Effects of Metabolites of *Gliocladium roseum* on Egg Hatching and Juvenile Mortality of *Meloidogyne incognita*

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Abstract: The filamentous fungi *Gliocladium* spp. has potential as a biocontrol agent against many pathogens. The objective of this study was to evaluate the effects of ferment filtrate and volatile metabolites of *G. roseum* on egg hatching and juvenile mortality of *Meloidogyne incognita*. The nematode egg hatching rates and juvenile activity for treatments with volatile metabolites and with 0×, 5×, 10×, 20× and 40× dilutions of the *G. roseum* culture filtrate were measured in this test. Results showed that *G. roseum* ferment filtrate inhibited nematode egg hatching and juvenile mortality by 80.4% and 32.0%, respectively. Dilution of ferment filtrate reduced the inhibition of nematode egg hatching and the juvenile activity. Volatile metabolite of *G. roseum* also inhibited nematode egg hatching and juvenile activity. *G. roseum* can be used as a biocontrol agent against plant parasitic nematodes.

Key words: *Gliocladium* spp.; Metabolites; *Meloidogyne incognita*

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粉红粘帚菌代谢物对南方根结线虫卵孵化及二龄幼虫的影响

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摘 要: 粉红粘帚菌(*Gliocladium* spp.)是一类对植物病原菌具有潜在生物控制作用的真菌, 为了探讨粘帚菌对南方根结线虫(*Meloidogyne incognita*)的抑制作用, 实验室条件下研究了粉红粘帚菌挥发性代谢产物和非挥发性代谢产物不同稀释度对南方根结线虫卵孵化及二龄幼虫活性的影响。结果显示非挥发性代谢产物在原液、5倍、10倍、20倍、50倍稀释浓度下, 12 d后对南方根结线虫卵孵化的相对抑制率分别为80.4%、12.0%、10.5%、6.7%和5.2%, 对二龄幼虫的矫正死亡率分别为32.0%、9.3%、2.0%、0.6%和0.1%, 与对照组相比差异显著; 挥发性代谢产物对南方根结线虫卵孵化的相对抑制率为22.5%, 对二龄幼虫的相对死亡率为17.4%。因此, 粉红粘帚菌的非挥发性和挥发性代谢产物对南方根结线虫卵孵化及二龄幼虫活性均有一定的抑制作用。

关键词: 粉红粘帚菌; 代谢物; 南方根结线虫

1 Introduction

Root-knot nematode (*Meloidogyne* spp.) is one of the most serious pathogens to economic crops, including oil plant, vegetables, fruit trees, tea, tobacco nd medicinal plants, it is also an important pathogen of soybean[1-3]. Infection caused by root-knot nematodes leads to significant yield losses of vegetables, like tobacco, melon, and so on, which cost about 78 billion US dollar worldwide annually[4]. In China, root-knot nematodes

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have been reported from greenhouses in the north and fields in the south. Control of *Meloidogyne* spp. is currently limited to application of soil nematicides, which are costly and detrimental to the environment and human health[5]. Therefore, alternative control measurements are necessary for pathogens management.

Major group of nematophagous microorganism belongs to actinomycetes, which have frequent worldwide occurrences. The microflora associated with cyst of *Heterdera glycines* in soybean cyst nematode soils in China was investigated and the number of species of the acntinomycetes was up to 128[6]. Actinomycetes were also observed in eggs and females of *Meloidogyne* spp.[7-8]. It has been reported that actinomycetes acted as antagonists of nematicidal metabolites[9-10].

Some species of soil fungi colonizing phytonematode eggs, females, and cysts have been known for many years, and their biocontrol potential has been widely studied[11]. Several species of soil fungi are identified as biological control agents against microbial diseases of plants and plant parasitic nematodes[12-15]. *Gliocladium* spp. has been widely studied as a biological control agent against microbial diseases of crops[16-17]. Zhang et al.[18] reported one strain of *G. roseum* had effect on nematode activities. This paper reports an investigation of the effects of the ferment filtrate and volatile metabolite of *G. roseum* strain ACM941, which is maintained by Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, on egg hatching and juvenile mortality of *M. incognita*. The objective of this research was to assess the biocontrol potential of the *G. roseum* on nematodes.

2 Materials and methods

2.1 Nematode inoculum preparation

The *M. incognita* nematode used in this study was isolated from diseased tomato roots and increased on susceptible tomato plants at Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Harbin, China. For the inoculum production, single egg mass was used to establish a population on tomato plants (*Lycopersicon esculentum* var. Roma VF). Egg masses in tomato roots were collected and eggs were extracted from egg masses using the method described by Hussey and Barker (1973) with some modifications, in which root pieces with galls were washed in running tap water for 3-5 minutes to get rid of soil and placed under a dissecting microscope. Roots were dissected with a dissecting needle, and egg masses and juveniles were picked up from the root surface and dissected roots with a forceps. The juveniles and egg masses were treated with 1% sodium hypochlorite (NaClO) for 1 min to surface-disinfest and release the eggs from masses. The eggs in NaClO solution were passed through a 200 μm aperture sieve and collected on a 30 μm-aperture sieve, and the juveniles were collected on a 200 μm-aperture sieve. Free eggs retained on the 30 μm-aperture sieve or juveniles on the 200 μm-aperture sieves were washed three times with distilled water to remove residual NaClO. The eggs and juveniles were diluted to 400 · mL^{-1} and stored at 4℃.

2.2 Fungal inoculum preparation

The *G. roseum* strain ACM908 was conserved in Northeast Institute of Geography and Agroecology, Chinese Academy of Science. The fungus was grown on potato dextrose agar (PDA), incubated at 25°C for 7 d, and then used to produce culture filtrate and volatile metabolites. To prepare the fungal culture filtrate, 15 pieces of fungal medium (4-mm diameter) from 10-day-old cultures on PDA were transferred to 100 mL potato dextrose broth (PDB) medium and cultured in an oscillator (HZQ-C Vapour-bathing Constant Temperature Vibrator, Harbin Dongming Medical Instrument Factory, Harbin, China) at 180 r · min^{-1} for 1 d (30°C) and used as a fungal stock culture, and then 4 mL of the stock culture were added to 120 mL of new-made PDB, incubated at 30°C with shaking at 180 r · min^{-1} for 6 days, and then centrifuged at 9 300 g at 4°C for 20 min. The fungal culture filtrate was made by filtering the fungal suspension with Seitz germproofing filter (The Peninsula Shanghai Industrial Co., Ltd., Shanghai, China) and stored at 4°C until used.

2.3 Plant material

Experiments were carried out with tomato (*Lycopersicon esculentum* var. Roma VF, susceptible to *M. javanica*) grown in a controlled-environment cabinet. The

air temperature was maintained at 27℃.

2.4 Effect of volatile metabolites of *G. roseum* on *M. incognita*

The effect of volatile metabolites of *G. roseum* to the egg hatching and juvenile activity was determined using the method described by Dennis (1971) with some modifications. Briefly, the plastic culture dish (35-mm diameter) with 0.5 mL of DE or DJ was covered by an identical plastic culture dish, in which *G. roseum* was cultured for 7 days so that the egg suspension or juvenile suspension was exposed to the volatile metabolite produced by *G. roseum*. The joint of the two identical plastic dishes was sealed with airproofed Parafilm. Then DE was incubated at 28°C for 12 days. The number of egg hatching was recorded after 12 days, and the DJ was cultured at 28°C for 24 hours, the mortality rate of juveniles was determined by the NaOH stimulus method described by Chen and Dickison (2000). Each experiment was repeated three times.

2.5 Effect of ferment filtrate of G. roseum on *M. incognita*

Fungal culture filtrate (2 mL) was added to the plastic culture dish containing 0.5 mL egg (about 100 eggs) suspension (DE) or juvenile suspension (DJ). Experiments were conducted with five concentrations of the fungal filtrate that were prepared using 0 ×, 1 ×, 5 ×, 10 ×, 20 × and 40 × dilutions with four replications. Experiments were repeated three times.

3 Results

3.1 Effect of volatile metabolites of *G. roseum* on *M. incognita* egg hatching

Under *in vitro* condition, the volatile metabolites of *G. roseum* showed significant effect on nematode egg hatching. Whereas maximum of egg hatching percentage in control was 66.4% at twelfth day but in treatments was 50.2% at the same day, which was more or less 14.6% lower than their controls. So the volatile metabolites of *G. roseum* had some inhibition on the nematode egg hatching. According to the examination of microscope, it showed no morphologic difference of the egg and juveniles of nematode between the treatments and the control, which indicated that the volatile metabolites of *G. roseum* had effect on the nematode egg hatching by inhibiting the nematode egg hatching but not affecting the nematode growth (Table 1).

Table 1 Effect of volatile metabolites of *G. roseum* on *M. incognita* egg hatching

Treatments	Rate of hatching eggs/%	Rate of relative inhibition/%
Volatile metabolites	50.2 ± 5.1 *	22.5 ± 8.2
CK	64.8 ± 2.3	

The data was analyzed by T test on SAS 8.12, and the result showed $t = 3.64$, $P = 0.0357 < 0.05$.

3.2 Effect of volatile metabolites of *G. roseum* on *M. incognita* juveniles activity

Under *in vitro* condition, the volatile metabolites of *G. roseum* showed significant effect on juveniles activity. Whereas maximum of nematode mortality percentage in control was 4.0% at the twelfth day and in treatments was 21.5% at the same day, which was more or less 16.6% higher than their controls. So the volatile metabolites of *G. roseum* could increase the nematode mortality (Table 2).

Table 2 Effect of volatile metabolites of *G. roseum* on *M. incognita* juveniles activity

Treatments	Juveniles mortality (%, $\bar{X} \pm s$)	Rectified juveniles mortality (%, $\bar{X} \pm s$)
Volatile metabolites	21.5 ± 11.7 *	17.4 ± 4.9
CK	4.9 ± 2.9	

The data was analyzed by T test on SAS 8.12, and the result showed $t = -2.96$, $P = 0.0133 < 0.05$

3.3 Foundation of the suppressive activity specification curve of ferment filtrate of *G. roseum*

In order to definite the maximum suppressive activity time of ferment filtrate, the suppressive activity specification curve of ferment filtrate of *G. roseum* was tested. The results indicated that the inhibition of ferment filtrate on nematode egg hatching and the juveniles' mortality were both increasing straightly at the fourth to sixth day, but it tended to be gentle at the seventh day. So the ferment filtrate at the sixth day had produced enough substance to inhibit the nematode egg hatching and juvenile activity, and the sixth day was the optimal ferment time (Fig. 1).

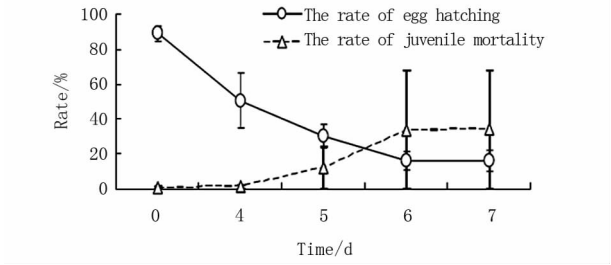
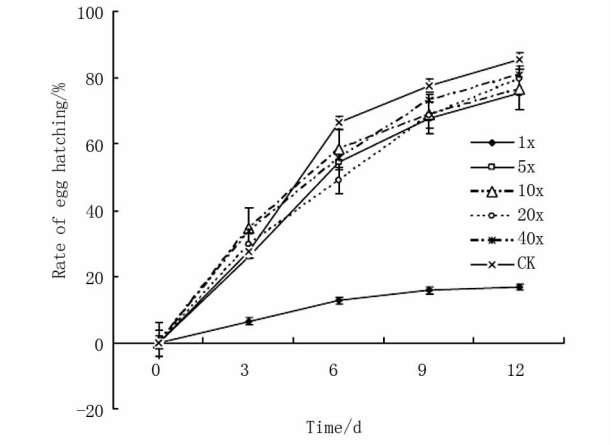


Fig. 1 The suppressive activity specification curve of ferment filtrate of *G. roseum*

3.4 Effect of ferment filtrate of *G. roseum* on *M. incognita* egg hatching

The culture filtrate of *G. roseum* showed significant effect on nematode egg hatching *in vitro*, especially the undiluted stock solution. All different dilutions showed inhibition on nematode egg hatching after 3 days, but the difference of the egg hatching rates between control and the treatments was obvious at the 12th day at which the nematode egg hatching rate for no-treatment control was 85.6%. The nematode egg hatching rates for treatments with 0 ×, 5 ×, 10 ×, 20 × and 40 × dilutions of the culture filtrate were 16.8%, 75.3%, 76.6%, 79.9% and 81.1%, respectively (Fig. 2).

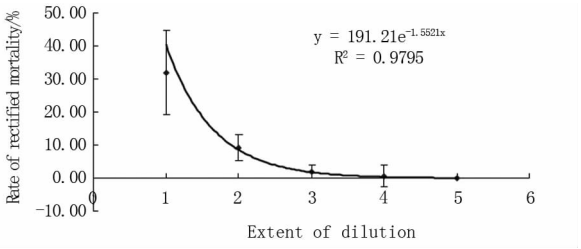


The extent of dilution of ferment filtrate was 1 ×, 5 ×, 10 ×, 20 ×, 40 ×, respectively. The egg hatching was detected each time per 3 days.

Fig. 2 Effect of ferment filtrate of *G. roseum* on *M. incognita* egg hatching

3.5 Effect of ferment filtrate of G. roseum on M. incognita juvenile activity

The juveniles' mortality was reduced with the increase of dilution of the culture filtrate. They were 5.2%, 38.7%, 14.0%, 7.1%, 5.7%, and 5.2% for the culture filtrates with 0 ×, 1 ×, 5 ×, 10 ×, 20 × and 40 × dilutions at the 12th day, respectively. And the results fitted to exponential relationship, and the exponential equation was fitted to $y = 191.21e^{-1.5521x}$, $R^2 = 0.9795$, which indicated ferment filtrate of *G. roseum* had inhibition on the nematode juvenile activity as well (Fig. 3).



The data(1, 2, 3, 4, 5) of abscissa was representative of the extent of dilution(1 ×, 5 ×, 10 ×, 20 ×, 40 ×).

Fig. 3 Effect of ferment filtrate of *G. roseum* at the sixth day on *M. incognita* juvenile activity

4 Discussion

Gliocladium spp. is a common fungus in agricultural soil, which has been studied as an important biocontrol agent against microbial diseases of crops. Some species of *Gliocladium* spp. have been used to manage/control *Sclerotinia* stem rot of soil-borne diseases of crops in greenhouse and cropland.

Results of this research indicate that metabolites and/or other compounds of *G. roseum* strongly inhibited the egg hatching and increased the mortality of *M. incognita*, however, there was no morphological difference of the egg and juveniles of nematode observed under the microscope. We speculated that the nematode egg hatching, but not its development, was inhibited by the volatile metabolite of *G. roseum*. The 6th day's culture filtrate had great inhibition on the egg hatching of *M. incognita*, and the egg hatching rate of the stock culture filtrate was from 85.6% in control down to 16.8%. The dynamic inhibition results indicated that the egg hatching rate of all different dilutions were higher than the group at the 3rd day but lower than the control at the 6th day. More than one metabolite in *G. roseum*'s culture filtrate could stimulate egg hatching and then inhibit the egg hatching.

Gliocladium spp. can produce some volatile substances that inhibit the growth of microorganisms[19]. Volatile metabolites reduced egg hatching 22.5% and increased juvenile mortality 17.4%, respectively. The biocontrol agent of *G. roseum* to the nematode has not been widely studied, but its metabolite inhibited the egg hatching of M. *incognita*. Strobel, et al. [19] reported that

volatile hydrocarbons and hydrocarbon derivatives were produced by *G. roseum*. It was also reported that a compound, gliotoxi, produced by *G. roseum* inhibited pathogenic fungi, especially *Bythium ultimum* and *Rhizoctonia solani*[20]. The active materials in the culture filtrate and the inhibitory mechanism of *Gliocladium* spp. to nematodes need to be further investigated.

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