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PATHOGENICITY OF *GLIOCLADIUM* ON THE SCLEROTIA OF *SCLEROTINIA SCLEROTIORUM*

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ABSTRACT

SANTOS, A.F. & DHINGRA, O.D. Pathogenicity of *Gliocladium* on the sclerotia of *Sclerotinia sclerotiorum*. Fitopatol. bras. (14):198-200. 1989.

Seventeen isolates of *Gliocladium* spp. obtained from 21 vegetable field soils by baiting with the sclerotia of *Sclerotinia sclerotiorum* were tested for pathogenicity and aggressiveness 'in vitro' and in field soil against the latter. Two isolates of *G. penicilloides*, one of *G. roseum* and two of an unidentified species killed over 80% of the sclerotia in 30 days. In the aggressiveness test, one isolate of *G. penicilloides* and one of the unidentified species killed 100% of the sclerotia in 15 days when inoculated with inoculum suspension of 10^6 or 10^8 conidia/ml. The same isolates killed 80 to 90% of the sclerotia in 60 days in field soil; thus showing their pontential for use in bio-control of the pathogen. Of all the isolates, *G. roseum* was the least aggressive.

RESUMO

Patogenicidade de *gliocladium* spp no escleródio de *sclerotinia sclerotiorum*.

Dezessete isolados de *Gliocladium* spp., obtidos de 21 tipos de solo de campo utilizando-se escleródios de *Sclerotinia sclerotiorum* como isca, foram testados quanto à patogenicidade e agressividade, "in vitro" e no campo, a *S. sclerotiorum*. Dois isolados de *G. penicilloides*, um de *G. roseum* e dois de espécie não identificada, mataram mais de 80% dos escleródios em 30 dias. No teste de agressividade, um isolado de *G. penicilloides* e um de espécie não identificada mataram 100% dos escleródios em 15 dias quando os escleródios foram inoculados com suspensão de inóculo de 10^6 ou 10^8 conídios/ml. Os mesmos isolados mataram 80 a 90% de escleródios em solos de campo, dentro de 60 dias, assim mostrando seu potencial para uso em bio-controle do patógeno. De todos os isolados o menos agressivo foi *G. roseum*.

INTRODUCTION

Sclerotinia sclerotiorum (Lib.). De Bary, a fungal pathogen of vegetable crops, causes heavy losses in certain vegetable seed production farms in the state of Minas Gerais, Brasil. In many of these farms, inoculum density of 8 to 10 sclerotia/kg soil and yield losses of 85 to 100%, have been observed. The soils in these fields are sandy, acidic (pH 4 to 5.5), low in organic matter, and with poor water retention capacity. Fumigation and application of soil fungicides did not control the disease to satisfaction; therefore, a search for bio-control agents was initiated. Considering the properties of these soils, we focused our attention on the fast growing fungal mycoparasites that could resist water deficit and grow well under acidic conditions. Besides *Trichoderma* spp., *Gliocladium* spp. also satisfy these requirements and are reported to be parasites of many sclerotial and nonsclerotial fungal pathogens. *Gliocladium virens* has been most studied as a possible bio-control agent for *Rhizoctonia solani* (Levis & Papavizas, 1985), *Pythium* and *Fusarium* spp. (Teyers & Dirks, 1985) and *S. sclerotiorum* (Tu, 1980; Lee & Wu, 1984). The following study was done to determine the potential of *Gliocladium* spp., occurring in the soils of Minas Gerais, in reducing the inoculum density of *S. sclerotiorum*.

MATERIALS AND METHODS

Isolates of *Gliocladium* spp. were obtained from 21 soils collected from vegetable fields in different agro-climatic regions, with or without the ocorrence of the disease caused by *S. clerotiorum*. Isolations were made using the following baiting technique. After adjusting the soil moisture to about 30% of its moisture holding capacity, 25 sclerotia, freshly harvested from corn-meal culture and without drying, were mixed with 500 g soil and incubated in polyethylene bags at room temperature. After one month, the sclerotia were recovered by wet

sieving, washed under running tap water for 15 ot 20 minutes, surface-sterilized in 1.7% sodium hypochlorite for 30 seconds and in 70% ethanol for one minute, and then rinsed in sterile distilled water. Sclerotia were then placed on autoclaved moist sand in clear plastic boxes (20 x 20 x 3 cm) and incubated at 25°C for 15 days. The fungi growing on the sclerotia were isolated and multiplied on potato-dextrose agar (PDA).

For initial pathogenicity tests, conidial suspension of isolates of *Gliocladium* spp. was prepared by washing the conidia from five-day old PDA cultures with sterile distilled water. Conidial counts were adjusted to 10^6/ml. Freshly prepared non-dried sclerotia were inoculated by immersion in the conidial suspension for one minute. Control sclerotia were immersed in sterile distilled water. Sclerotia were then placed on autoclaved moist sand as described earlier and incubated at 25°C. After 30 days, they were removed, washed in running tap water, surface-sterilized and plated on 1.5% water agar, and incubated at 20°C for 15 days to check their viability.

The isolates that killed over 80% of the sclerotia in the above experiment were studied for aggressiveness 'in vitro' and in a field soil. 'In vitro' test was done as described earlier, except that the sclerotia were inoculated with suspensions of 10^4, 10^6 or 10^8 conidia/ml and the incubation period was reduced to seven or 15 days. The field test was done in a vegetable seed production farm where the pathogen was well established. Holes of 15 cm diameter and 10 cm deep were dug 2 meters apart. The soil was infested with 10 sclerotia of *S. sclerotiorum* and with respective isolate of *Gliocladium* at the concentrations of 10^4 , 10^6 or 10^8 conidia/g of soil. The soil was then placed in a nylon mesh (1 mm) cylindrical bag and placed in the respective holes. After 20, 40 or 60 days the bags with soil were removed. Sclerotia recovered by wet sieving were tested for viability and infection by *Gliocladium* sp.

All studies had three replications and were repeated twice. The field experiment was repeated after one month at a different site of the same field.

RESULTS

Of the 17 isolates of *Gliocladium* isolated from sclerotia and tested for pathogenicity 'in vitro', two isolates of *G. penicilloides*, one of *G. roseum* and two of an unidentified species killed 94 to 98% of the sclerotia. The other isolates either did not affect the germination or reduction in viability was too low to demand further testing. The isolates that killed the sclerotia grew profusely on them, causing a dry rot.

In the aggressiveness test, during the first seven days of incubation, 57 to 68% and 67 to 92% of sclerotia were killed when inoculated with 10^4 and 10^8 conidia/ml, respectively (Table 1). Isolates G-1 and G-2 of *Gliocladium* caused maximum decline in viability. The death rate of sclerotia inoculated with suspension of 10^6/ml was similar to that of 10^8/ml. However, after 15 days of incubation 94 to 100% sclerotia were killed by all isolates except *G. roseum*, with no

TABLE 1 – Percent germination of sclerotia of *Sclerotinia sclerotiorum* inoculated by immersion in a conidial suspension of *Gliocladium* spp. and incubated on sterile moist sand at 25°C[1,2].

Isolate	Incubation period (days)	Inoculum Concentration/ml			Mean
		10^4	10^6	10^8	
G. penicilloides (Gp-1)	7	43	35	33	37
	15	5	5	2	4
G. penicilloides (Gp-2)	7	35	34	30	33
	15	0	0	0	0
G. roseum	7	38	16	13	21
	15	13	12	10	12
Gliocladium sp. (G-1)	7	26	23	13	21
	15	6	0	0	2
Gliocladium sp. (G-2)	7	22	10	8	13
	15	5	5	2	4
Control	7	89	89	89	89
	15	91	91	91	91

1. Average of two experiments and three replications. The numbers are rounded to the nearest whole number.
2. Considering large differences among treatments and small differences among replications, statistical analysis was discarded.

TABLE 2 – Percentage germination of sclerotia of *Sclerotina sclerotiorum* recovered from field soil infested with conídia of *Gliocladium* to a depth of 10 cm 1,2.

Conidia/g	Incubation period (days)	Control	G. penicilloides		*G. roseum*	Gliocladium sp.	
			Gp-1	Gp-2		G-1	G-2
10^4	20	89	67	63	67	60	63
	40	63	47	57	57	27	20
	60	63	43	43	33	27	10
	Mean	72	52	54	52	38	31
10^6	20	89	57	67	66	53	57
	40	63	43	40	67	50	53
	60	63	33	30	47	40	20
	Mean	72	44	46	60	48	43
10^8	20	89	57	57	57	53	63
	40	63	30	37	47	27	43
	60	63	17	40	27	17	10
	Mean	72	35	45	44	32	39
Mean of isolate		72	44	48	52	39	38

1. Average of three replications and two experiments. The numbers are rounded to the nearest whole number.
2. Tukey for comparison of treatments with control: 14.

significant difference among concentration of inoculum and isolates (Table 1).

In the field test viability of sclerotia in soil infested with *Gliocladium* declined significantly and constantly with time. The effect of inoculum concentration was more pronounced at 60-day incubation period than earlier. However, averaging the effect of isolates and incubation period, did not show significant difference among inoculum concentrations in reducing the sclerotial viability. On the other hand, averaging the effect of isolates and inoculum concentrations, showed that the sclerotial death increased with time, with maximum decline occuring between 40 and 60 days (Table 2).

The rate of sclerotial death in the soil was more rapid with isolates G-1 and G-2, even at lower inoculum concentrations. On an average, these isolates were more aggressive than Gp-1 and Gp-2. *Gliocladium roseum* was the least aggressive. Respective *Gliocladium* spp. were recovered from all non-germinating and majority of germinating sclerotia.

DISCUSSION

This study shows that some species and strains of *Gliocladium* parasitize the sclerotia of *S. sclerotiorum*. This confirms or adds to the list of mycoparasites that show potential for controlling this and some other soil-borne pathogens (Santos & Dhingra, 1982; Huang, 1978, 1980; Huang & Hoes, 1976; Tu, 1980; Uecker et al., 1978). *Gliocladium catenulatum* and *G. virens* have been reported to be aggressive myco parasites of *S. sclerotiorum* (Zazzerini & Tosi, 1985; Lee & Wu, 1984). This study reports, for the first time, *G. penicilloides* as an aggresive pathogen of *S. sclerotiorum*. *Gliocladium catenulatum* and *G. virens* were not found in the soils sampled. From the small sample of isolates tested, it was obvious that species or strains of *Gliocladium* nonpathogenic to sclerotia of *S. sclerotiorum* are more common than the pathogenic ones in the soils of Minas Gerais. However, within the pathogenic strains, there is a great difference in the aggressiveness. The isolates G-1 and G-2, that showed high aggressiveness, appear to be promising for bio-control of *S. sclerotiorum*.

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EFEITO DE REGIÕES E ÉPOCAS DE PRODUÇÃO NA QUALIDADE SANITÁRIA DE SEMENTES DE FEIJOEIRO NO ESTADO DE SÃO PAULO*

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RESUMO

FURLAN, S.H. & MENTEN, J.O.M. Efeito de regiões e épocas de produção na qualidade sanitária de sementes de feijoeiro no Estado de São Paulo. Fitopatol. bras. (14):200-205. 1989.

Com o objetivo de conhecer regiões e épocas de cultivo mais adequadas à produção de sementes de feijoeiro no Estado de São Paulo, utilizou-se o parâmetro sanidade como principal, seguido da germinação e envelhecimento acelerado como indicativos da qualidade das sementes. Sementes genéticas, básicas e certificadas produzidas nas épocas da seca de 1984, das águas de 1984/85, e do inverno de 1985 provenientes de 46 municípios foram avaliadas. Os testes de sanidade envolveram o método de papel de filtro comum e o método de papel de filtro com congelamento. De maneira geral, a sanidade das sementes foi boa, considerando-se a baixa freqüência de fungos potencialmente importantes à cultura como *Colletotrichum lindemuthianum*, havendo, no entanto, efeito de regiões dentro de cada época de produção. Houve, também, o efeito de regiões e épocas de produção na germinação e envelhecimento acelerado das sementes. As melhores áreas para

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