. virens*, *T. virens* strain 16-1 effectively suppressed the damping off disease and recorded the minimum damping-off disease incidence of 18-20% in both the experiments and also it improved the plant growth by recording the maximum plant height of 12.38 cm (Trail I) and 12.35 cm (Trail II). This clearly revealed that gliotoxin producing Q strains of *T. virens* effectively suppressed the damping off disease incidence and improved the plant growth (Table 4).

Pythium is one of the most important diseases of tomato which infects root and seedling of vegetables and horticultural crops grown in greenhouse and field conditions. Previously, gliotoxin producing *T. virens* has been proven to be an effective biocontrol agent for the management of various soil-borne pathogens and it is formulated and marketed as SoilGard (Lumsden *et al*., 1992; Belbase *et al*., 2018). Howell and Puckhaber (2005) reported that strains of *T. virens* belonging to the P group are ineffective as biocontrol agents of seedling diseases in cotton whereas Q group are effective biocontrol agents of cotton seedling disease. Mutants of *T. virens* Q strains (that do not produce gliotoxin) are ineffective as mycoparasite against oomycetous fungus *P. ultimum* and the necrotrophic fungal pathogens, *Sclerotinia sclerotium* and *Rhizoctonia solani* and mutant *T. virens* strain lacking gliotoxin production is less effective in suppression of damping off of cotton compared to the isogenic wild type strain of *T. virens*. Similarly, mutant strains of *T. virens* lacking gliotoxin production were less effective for the control of damping off disease caused by *Pythium* spp. and *R. solani* in cotton and zinnia plants compared to its isogenic wild-type strain (Whilhite *et al*., 1994).

CONCLUSION

Thus, the present study clearly revealed that gliotoxin production by Q strains of *T. virens* could be one of the important mechanisms in inhibition of *P. aphanidermatum* and the gliotoxin producing *T. virens* could be used as an effective biocontrol agent for the suppression of damping off disease in tomato.

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