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Effects of *Aphelenchus avenae* on Suppression of Soilborne Diseases of Ginseng

Young Ho Kim*
Korea Ginseng and Tobacco Research Institute, Taejon, 305-345, Korea

*Aphelenchus avenae*에 의한 인삼 토양병의 억제효과

김 영 호*
한국인삼연초연구원

ABSTRACT : The monoxenic culture of the fungivorous nematode, *Aphelenchus avenae*, was applied for the control of soil-borne ginseng pathogens such as *Fusarium solani* and *Rhizoctonia solani*. Fungivorous nematode populations were measured in a field to examine relationships between the nematode populations and suppression of ginseng root diseases. Inoculation of *A. avenae* (5000 nematodes per petri-dish) reduced the colonization of the *Fusarium* mycelium on root discs of ginseng and carrot by 80.0% and 60.5%, respectively. *A. avenae* also significantly reduced the occurrence of damping-off of ginseng by *R. solani* pathogenic to ginseng, and no plant damage by the nematode was noted. In a 3-year-old ginseng field infested with *Cylindrocarpon destructans*, plant missing caused by root rot was positively correlated to the density of potato rot nematode, *Ditylenchus destructor*, but it was reduced with the population of *A. avenae*, suggesting that *A. avenae* might inhibit the occurrence of ginseng root rot.

***Key words* : *Aphelenchus avenae*, *Ditylenchus destructor*, *Fusarium solani*, *Rhizoctonia solani*, ginseng root rot.**

Ginseng (*Panax ginseng* C. A. Meyer) root rot is one of the most important limiting factors in ginseng cultivation. It can be caused by various soil microorganisms (17), and generally a disease complex comprising more than one organism for the disease development. Important organisms related to the ginseng root rot are fungal root pathogens such as *Cylindrocarpon destructans* (2), *Fusarium solani* (3) and *Phytophthora infestans* (11), and the potato rot nematode, *Ditylenchus destructor* (10, 12). *Rhizoctonia solani* is the causal agent of damping-off and also induces ginseng root rot (17).

The soil is very complicated medium in which microorganisms interact with one another. In ginseng, contrary to other annual crops of which the soil ecosystem is disturbed frequently by plowing and cultural practices, relationships of soil microorganisms in the ginseng field soil may be stabilized due to the least cultural practices during the ginseng cultivation. Shading of the ginseng bed may also provide stable soil conditions for microbial activities by preventing severe fluctuations in temperature and moisture. The soil conditions may favor the natural enemies of ginseng root rot pathogens with a proper medium for microbial interactions.

Soil nematodes participate in many interactions affecting crop plants (5). Some nematodes directly parasitize plants, or give harmful effects on root health by inciting or aggravating root rot, while others such as fungivorous nematodes feed on pathogenic fungi, and thus suppress root diseases. The ability of fungivorous nematodes to control pathogenic fungi has been demonstrated in many studies. For example, *Aphelenchus avenae* reduced the population of *R. solani*, *Alternaria tenuis* and the mycelium of several other fungal species (9, 13). Disease severity of *R. solani* was limited by coating seeds with *A. avenae* (1). Therefore, this study was aimed to investigate the effectiveness of the fungivorous nematode, *A. avenae*, on the suppression of ginseng

*Corresponding author.

root diseases.

MATERIALS AND METHODS

Culture of *A. avenae*. *A. avenae* was isolated from a ginseng field. Viable nematodes extracted by Baermann funnel were transferred to and multiplied on potato dextrose agar medium (PDA) on which *F. solani* was growing. Nematodes cultured on the medium were extracted by the Baermann funnel, and collected on a 400-mesh sieve to remove fungal spores. The nematodes were suspended in sterilized water and transferred into the 2-week-old fungal cultures (*Pythium ultimum, F. solani, R. solani, Alternaria alternata*, and *Botrytis cinerea* which had been isolated from diseased ginseng plants) on PDA. Fifty nematodes were inoculated on each fungal culture, and incubated at 27℃. Fifteen days after inoculation, nematodes were extracted by the Baermann funnel and counted under a stereomicroscope. Six replications were used for each fungal culture.

Suppression of *F. solani* colonization on root discs. The nematodes cultured on *A. alternata* were collected on the 400-mesh sieve, and suspended in sterilized water. Ginseng root and carrot discs (ca. 1 cm in diameter and 0.5 cm in thickness) were placed on a filter paper soaked with *F. solani* spore suspension (10^5 spores/ml) in a 8-cm-diameter petri dish. The nematode suspension (3 ml, containing about 5,000 nematodes) was poured on the filter paper. After five days of incubation at room temperature (20~25℃), root discs colonized by the fungal mycelium were examined visually. Four replications, having 15 or 5 root discs in a plate for carrot or ginseng respectively, were used in this experiment.

Suppression of *R. solani* damping-off. *R. solani* (AG 2-1) isolated from a diseased ginseng seedling and pathogenic to ginseng was cultured on PDA for 15 days at the room temperature, and the fungal culture was mixed with sterilized river sand. Radish (*Raphanus sativus* L.) seeds were planted in the soil in a 15-cm-diameter petri-dish, followed by pouring the nematode suspension (1000 nematodes per petri-dish) into the soil, and grown at the room temperature. Five replications with 20 seeds in each petri-dish were used for each treatment. Five days later, pre-emergence damping-off was examined. Also ginseng seeds (dehisced by stratification) and 2-year-old roots were planted in 25×70×15 cm plastic pots (30 seeds or 14 roots in a pot) containing soil (sand : Yakto <ginseng fertilizer> = 1 : 1) mixed with the 10-day-old *R. solani*-potato dextrose broth culture (50 ml per pot). About 16,000 nematodes were inoculated into the soil of each pot, having 3 replications for ginseng seed and 6 replications for 2-year-old ginseng root. The pots were placed in a greenhouse with polyethylene net shading at about 15~22℃ during the experiment. One month after planting, the occurrence of damping-offs was examined.

Field experiment. In 1987, a 3-year-old ginseng field at the Suwon Experiment Station was severely damaged by ginseng root rots from which *C. destructans* was isolated. In this field, 10 locations, each of which included a pair of neighboring 1.6-m^2 sectors were randomly selected, and the incidence of plant missing was examined at the harvest time in September, 1987. Soil was collected from each sector, and the nematodes were extracted from 100 ml of soil (with 3 replications) by the Baermann funnel and counted. Severity of disease based on the plant missing rate was divided into 4 grades; weak (0~25 %), medium (25~60%), moderately severe (60~85%) and severe (85~100%), for which nematode populations were plotted. Also correlation coefficients between nematode populations and plant missing rates were measured.

RESULTS

Growth of *A. avenae* on fungal cultures. The fungivorous nematode, *A. avenae*, grew rapidly on all of the fungal cultures used in this experiment, showing numerous nematodes in the fungal cultures (Fig. 1A). The nematode growth rate was highest on the *A. alternata* culture, and lowest on the *P. ultimum* culture, in each of which the multiplication rates were 2,248 and 507 folds in 15 days (Fig. 2). The nematode increased by 610, 673, 1,494 folds in *R. solani*, *F. solani* and *B. cinerea* cultures, respectively.

Nematode feeding was observed under a light microscope (Fig. 1B). The nematode protruded stylet into fungal hypha, and absorbed cellular materials. Feeding of fungal spores was not observed in this experiment. All of the fungal colonies were damaged by the nematode, composed of thinner mycelia than healthy ones. In *B. cinerea*, formation of sclerotia was inhibited by the nematode.

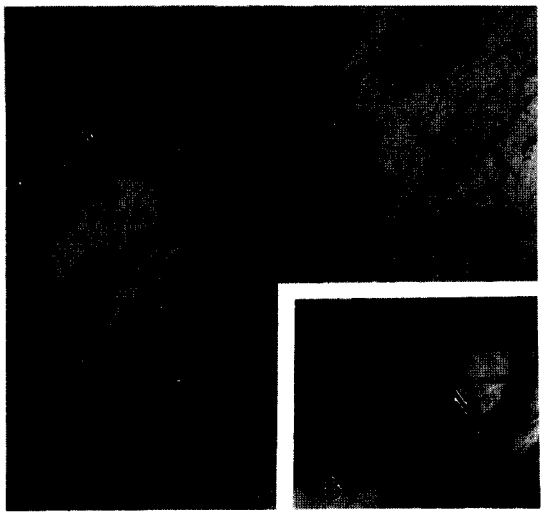

Fig. 1. *Aphelenchus avenae* nematodes (N) cultured on potato dextrose agar medium with *Alternaria alternata* (1A) (×40), and a nematode (N) feeding on the fungal hypha (H) (1B) (×100).

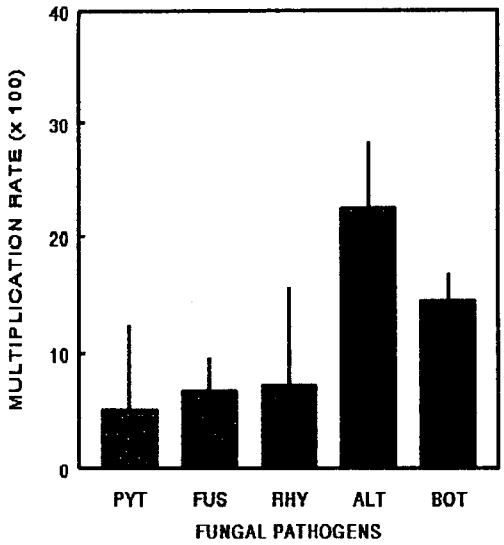

Fig. 2. Multiplication of *Aphelenchus avenae* for 20 days on the cultures of fungal pathogens at 27℃. Each bar and vertical line represents the average and standard deviation of 6 replications. PYT : *Pythium ultimum*, FUS : *Fusarium solani*, RHY : *Rhizoctonia solani*, ALT : *Alternaria alternata*, and BOT : *Botrytis cinerea*.

Suppression of *F. solani* colonization on root discs. Mycelium was formed on all of the ginseng root discs and most of carrot discs in the plates inoculated only with *F. solani*, while the mycelial colonization was greatly inhibited by the nematode inoculation (Fig. 3). The control efficacies by the nematode were 80.0% in ginseng and 60.5% in carrot, respectively. In the nematode alone, 10% of the ginseng root discs and 5% of the carrot discs were colonized by mycelium, which might be derived from the contamination of the nematode suspension.

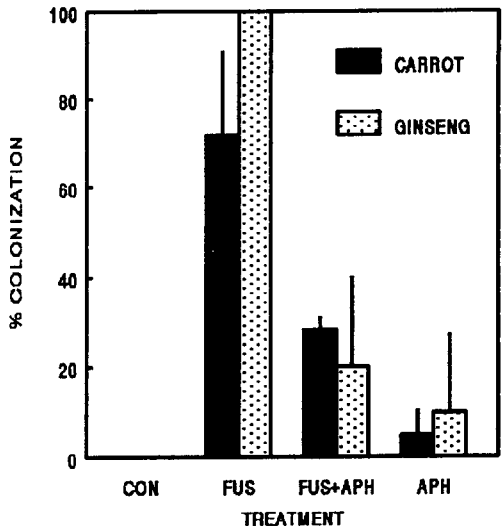

Fig. 3. Colonization of carrot and ginseng root discs by *Fusarium* mycelium in 5 days at room temperature. Bars and vertical lines represent averages and standard deviations of 4 replications.

Suppression of *R. solani* damping-off. In the petridish experiment, pre-emergence damping-off (including failure of seed germination) of radish was significantly inhibited by the application of the nematode (Fig. 4). The percentages of damping-off was reduced from 41.0% to 16.0% by the nematode. No indication of harmful effects of the nematode itself on the seed germination was noticed.

In ginseng, the suppression of damping-off by the nematode was less than that in radish; the incidence of damping-off was slightly reduced by the nematode. The incidence of damping-off of ginseng was reduced from 80.0% to 66.7% during seed germination and from 63.9% to 58.3% for 2-year-old ginseng root (seedling) (Fig. 5). In the controls damping-offs of seed and root were 24.4% and 25.4%.

Field experiment. In the 3-year-old ginseng field damaged by rusty root rot, abundant plant parasitic and fungivorous nematodes were observed. The densities of the nematodes were as follows: *D. destruc-*

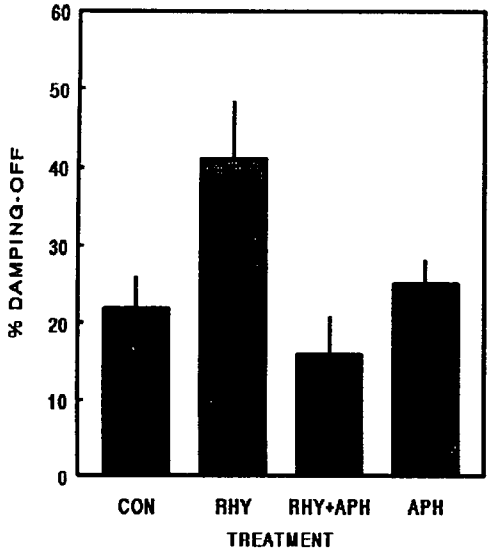

Fig. 4. Damping-off of radish 5 days after planting at room temperature. Bars and vertical lines represent averages and standard deviations of 5 replications. CON : control check (with no inoculation of *Rhizoctonia solani*, RHY : *R. solani* only, APH : *Aphelenchus avenae* only, RHY+APH : coinoculation of *R. solani* and *A. avenae*.

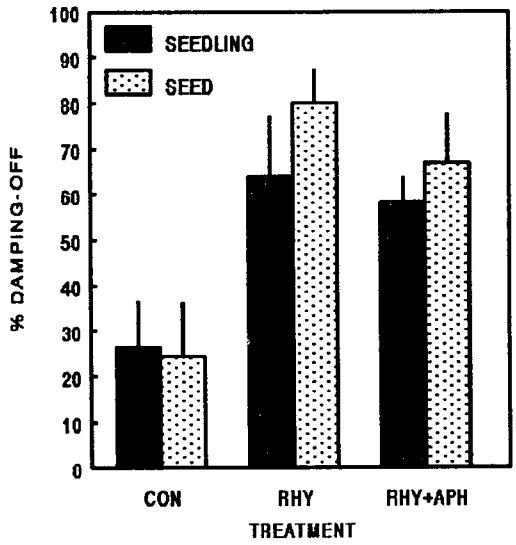

Fig. 5. Damping-off of ginseng seed and 2-year-old seedling one month after planting and transplanting, respectively, in a greenhouse. Bars and vertical lines represent averages and standard deviations of 3 (for seed) and 6 (for seedling) replications. CON : control check with no inoculation of *Rhizoctonia solani*, RHY : *R. solani* only, RHY+APH : coinoculation of *R. solani* and *Aphelenchus avenae*.

Table 1. Numbers of fungivorous nematodes in a 3-year-old ginseng field and correlation coefficients between nematode populations and plant missing[a]

Nematode(s)	No. nematodes/ 100 ml soil[b]		Correlation coefficient[c]
	Average	Range	
Aphelenchus avenae	21	0~ 72	−0.792**[d]
Ditylenchus destructor	22	0~ 52	0.735*
Aphelenchoides spp.	8	0~ 16	−0.244 NS
Tylenchus spp.	50	12~158	0.329 NS

[a] Three soil samples were collected from and plant missing was examined in each of 10 locations in a 3-year-old ginseng field in Suwon, Korea, in September, 1987.
[b] Nematodes were extracted by Baermann funnel from 10 locations of the ginseng field.
[c] Correlation coefficient between the nematode population and the plant missing rate.
[d] *Significant at p=0.05, **Significant at p=0.01, and NS : not significant at p=0.05.

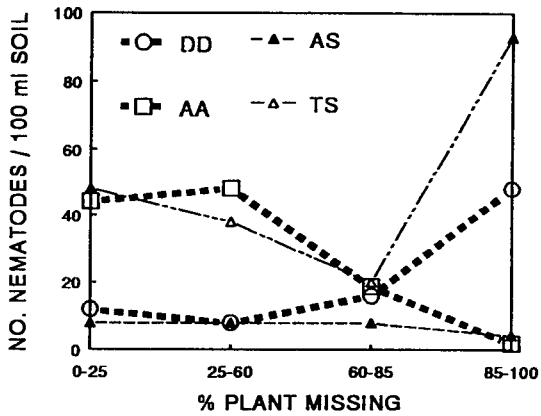

Fig. 6. Plotting of incidence of plant missing in a 3-year-old ginseng field severely damaged by ginseng root rot (probably caused by *Cylindrocarpon destructans*) and the nematode populations (DD : *Ditylenchus destructor*, AA : *Aphelenchus avenae*, AS : *Aphelenchoides* spp., and TS : *Tylenchus* spp).

tor, 0~52 (22 in average); *A. avenae*, 0~72 (21 in average); *Aphelenchoides* spp., 0~16 (8 in average); and *Tylenchus* spp., 12-158 (50 in average) per 100 ml of soil (Table 1). The locations with higher plant missing rates had higher densities of *D. destructor* and lower densities of *A. avenae* (Fig. 6). The Populations of *Aphelenchoides* spp. and *Tylenchus* spp. appeared to have no significant relationships to the plant missing rate. Correlation coefficients between

nematode density and plant missing rate (caused by root rot) were 0.735 for *D. destructor*, −0.792 for *A. avenae*, −0.244 for *Aphelenchoides* spp., and 0.329 for *Tylenchus* species (Table 1). Both *D. destructor* and *A. avenae* were significantly related to the plant missing. Most of the remaining roots were diseased in part with rusty rot, from which *C. destructans* was readily isolated.

DISCUSSION

Among fungivorous nematodes such as *A. avenae*, *Aphelenchoides* spp., *Tylenchus* spp., and *Ditylenchus* spp., only *A. avenae* has been considered as a biological control agent against plant pathogenic fungi (5). Other fungal feeding nematodes are facultative plant parasites. *D. destructor* feeds on onion bulbs, potato tuber, and ginseng root (10, 16). *Tylenchus* spp. are considered polyphagous, feeding on mosses and plant roots, and *Aphelenchoides* spp. parasite leaves of many herbaceous plants such as strawberry, begonia, rice, chrysanthemums, etc. (14). *A. avenae* was found in a diseased portion; however, it was suggested that the nematode was not associated with the disease as a causal agent, but as a feeder of microorganisms associated with the disease (13). *A. avenae* feeds on many different species of fungi, but other microorganisms are poor food sources for the nematode (9, 15). The nematode population increased very rapidly in all of the fungal cultures used in this experiment. The nematode growth was most rapid in the *A. alternata* culture. However, the nematode growth seemed not to be specific to fungal species because its rate varied greatly within a fungal species. In another experiment, the growth rate differed from that of this experiment (unpublished). It may be dependent on the amount and cultural conditions of fungal mycelium.

In this study, disease severity of *F. solani* and *R. solani* was limited by the treatment of the nematode in petri-dish and pot tests. Colonization of *F. solani* was blocked by the nematode, which suggests that the nematode may inhibit disease development by preventing the fungus from occupying root surfaces. In the field experiment, plant missing possibly caused by *C. destructans* was reduced with the increase of the *A. avenae* population. *A. avenae* may feed on the mycelium of *C. destructans* and thus might have reduced the severity of ginseng root rot.

The nematodes can be easily cultured, and have a dormant state in which large numbers of the nematodes can be obtained and readily applied to soil. Therefore, the nematode may be highly potent to be used as a control agent for pathogenic fungi. In this study, the control efficacy of the nematode for damping-off of ginseng was reduced compared with that of radish caused by *R. solani*. This is probably because the ginseng test was conducted in a larger scale than that of radish. It is generally accepted that the control efficacies of biological control agents of plant diseases fluctuate greatly depending upon crops, fields, environmental conditions, and species and strains of target organisms. Application of soil amendments should be considered to improve control efficacies of the biological control agent. The fungivorous nematode can also be mixed with other biocontrol agents and chemicals for integrated control of soil pests (8).

No direct harmful effect of *A. avenae* on ginseng plants was noted in this experiment. However, as fungivorous nematodes suppress mycorrhizae on many plants, and feed on beneficial fungi which stimulate plant shoot and root growth (6, 7), indirect harmful effects of the nematode on ginseng should be examined before using *A. avenae* as a biological control agent.

요 약

곰팡이 배지에서 배양한 식균선충(*Aphelenchus avenae*)의 인삼 뿌리 병원균인 *Fusarium solani*와 *Rhizoctonia solani*에 대한 방제 효과와, 포장에서 선충의 밀도와 인삼 뿌리썩음병의 발생을 조사하여 식균선충과 뿌리썩음병의 발생과의 관계를 조사하였다. 인삼 뿌리와 당근 절편에 *A. avenae*를(petri-dish당 5,000 마리) 접종한 결과 *F. solani*의 균사 형성을 각각 80.0%, 60.5% 저해하였다. 또한 *A. avenae*는 인삼의 모잘록병균인 *R. solani*에 의한 모잘록병 발생을 감소시켰다. *Cylindrocarpon destructans*에 의해 뿌리썩음병이 심한 3년근 인삼포에서의 뿌리썩음병에 의한 결주의 발생은 *Ditylenchus destructor*의 밀도와는 양의 상관(r=0.735)이 있었으나 *A. avenae*의 밀도가 높아짐에 따라 결주율이 낮아져 부의 상관(r=−0.792)를 나타내었다. 위와 같은 결과로 보아 *A. avenae*는 인삼 뿌리병의 발생을 감소시키는 생물적 방제인자의 하나로 생각된다.

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