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# Nematicidal Activity of Chaetoglobosin A Poduced by *Chaetomium globosum* NK102 against *Meloidogyne incognita*

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**ABSTRACT:** The nematicidal activity of *Chaetomium globosum* NK102, culture filtrates, and chaetoglobosin A (ChA) purified by HPLC was evaluated on *Meloidogyne incognita*. The results showed that *C. globosum* NK102 significantly repelled second-stage juveniles (J2s). Both filtrates and ChA demonstrated strong adverse effects on J2 mortality with 99.8% at 300 μg ChA/mL ($LC_{50}$ = 77.0 μg/mL) at 72 h. ChA and filtrates did not affect egg hatch until 72 h of exposure. All filtrate treatments inhibited the J2 penetration even in 12.5% dilution treatment. Similarly, ChA (300 and 30 μg/mL) showed a significant inhibitory effect on J2 penetration. The number of eggs per plant was significantly reduced in the treatment of 30 mg ChA/kg soil by 63% relative to control plants, indicating the apparent negative effect on reproduction of *M. incognita*. The study demonstrated the nematicidal activity of ChA and suggested that it could be a potential biocontrol agent for integrated management of *M. incognita*.

**KEYWORDS:** *chaetoglobosin A (ChA), Meloidogyne incognita, Chaetomium globosum, mortality*

## INTRODUCTION

*Meloidogyne* spp., one of the most important plant parasitic nematodes, has caused great damage to a wide range of crops worldwide. [1] The root-knot nematode (RKN), *Meloidogyne incognita*, is one of the most important plant-damaging species. The global annual loss of crops by RKN is multiple billions.[2] Many nematicides hardly prevent increasing nematode infestation.[3] Moreover, due to environmental and human health concerns, regulation on the use of several widely applied nematicides such as methyl bromide has been tightened. Thus, the search for alternative nematicides, for example, biocontrol agents and natural compounds, has drawn great attention in recent years.[4] Many fungi have shown potential antinematode activities.[5−10]

*Chaetomium globosum* is one of the most prevalent fungi in the environment. It is often found on decaying plant debris and in living plants as an endophyte or a pathogen. *C. globosum* produces abundant secondary metabolites such as chaetoglobosins.[11] It is clear that chaetoglobosins inhibit movement and proliferation of mammalian cells due to their ability to bind to actin filaments.[12] Of them, chaetoglobosin A (ChA) is the major component of the secondary metabolites and has strong cytotoxicity.[13]

Lately, *C. globosum* has been used in agricultural applications, for instance, as a biocontrol agent against other plant pathogenic microbes and even aphids.[14,15] In addition, a few studies have reported the nematicidal attribute of *C. globosum*. A culture broth filtrate of *C. globosum* was inhibitory against egg hatch of both *M. incognita* and *Heterodera glycines*.[16] The culture filtrates of the fungus *Chaetomium* strain Ch1001 caused significant mortality of second-stage juveniles of *M. incognita*. This fungus significantly reduced the number of root galls in cucumber seedlings.[17]

However, studies regarding the nematicidal activities of the natural compounds from *C. globosum* have been rarely reported and discussed. So far, only one nematicidal compound, flavipin, was reportedly extracted from a strain *C. globosum* isolated from soybean cyst nematode.[18] Because the production of secondary metabolites can also be a strategy used by nematophagous fungi to infect or kill nematodes, the natural chemicals from nematophagous fungi could be potential alternatives of chemical nematicides and fumigants for controlling *M. incognita*. In addition, with regard to its ubiquitous distribution in soil and its characteristics as a biocontrol agent against both plant pathogenic microbes and even pest insect aphids, exploration of nematicidal activity will be another competitive priority of *C. globosum* as a biological agent for future commercialization and field application.

To find potential nematicidal compounds from *C. globosum*, we found an isolate, *C. globosum* NK102, and isolated ChA from its culture filtrates. To further investigate the potential nematicidal activity of *C. globosum*, the objective of this study was to (1) test the nematicidal activity of the chemical ChA isolated from *C. globosum* NK102 against *M. incognita* and to (2) determine if the culture filtrate of *C. globosum* NK102 has nematicidal activity against *M. incognita*. To our knowledge, this is the first report of the nematicidal activity of chaetoglobosin A against *M. incognita*.

## MATERIALS AND METHODS

**Fungal Strain and Culture Filtrate Preparation.** The fungal strain NK102 used in this study was isolated from the bark of *Cupressus* sp. and classified to be *C. globosum* by sequence homology of the 18S rDNA and morphological structure (unpublished data). Growth conditions have been described previously.[19] Briefly, the strain was cultured in 500 mL flasks with 200 mL of S-7 medium[20] or potato dextrose broth (PDB) at 28 °C, shaken at 180 rpm. Each flask was inoculated with $1 \times 10^5$ spores obtained from a 2-week-old fungal colony. The fungal mycelium was separated from the culture fluid after 8

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days by filter paper under vacuum. Then the remaining liquid medium was centrifuged, and subsequently the supernatant was filtered through a sterile 0.22 $\mu$m polyethylene filter.

**HPLC Purification of ChA from the Culture Filtrate.** The supernatant obtained above was extracted with equal volumes (200 mL) of chloroform and methanol (10:1 v/v). The organic phase was evaporated at 55 °C, and the residue was dissolved in 2 mL of methanol and centrifuged at 1200 rpm for 10 min. The supernatant was then filtered through a 0.22 $\mu$m filter. Samples in 20 $\mu$L of methanol were loaded to high-performance liquid chromatography (HPLC) by injection (HPLC, Agilent 1100, Agilent Technologies, Santa Clara CA, USA) with a Kromasil C18 ODS column (4.6 × 250 mm, AKZO Nobel, Luzern, Switzerland). Elution was performed with methanol/sterile distilled water (SDW) (7:3, v/v). A variable-wavelength recorder was set at 227 nm to detect the compounds eluting from the column at a flow rate of 1 mL min$^{-1}$. The candidate peak of chaetoglobosin A (ChA) at approximately 14.5 min as determined by comparison to the standard sample was collected. The standard curve of ChA (Sigma, St. Louis, MO, USA) was made at 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL. HPLC purification was conducted three times. HPLC-purified ChA was used in all subsequent experiments for nematicidal activity evaluation against *M. incognita*.

**Nematode Egg and J2 Preparation.** Eggs were obtained from greenhouse-grown *M. incognita*-infected pepper (*Capsicum annuum* L.) plants by stirring with 1% NaClO solution, rinsing thoroughly with tap water, and centrifugation with 38% sucrose solution. Second-stage juveniles (J2s) of *M. incognita* were hatched from egg masses collected from pepper.

**J2 Chemotaxis Assay.** Chemotaxis assay was performed according to the modified method of Tajima et al.[21] A 1 cm$^2$ agar block chopped from a 1-week-old NK102 fungal colony on PDA was inoculated on the test location of 9 cm Petri dishes containing 1.5% HEPES agarose (2.38 g/L HEPES, 0.25% Tween 20, pH 7.2), while a 1 cm$^2$ PDA was transferred to the control location, on the opposite sides of the dish (Figure 1). Two hours later, 200 *M. incognita* J2s were added to the center of the Petri dishes (Figure 1), and the dishes were incubated in a dark cabinet at 25 °C for 4 h. Then the numbers of J2s in A, B, and C were counted under an inverted microscope (Olympus CKX41, Tokyo, Japan). Petri dishes without the medium served as control. The experiment consisted of seven replications and was repeated once at 25 °C. The numbers of J2s in zones A and B as percentages of the total (nematodes in zones A, B, and C) were compared using Student's *t* test ($P < 0.05$).

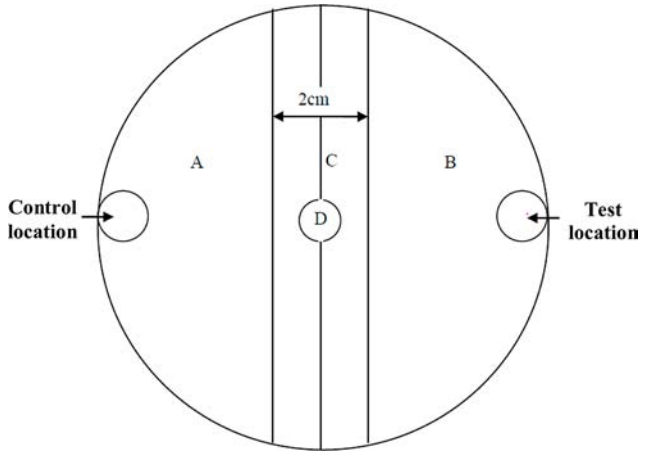


**Figure 1.** Configuration of chemotaxis assay plate. About 1 cm$^2$ of PDA medium with or without NK102 fungal colony was placed in the test or control location of the Petri dish, opposite one another. Two hours later, about 200 second-stage juveniles (J2s) of *M. incognita* were added to the center of the Petri dish (D), and the dishes were maintained in a dark cabinet at 25 °C for 4 h. Then the numbers of J2s in A, B, and C were counted under an inverted microscope (Olympus CKX41). Petri dishes without the medium served as the control. The experiment consisted of seven replicates and was repeated once at 25 °C.

**Effects of Culture Filtrates and ChA on Egg Hatch.** HPLC-purified ChA was diluted with a mixture of methanol (MeOH) and SDW (3:7, v/v) to get the initial concentrations of 300, 30, and 3 $\mu$g/mL, respectively. MeOH/SDW (3:7, v/v) was used as an untreated control. The culture filtrates of *C. globosum* were used at concentrations of 100, 50, 25, and 12.5%. S-7 medium without fungal inoculum was used as control. SDW was included as a negative control in both the ChA and fungal filtrate experiments. Each treatment had six replicates.

Egg suspension (0.05 mL, 200 eggs/well) was added onto a 35 $\mu$m sieve fastened by a 1.5 mL modified Eppendorf.[22] The sieves containing eggs were placed individually into a single well of 24-well tissue culture plates, where only the center well of each plate was used. After that, approximately 1.7 mL of fungal culture filtrate at various dilutions was added to the corresponding well. The 24-well culture plates were incubated in the dark at 25 °C. In the course of the experiment, the tested solution was replaced at days 3 and 7. The numbers of J2s were counted at days 3, 7, and 14, and hatch rate was measured.

**Effects of Culture Filtrates and ChA on J2 Mortality.** HPLC-purified ChA solutions (3, 30, and 300 $\mu$g/mL) and MeOH/SDW (3:7, v/v) were used for evaluation of its effect on J2 mortality. Fungal culture filtrates (100, 50, 25, and 12.5%), S-7 medium, and SDW were included for filtrate evaluation.

J2 suspension (about 100 nematodes in 0.02 mL) was added into one well of a 24-well tissue culture plate filled with 1.0 mL of fungal culture filtrate at various dilutions. Each treatment included six replications. The plates were maintained at 25 °C. Nematode mortality was recorded at 24, 48, and 72 h after exposure under an inverted microscope (CK41 Olympus). J2 survival was judged by stimulation of 1.0 M NaOH treatment.[23] The activities were expressed as $LC_{50}$ (concentration of the compound that causes 50% lethality).

**Effects of Culture Filtrates and ChA on J2 Penetration.** J2s were preincubated in corresponding HPLC-purified ChA solutions (300, 30, and 3 $\mu$g/mL) and SDW at 25 °C for 4 h. Likewise, J2s were preincubated in the filtrates (100, 50, 25, and 12.5%), S-7 medium, and SDW, respectively.

The pot experiment was performed in the greenhouse of the College of Life Sciences, Nankai University. After drying at 80 °C for 8 h, the clay loamy soil was thoroughly mixed with an equal volume of vermiculite and then fertilized with 0.74 g of $K_2SO_4$, 0.48 g of $(NH_4)_2HPO_4$, and 0.60 g of $CO(NH_2)_2$ per kilogram of dry soil. One 9-day-old cucumber seedling (*Cucumis sativus* var. Jinchun No. 4) was transplanted to the pot filled with 200 g of soil mixture. Two days after transplant, ca. 1000 pretreated J2s were inoculated into the pots through three holes (2 cm deep) made at the base of the plant. Nine days after inoculation, roots of the plants were harvested and frozen at −20 °C for 12 h and then whipped with a food processor to release J2s. The number of J2s was counted under an inverted microscope (CK41 Olympus). Each treatment included six replicates.

**Effects of ChA on *M. incognita* Reproduction.** For the reproduction assay, HPLC-purified ChA from the culture of *C. globosum* NK102 was directly added into the pots with 800 g of soil mixture described above at concentration of 30, 3, and 0.3 mg/kg dry soil. Likewise, one 9-day-old cucumber seedling was transplanted in each pot. On the third day, 1000 eggs in 2 mL of suspension were inoculated into the soil through holes around the plant. All of the pots were arranged randomly with six replicates per treatment. The plants were harvested 35 days after inoculation. Eggs were collected from roots and counted under an inverted microscope (Olympus CK41). Then the shoots and roots were weighed before drying at 105 °C for 30 min and at 80 °C for 48 h.

**Statistical Analysis.** Mortality values for the in vitro bioassay against the *M. incognita* were corrected by Abbott's formula.[24] The lethality concentration ($LC_{50}$) was calculated by Probit analysis. Data from the chemotaxis assay was analyzed using Student's *t* test ($P < 0.05$). Data from all assays except the chemotaxis assay were analyzed by one-way variance with SPSS 17.0. Means among treatments were compared by Fisher's least significant difference (LSD) test at $P = 0.05$ level.

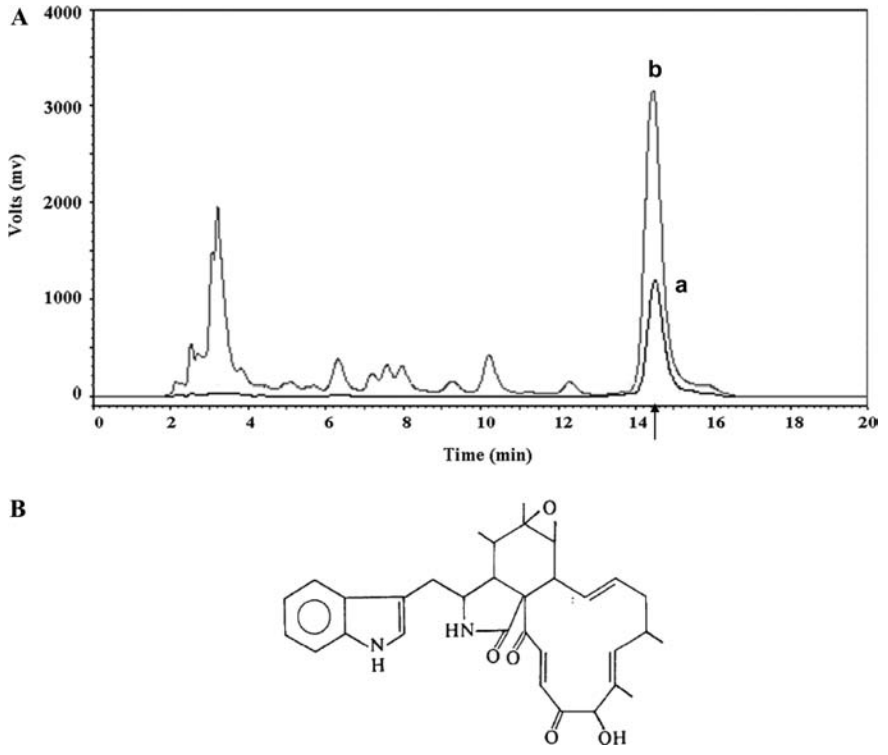


**Figure 2.** Structure of ChA (B, reported by Silverton et al.[25]) and detection of ChA by HPLC in *C. globosum* NK102 (A). Peak a is the standard ChA (a), and peak b is ChA produced by *C. globosum* NK102.

## RESULTS AND DISCUSSION

**Purification of ChA Produced by *C. globosum* NK102.** There were a great number of secondary metabolites in *C. globosum* NK102 extracts (Figure 2A). The putative ChA component predominated with a 52% peak area in the culture filtrate. The retention time of the substance is 14.480 min (b), which was close to that of the authentic ChA (14.490 min, a) (Figure 2A). The concentration of the ChA was determined by making a standard curve. The maximum production of ChA in S-7 medium was found to occur on the ninth date with 112.7 mg/L in the culture broth, which was much higher than that in PDB medium (50.5 mg/L). Additional characterization with LC-MS and NMR confirmed the molecular weight at 528 (unpublished data) and a structure identical to that of authentic ChA reported by Silverton et al.[25] (Figure 2B). NK102 could steadily produce ChA at a high yield in S-7 medium. The high yield of ChA in S-7 medium has not been previously reported. Thus, this strain and the medium are usable for mass production of ChA. The high amount of ChA produced by this procedure would benefit its future application for nematode control.

**Effects of *C. globosum* NK102 on J2 Chemotaxis.** As shown in Figure 3, the proportion of J2s in the zone inoculated with NK102 was 10.3%, significantly lower than the proportion of J2s (33.3%) in the zone inoculated with PDA medium only, whereas no difference in the proportion of *M. incognita* J2s between the zones of CK was observed (Petri dishes without the medium addition). These data suggested that *C. globosum* NK102 possessed a negative effect on nematode chemotaxis. Because the chemicals excreted by NK102 might contribute to a repellency effect toward *M. incognita*, the adverse effect of *C. globosum* NK102 on nematode chemotaxis might reduce the extent of host plant damage caused by nematodes. Nematodes preferred roots without microorganisms, and this could partially account for the controlled abundance of the ectoparasitic nematode *Tylenchorhynchus ventralis* by local interactions with soil microorganisms in coastal foredune soil.[26]

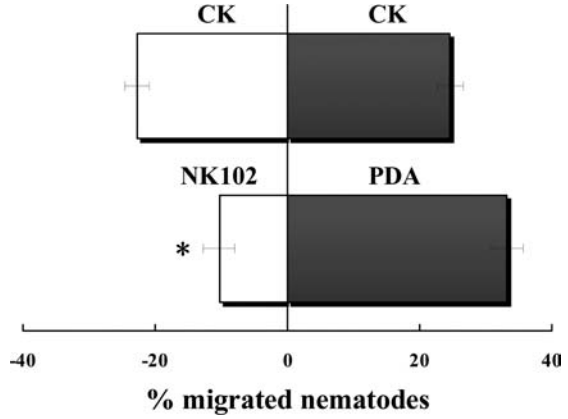


**Figure 3.** Repellence of *M. incognita* second-stage juveniles to *C. globosum* NK102 and PDA medium. The paired test was applied for analyzing nematode proportion means between the two sides. Data are shown as the mean ± SE. CK, no medium was added. The asterisk (*) indicates a significant difference determined by Student's *t* test ($P < 0.05$).

**Effects of Filtrates and ChA on Egg Hatch.** In general, there was no significant adverse effect on *M. incognita* eggs in all dilutions of *C. globosum* NK102 filtrates on days 3, 7, and even 14 (Figure 4A). In contrast, the treatments of ChA inhibited hatching after 7 days of exposure, as compared to control (Figure 4B). After 14 days of exposure, the treatments at concentrations of 300 and 30 μg/mL of ChA had adverse effects on hatching of 57.8 and 63.7% (Figure 4B), indicating that the inhibitory effect of ChA may have approached saturation over 30 μg/mL. This was inconsistent with the findings by Meyer et al.,[16] who found that *C. globosum* was capable of producing compounds active

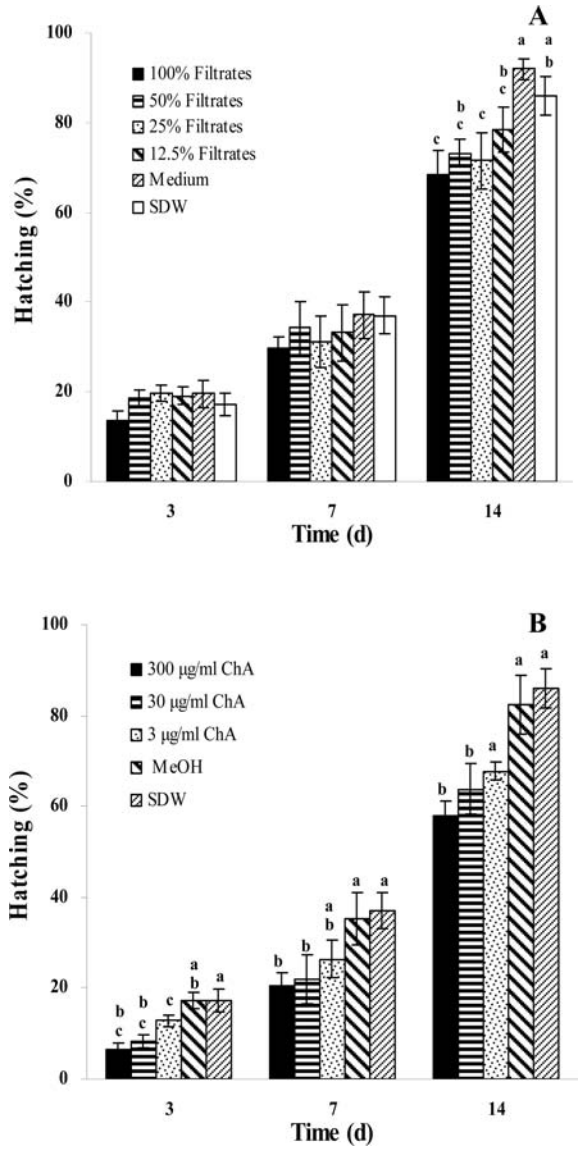


**Figure 4.** Effects of cultural filtrates of NK102 (A) and ChA isolated from NK102 (B) on egg hatch of *M. incognita* at 3, 7, and 14 days after inoculation. Medium, S-7 medium. Each value represents the mean ± standard error with six replicates each. Means (±SEs) followed by the same letters within bars are not significantly different for each treatment concentration ($P = 0.05$) in Tukey's HSD test.

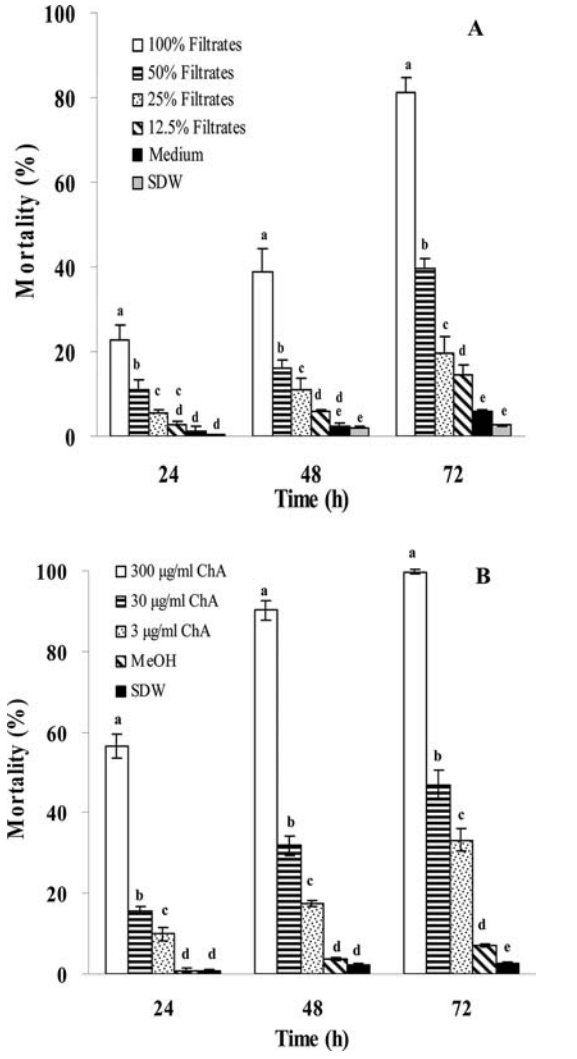


**Figure 5.** Effects of cultural filtrates of NK102 (A) and ChA isolated from NK102 (B) on mortality of second-stage juveniles (J2s) of *M. incognita* at 24, 48, and 72 h after inoculation. 100%, original preparation (1×); 50%, 25%, and 12.5%: original preparation was diluted 2×, 4×, and 8× by sterile distilled water (SDW), respectively. Medium, S-7 medium. SDW was included as control. Each value represents the mean ± standard error with six replicates each. Means (±SEs) followed by the same letters within bars are not significantly different for each treatment concentration at $P = 0.05$ in Tukey's HSD test.

against nematode egg hatch. *C. globosum* was isolated from eggs of *H. glycines* in their studies, and this fungus showed nematicidal activity against *M. incognita* as well. There might be an efficient way to explore isolates with high ChA production by collecting more isolates of *C. globosum* from the eggs of *M. incognita* and analyzing the yield of ChA in their filtrates.

**Nematicidal Activity of ChA and Crude Extracts of NK102.** Filtrates of *C. globosum* NK102 showed a nematicidal activity toward *M. incognita* J2s even at 12.5% dilutions after 24 h of exposure (Figure 5A). The highest toxicity observed at the original filtrate treatment (100%) after 72 h reached 81.3%. Okada et al.[27] found that the nematodes *Filenchus misellus* and *Filenchus discrepans* could hardly reproduce in the presence of *C. globosum* on PDA. Different stains might show different nematicidal activities, and thus we screened more isolates to explore their potential of nematicidal activity in the future.

The HPLC-purified ChA displayed strong nematicidal activities after 48 h of exposure with 90.2% J2 mortality at 300 μg/mL ($LC_{50}$ = 126.5 μg/mL), and almost all nematodes (90.2%) were killed after 72 h of treatment ($LC_{50}$ = 77.0 μg/mL) (Table 1 and Figure 5B). Moreover, the differences in J2 mortality were significant among different dilution treatments.

From an ecological prospective, the approach to reducing population density of plant parasitic nematodes under the economic threshold would be acceptable. Therefore, a significant reduction of J2 mortality at an appropriate concentration of ChA

**Table 1. $LC_{50}$ and $R^2$ Values of ChA against *M. incognita* at 24, 48, and 72 h**

| | 24 h | 48 h | 72 h |
|---|---|---|---|
| $LC_{50}$ (μg/mL) | 262.6 | 126.5 | 77.0 |
| $R^2$ | 0.99 | 0.98 | 0.98 |


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can be used for effective control of nematodes, even though J2 mortality at this level of ChA (30 μg/mL) may not be the highest.

**Effects of Culture Filtrates and ChA on Penetration of Cucumber by J2s.** Compared with controls of SDW and S-7 medium without inoculation, the culture filtrates of *C. globosum* NK102 could decrease the penetration of J2s significantly (Figure 6A). Both filtrates and ChA treatments showed a dose-dependent pattern against J2 penetration. Compared to the negative control (SDW), ChA caused a suppression of penetration of cucumber by J2s, with 59.4% at the concentration of 300 μg/mL and 31.7% at 30 μg/mL, respectively (Figure 6B).

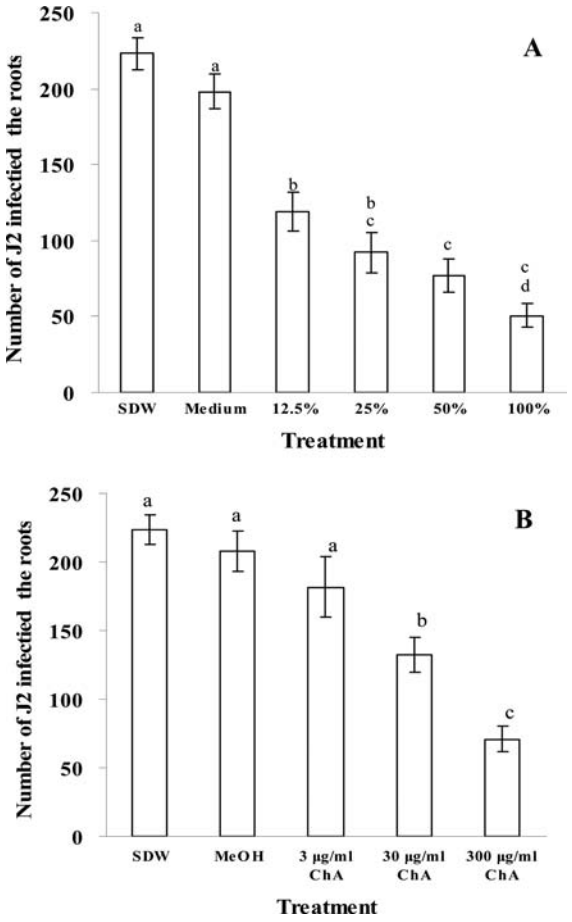


**Figure 6.** Effects of culture filtrates (A) and ChA isolated from NK102 (B) on infectivity of *M. incognita* on cucumber roots. The penetration of *M. incognita* J2s was evaluated 9 days after inoculation. 100%, original preparation (1×); 50%, 25%, and 12.5%, original preparation was diluted 2×, 4×, and 8× by sterile distilled water (SDW), respectively. SDW, sterile distilled water; medium, S-7 medium. Means (±SEs) followed by the same letters within bars are not significantly different for each treatment concentration at $P = 0.05$ in Tukey's HSD test.

Similarly, filtrates diminished the penetration of J2s, even at a low level of 12.5% filtrate treatment. Neither MeOH nor S-7 medium had any apparent effect on J2 penetration compared to SDW control. Penetration is one of the critical stages of the life cycle of *M. incognita*. It is interesting that some compounds might have toxic effects on the nematode that may not result in J2 death but still result in decreased infection. The capability of disrupting penetration of the nematode elucidated the antinematode activity of ChA.

**Effect of ChA on *M. incognita* Reproduction in Cucumber.** After 35 days, ChA treatment at the rate of 30 mg/kg significantly reduced egg reproduction of *M. incognita* by 63% of the control treatments (Table 2). Consistently, plant growth was significantly improved at the level of 30 mg/kg, with a 3.4-fold increase in root biomass and a 1.9-fold increase in shoot biomass relative to the control (Table 2). The results confirmed that ChA possesses nematicidal activity. In contrast, a fungus compound, flavipin, isolated from *C. globosum* did not have adverse effects on the reproduction of *M. incognita*.[18]

The life cycle of *M. incognita* includes several stages, for example, egg hatch, J2 chemotaxis, J2 penetration, and juvenile development within root tissue. In this work, we elucidated that the culture filtrate of *C. globosum* NK102 had nematicidal activities against the root knot nematode *M. incognita*. The suppressive effects on chemotaxis, J2 mortality, J2 penetration, and reproduction revealed the strong nematicidal activity of ChA produced by the fungus. It is significant that ChA has a strong nematicidal activity, even at low levels of 3 mg/kg or 3 mg/L, via the killing of J2s, inhibiting egg hatch, and J2 penetration. The minimum inhibitory concentrations (MICs) of fervenulin isolated from *Streptomyces* sp. CMU-MH021 against egg hatch of *M. incognita* was 30 mg/L, and an increase in juvenile mortality of *M. incognita* was observed at 120 mg/L.[28] Compared with carbofuran ($LC_{50}$ = 72.29 μg/mL),[29] a synthetic insecticide widely used in the field, ChA exhibited the same level of toxicity ($LC_{50}$ = 77.0 μg/mL at 72 h) against *M. incognita*. In addition, ChA showed strong adverse effects on J2 mortality but slightly significant adverse effect on egg hatch of *M. incognita*. Niu et al.[30] reported similar results, in which they found a nematodetoxic aurovertin-type metabolite from a *M. incognita* parasitic fungus, *Pochonia chlamydosporia*. The antimammalian cell action mode of ChA has been studied intensively.[12,31,32] This would benefit the comprehensive evaluation of ChA action as a nematicide in the future. The nematicidal activity of ChA may be due to its inhibitory property against the polymerization of monomeric actin (G-actin) to polymeric form (F-actin), thereby inhibiting cell functions requiring cytoplasmic microfilaments. To our knowledge, this is the first report of nematicidal activities of ChA against *M. incognita*. This work confirmed that ChA may be used as a nematode control agent in combination with other techniques for plant-parasitic nematode management. The field experiment and evaluation on other plant-parasitic nematodes is

**Table 2. Size of Glasshouse-Grown Cucumber Plants and *M. incognita* Egg Populations at 35 Days after Inoculation with *M. incognita* (1000 Eggs per Plant) Treated with ChA Isolated from NK102[a]**

| ChA (mg/kg) | root dry weight (g) | shoot dry weight (g) | shoot height (cm) | eggs per plant |
|---|---|---|---|---|
| 0 | 0.32 ± 0.05c | 3.49 ± 0.76b | 25.28 ± 2.53b | 530.5 ± 50.24b |
| 0.3 | 0.31 ± 0.04c | 3.61 ± 0.43b | 23.95 ± 2.80b | 507.8 ± 62.13b |
| 3 | 0.63 ± 0.07b | 2.81 ± 0.38b | 24.13 ± 2.64b | 461.0 ± 84.79b |
| 30 | **1.09 ± 0.13a** | **6.54 ± 0.60a** | **39.73 ± 4.09a** | **194.0 ± 46.31a** |

[a]Means (±SE) within columns followed by the same letter are not significantly different at $P < 0.05$.


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needed for future field application. Given the antagonism of *C. globosum* against plant pathogenic microbes and pest insects, the demonstrated nematicidal attribute of *C. globosum* NK102 reported in the present study would make *C. globosum* more attractive as a biological control agent.

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### Notes

The authors declare no competing financial interest.

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