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Control of root-rot diseases of *Phaseolus vulgaris* using gliotoxin

Aliaa, R. El-Shami

Chemistry of Natural and Microbial Products Dept., NRC, El-Bohoos – Street, Cairo, Egypt
E-mail: erhamed@yahoo.com

ABSTRACT

Effect of the antifungal antibiotic gliotoxin on root-rot diseases caused by *Fusarium solani* and its influence on population of fungal flora in soil were investigated. Bean seeds were treated with different concentrations of gliotoxin before sowing. The results obtained from the green house application of bioagent indicated that soaking seeds in different concentrations of gliotoxin from 1µg/mL to 15µg/mL (for 60 minutes) significantly reduced the percentage of damping off and root rot as compared with control (pathogen only). Also 10µg/mL of gliotoxin was significantly decreased the population of fungal flora as compared with control.

Keywords: Biocontrol, *Fusarium solani,* Gliotoxin, Population of Fungal flora

INTRODUCTION

Root-rot diseases caused by soil-borne fungi are the most important diseases of many crops. Several fungi were recorded as a causal pathogens of root-rot and wilt diseases such as *Rhizoctonia solani, Fusarium solani* (Abdallah, 1969; Abou-zeid *et al*., 1990 and Abou-zeid *et al*., 1997).

Fusarium wilt disease caused by pathogenic forma specials of the soil inhabiting fungus fusarium, *Fusarium oxysporum* can cause severe loses in a wide variety of crop plants (Larkin and Fravel, 1998).

Schroth and Cook (1963) tested three bean varities for variation in susceptibility to pre-emergence damping off caused by *Rhizoctonia solani*, *Fusarium solani*, and *Pythium* spp, for amount of seed exudation and suggested that exudates influence the incidence of pre-emergence damping off by providing fungi with nutritive substances necessary for germination and growth in soil.

Gliotoxin is a potent crystalline antibiotic was produced during the growth of the imperfect fungus *Gliocladum fimbriatum* (Johnson *et al*., 1943). Production of the antifungal antibiotics gliotoxin has been associated with its efficacy as a biocontrol agent of soil borne diseases (Howell and Stipanovic, 1995).

Gliotoxin is an immuno-suppressive cytotoxin produced by pathogenic fungal species for this reason. It is one of the mycotoxins which must be systematically searched for biological control (Grovel *et al*., 2006).

Osamu *et al*. (2006) stated that fungal secondary metabolites such as gliotoxin were produced by different types of fungi such as *Aspergillus, Fusarium* and *Trichoderma*. This work aimed to study the effect of different concentrations of gliotoxin on root-rot diseases, fungal population and determination the optimum concentration for controlling root-rot diseases in *phaseolus vulgaris* plant.

MATERIAL AND METHODS

Fungal strains

Aspergillus fumigatus Fersenius NRC (147) and *Fusarium solani* (Mart.) Sacc NRC (215) were obtained from NRC Microbial collection unit.

Media and growth conditions

The antifungal antibiotic gliotoxin was produced by *A.fumigatus* NRC147 strain in a fermentation medium under culture conditions adopted by El-Shami. (2001). Isolation, purification and crystalisation of the compound also have been described.

Different concentrations of the toxin were prepared (1, 5, 10, and 15 µg/mL ethyl alcohol).

In vitro studies

The antimicrobial activities of gliotoxin against *Fsarium solani* (pathogenic fungus) was roughly estimated by filter paper disc method (Murray *et al*., 1995) using an inoculum containing 10^6 fungal cells/mL distilled water spread on Czapek Dox Agar (0.25 mL inoculum/plate) Four sets of filter paper disks 5 mm in diameter were saturated with the different concentrations of gliotoxin (1, 5, 10 and 15 µg/mL). Another set of discs were soaked in ethyl alcohol to serve as control. The prepared discs were dried and firmly applied to the surface of Czapek Dox Agar plates inoculated with the pathogenic fungus. Then the plates were incubated at 28-30 °C for 48-72 h. Diameters of inhibition zones were measured in mm.

* **Corresponding author**

In situ studies

Pot experiments were carried out in the green house of the National Research Centre (N.R.C), Dokki, Cairo to study the activity of gliotoxin in suppressing the pathogenic fungus (*Fusarium solani)* the causal agent of wilt and / or root rot of bean seedlings. Seeds of *Phaseolus vulgaris* abtained from the Agricultural Research Centre in Giza were used. Seeds were surface sterilized using 0.5 % w/v sodium hypochloride (NaOCl) for 5min and washed 3 times by sterilized distilled water, then soaked in four concentrations of gliotoxin 1, 5, 10, 15 µg/mL 60 min. Another group of seeds were soaked in sterilized distilled water served as control. The experiment was done and evaluated by Hamed (2001).

The effect of the antibiotic gliotoxin on root rot and wilt disease was evaluated under green house conditions during seedling stage after 15 days from sowing and on plant maturity after 45 days.

Population of fungal flora in soil

The effective concentration of gliotoxin was found to be 10 µg /mL. The pot experiments were repeated using unsterilized soil and treated with 3% wheat meal-sand medium (WSM) inoculated with the pathogen and seeds soaked in 10 µg/mL gliotoxin. Another three replicates of pots served as control (seeds were soaked in distilled water). Population of fungal flora in soil were estimated after six weeks from planting. Soil suspensions were prepared by mixing 10 gm of soil with 90 ml sterile water. The soil water was subjected to serial dilution (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}) .Three replicate plates were prepared from each concentration. One mL of each sample was poured into sterilized plates and 20 mL sterilized medium (Czapek-Dox-Agar) previously prepared. The same steps were done to the control soil. The plates were incubated at 28 °C and fungal count was estimated after 5 days and again after 8 days to count the slow growing fungal species. The isolated fungi were identified in the National Research Center, Chemistry of Natural and Microbial Products Dept. Microbial Culture Collection Unit (MCCU) according to their morphological characters (Barent, 1960). The results were statistical analyzed using Co. Stat. Progrm, Software. One way analysis was made and the treatments mean were compared by LSD at 5 and 1% probability (Snedecor and Cochrou, 1990).

RESULTS AND DISCUSSION

The results *in vitro* showed that the different concentrations of gliotoxin had strong antimicrobial activities against *F. solani*. In general, concentration 10 µg/mL was sufficient to stop the growth of the tested organism as shown in Table 1 and Figure 1.

This result was in agreement with Johnson et al. (1943), Boutibonnes et al. (1984), and El-Shami (2001) lend a strong support that gliotoxin exhibited strong antimicrobial potentialities aganist gram negative bacteria, gram positive bacteria, yeast and filamentous fungi.

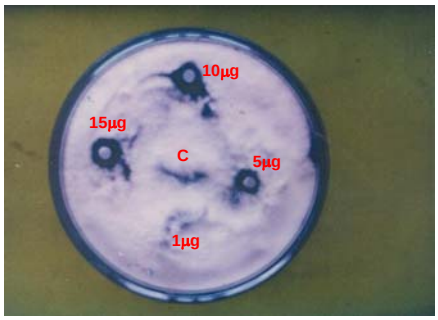

Figure 1: Antimicrobial activities of different concentrations of gliotoxin on *F. solani*
(C) Control
(1) Gliotoxin 1 µg/mL
(2) Gliotoxin 5 µg/mL
(3) Gliotoxin 10 µg/mL
(4) Gliotoxin 15 µg/mL

Table 1: Antimicrobial activities of gliotoxin against *Fusarium solani*

Concentration of gliotoxin (µg/mL)	Inhibition zone (mm)
Control	0
1	0
5	8
10	10
15	12
L.S.D. 5%	2.82
L.S.D. 1%	4.01

From the results in Table 2, it is obvious that increasing the gliotoxin concentration until 10 µg/mL reduced the percentage of damping-off and wilt as compared with the control (untreated seeds).

Highly reduction in infection with pre-emergence damping off and root-rot disease of bean plants was observed when treated seeds with 10µg/ml gliotoxin were cultivated in *F. solani* infected soil. Gliotoxin is likely to be responsible for biocontrol activity *in vivo* (Brian and Hemming, 1975). The continuous increase in gliotoxin up to 15 µg/mL leads to increasing the percentage of damping off and wilt another time this result might be reflectance of the toxic effect of gliotoxin on bean seeds. The result coincided with Haraguchi *et al*. (1995) who said that gliotoxin, inhibited tobacco cell growth by inhibiting acetolactate synthtase activity which is the first step in the biosynthesis of branched chain amino acids in cultured tobacco cells.

Gliotoxin was found to be active against *Rhizoctonia bataticola* (0.03 mu g ml (-1)), *Macrophomina phaseolina (*1.76 mu g ml (-1)), *Pythium debaryanum* (29.38 mu g ml (-1)), *Pythium aphanidermatum* (12.02 mu g ml (-1)), *Sclerotium rolfsii* (2.11 mu g ml (-1)), *Rhizoctonia solani* (3.18 mu g ml (-1)) [Singh-shyamli *et al*., 2005).

The results in Table 3 shows a significant decrease in the population of fungi when the soil was treated with 10 µg/mL gliotoxin after 6 weeks of planting.

Table 2: *In situ* effect of gliotoxin against *Fusarium solani* on bean plant during seedling stage

Treatment	% Dumping off	% root-rot
1 µg/mL	76	54.5
5 µg/mL	47.6	40
10 µg/mL	23.8	12.5
15 µg/mL	47.1	33.3
Control (untreated seeds)	90.5	100
L.S.D. 5%	4.86	5.33
L.S.D. 1%	6.92	7.59

Table 3: Fungal population in soil for control and treated seeds with 10 µg/mL *gliotoxin*

Species isolated	Control	Treated
Alternaria		
A. dianthi	+	-
Aspergillus		
A. flavipes	++	+
A. fumigatus	+	+
A. niger	+++	+
A. usfus	+	+
A. versicolor	+	+
Cephalosporium		
C. acremonium	+	+
Fusarium		
F. oxysporum	+++	+
F. solani	+++	+
Penicillium		
P. citrinum	+	-
P. notatum	+++	+
P. stoloniferum	+	+

-ve (N° of cells = 0)
+ve (N° of cells = 1 x 10^4: 2 x10^4)
++ve (N° of cells = 3 x10^4)
+++ve (N° of cells > 4 x10^4)

Gliocladium virens and *A. fumigatus* are filamentous fungi formulated for the bilogical control of damping off diseases of plants seed parts of its antagonistic activity. It is due to the production of an epidithiodioxy-piperazine antibiotic (gliotoxin) (Wilhite and Straney, 1996).

Production of the antifungal antibiotic gliotoxin by the biocontrol fungus *G.virens* has been associated with its efficacy as a biocontrol agent of seedling diseases infected by *R.solani, Fusarium solani* and *Pithium ultimum* (Howell and Stipanovic, 1995).

The antifungal activity of some compounds is due to their ability to affect the function or the structure of the plasmalema and other membranes of the fungal cell. These compounds often indicated as antibiotics, may include small molecules and peptides, proteins, enzymes and chemicals pesticides. Cell wall degrading enzymes and cell membrane degrading enzymes produced by plants, bacteria and fungi are also powerful antifungal agents *in vitro* (Mauch *et al*., 1988; Lorito *et al*., 1994; Lorito *et al*., 1993). Some active compounds cell membrane and cell wall degrading enzymes are able to interact synergistically in the inhibition of pathogenic fungi and it has been suggested that this synergism is involved in both plant defense and microbial biocontrol mechanisms (Schirmbok *et al*., 1994).

Gliotoxin, which selectively attacks thiol groups located on cell membranes (Johnes and Hancock, 1988), and may be strongly enhance the fungicidal effect of commonly used inhibitors of sterol synthesis, which alter membrane integrity and structure (Lorito *et al*., 1994).

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