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In Vitro Culture and Factors Affecting Population Changes of *Ditylenchus destructor* of Ginseng

Young Ho Kim* and Seung Hwan Ohh
Korea Ginseng & Tobacco Research Institute, Taejon 305-345, Korea

인삼의 *Ditylenchus destructor* 선충의 인공배양 및 밀도에 영향을 주는 요인

김영호* · 오승환
한국인삼연초연구원

ABSTRACT : *Ditylenchus destructor* isolated from diseased ginseng roots was cultured on ginseng calli, fungal mycelium (*Fusarium solani*), carrot discs and radish sprouts. Effects of temperature, organic material and flooding on the nematode population changes were examined. *D. destructor* multiplied readily on the culture media except radish sprout medium, and was cultured best on the fungal culture at 20℃. Feeding of the fungal hypha and radish root hairs, molting and mating in the fungal culture medium were observed. Addition of organic materials (perilla, sesame, soybean and ginseng leaves) in soil significantly increased *Aphelenchus avenae* and saprophytic nematode populations, while *D. destructor* populations changed little and the nematode population growths were limited by the organic amendments (except sesame leaves). The nematode populations in soil including *D. destructor* were decreased by flooding. The results indicate that *D. destructor* may survive but not multiply readily in soil without host plants and that it can be effectively controlled by flooding.

***Key words* : *Ditylenchus destructor*, *Aphelenchus avenae*, *Panax ginseng*, organic amendment, flooding.**

Potato rot nematode (PRN), *Ditylenchus destructor* Thorne, is a polyphagous nematode, parasitizing various hosts including potato, bulbs and peanut (5, 8, 11). In ginseng (*Panax ginseng* C. A. Meyer) PRN was first reported in 1976 by Choi (2). The nematode was found in Kimpo, Anseong, Seosan, Geumsan and Punggi, which are important ginseng cultivation areas in Korea. He also described the morphological characteristics of PRN and suggested that ginseng root rot might be related to the nematode as in potato. Later in 1983, morphological characteristics of PRN were described and its pathogenicity and damage to ginseng were confirmed by Ohh *et al.* (16). According to Ohh *et al.* (16), 36% of the ginseng fields in Dongseong area of Cheolweon-gun, Korea, were extensively damaged by PRN. Thereafter PRN damages in ginseng fields have often been observed. Out of 61 ginseng fields which were abolished in 1986, 5 were notably damaged by the nematode (13).

In ginseng, root rot is caused by multiple organisms, and is one of the most important diseases limiting successful ginseng cultivations. Major causal organisms are PRN (16), and fungal pathogens such as *Cylindrocarpon destructans*, *Fusarium solani*, *Phytophthora cactorum*, *Rhizoctonia solani*, and *Sclerotinia* sp. (3, 4, 17, 24). The severity of root rot was proportional to the population density of PRN inoculated on ginseng plants (16), and this may hold true for the damages by the dual infection of both the nematode and fungal pathogens. In this study, effects of culture media, temperature, organic amendment and flooding on the nematode population changes were investigated to provide information used to manage the nematode for controlling ginseng root rot.

*Corresponding author.

MATERIALS AND METHODS

Nematode preparation. Four 4-year old ginseng roots (showing apparent disease symptoms), grown in Suwon Experiment Station of Korea Ginseng & Tobacco Research Institute, were chopped in about 1 cm pieces and placed on funnels for 5 days at room temperature to extract nematodes. Extracted nematodes were surface disinfected in 500 ppm streptomycin sulfate and 50 ppm mercury chloride solution for 2 minutes, washed in sterilized water, and transferred into fresh sterilized water. About three thousand *D. destructor* adults and juveniles were collected under a stereomicroscope, and used as nematode inoculum.

***In vitro* culture of *D. ditylenchus* and observation of the nematode behavior.** Four culture media, fungal mycelium, carrot disc, callus and radish sprout, were used for PRN culture in this study. *F. solani* cultures grown in PDA media in petri-dishes (8 cm in diameter) for 10 days at 25℃ and ginseng root calli grown on Murashige and Skoog media for 30 days in 250 ml flasks were inoculated with 300~400 nematodes on each media. About 200 nematodes were inoculated on the surfaces of fresh carrot discs (about 5 cm in diameter and 5 mm thick) in the petri-dishes, and on 1.5% water agar media in petri-dishes (8 cm in diameter) where radish (*Raphanus sativus* L.) sprouts were grown for 5 days. After inoculation, the plates and flasks were placed at 25℃, and often observed visually until the nematodes were extracted by Baermann funnel and the population growth was examined at 30 days later. For carrot discs the nematodes population was examined 90 days after inoculation, because little population growth was noted at 30 days after inoculation. Each medium was replicated 3 (for ginseng callus) or 5 times (for others).

Effects of temperature on multiplication of *D. destructor*. About 110 nematodes were inoculated on the *F. solani* cultures mentioned above, and the cultures were incubated at 5, 10, 15, 20, 25 and 30℃ in the dark. For each temperature, 6 cultures were examined. Forty-five days after inoculation, the agar media were copped and nematodes were extracted from the cultures by Baermann funnel, and the numbers were calculated.

Effects of temperature on mobility of *D. destructor*. Ten to twenty nematodes in a drop of the nematode suspension were placed on the center of a petri-dish (8 cm in diameter) containing 1.5% agar medium, and the petri-dishes were placed in different chambers at 5, 10, 15, 20, 25 and 30℃ in the dark. Twenty four hours later, the distances between nematodes and the center of the petri-dish were measured. Four replications were used for each temperature.

Effect of organic matter on population change of *D. destructor* in field soil. Plant materials such as perilla (*Perilla sikokiana* Nakai) leaves, sesame (*Sesamum indicum* L.) leaves, ginseng leaves and soybean (*Glycine max* Merr.) leaves were dried and ground to powder, each of which was amended 1% (w/v) to loamy soil (ca. 25% moisture content) naturally contaminated with PRN (6 nematodes/50 ml), and *Aphelenchus avenae* (13 nematodes/50 ml) and saprobes (110 nematodes/50 ml). The soil was placed in a 28×21×17 cm-plastic container, tightly sealed with a lid to prevent drying, and incubated at room temperature (20~25℃). The soil without organic matter was used as the untreated control. For each plant material, 5 replications were used. Nematode populations were examined by Baermann funnel after 20 days and 6 months of incubation.

Effect of water saturation on population change of *D. destructor*. Ginseng field soil heavily contaminated with PRN (25% moisture content) contained in 250 ml flasks were saturated, and placed in dark chambers of 5, 10, 15 and 30℃ and at room temperature (20~25℃<23℃ in average>) in a plastic container. Twenty days later, nematodes were extracted by Baermann funnel and counted.

RESULTS

Population growth of *D. destructor* on culture media. PRN populations examined after 30 days varied depending on the culture media; however, the viable nematode populations were about 10,000 in the fungal culture and about 3000 in the ginseng callus (Fig. 1). Little nematode population growth was observed on the carrot disc 30 days after inoculation. PRN population on the carrot disc was checked again after 90 days, and the average nematode population was about 3000 per carrot disc. There was no indication of the PRN population increase on radish sprout media. The surfaces of the fungal culture media infested with PRN turned watery. In

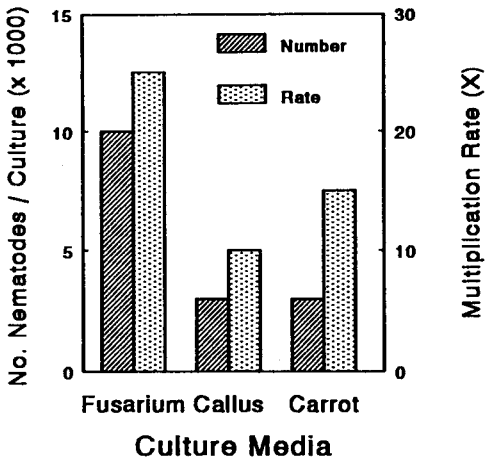

Fig. 1. *In vitro* population growth of *Ditylenchus destructor* on *Fusarium solani* culture medium (Fusarium) and ginseng callus medium (Callus) for 30 days, and on carrot disc medium (Carrot) for 90 days of incubation. Bars represent averages of 5 replications for *F. solani* culture and carrot disc media and 3 replications for ginseng callus medium.

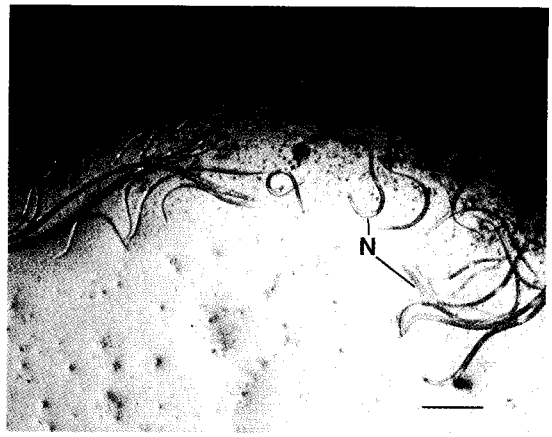

Fig. 2. *Ditylenchus destructor* nematodes cultured for 30 days on a *F. solani* culture, showing numerous nematodes (N) on the medium. The opaque portion is composed of fungal mycelium, and the other light area is a mycelial blank due to the nematode feeding. Bar= 250 μm.

some portions, mycelial blanks were formed, through which aggregated nematodes were often observed under the light microscope (Fig. 2). The infected ginseng calli and carrot discs became slimy, and the cell contents appeared to be extensively deprived when viewed under the light microscope.

Observation of *D. destructor* behavior. Various nematode behaviors were observed on fungal culture media, including feeding (Fig. 3a), molting (Fig. 3c), mating (Fig. 3d), oviposition and hatching (not photographed). Radish root hairs were fed on by the nematode (Fig. 3b). In the fungal culture, the nematode initially probed hyphal surface, followed by insertion of the stylet into the cell and feeding. Feeding on fungal spores was not observed in our experiment. When molting, a nematode became sluggish, and the old cuticle was detached and sloughed off the nematode body. In mating, female and male nematodes were contacted with each other, arranging their bodies in the opposite direction. Soon after the spicule was inserted, the uterine area was bulged momentarily, indicating a seminal emission.

Effects of temperature on nematode reproduction and mobility. The PRN population density in the *Fusarium* culture was highest when the culture was incubated at 20℃ for 45 days, while there was no population growth at 5 and 10℃ (Fig. 4). The PRN population increased 20.3 × at 20℃. At 25℃ and 30℃ the PRN populations increased 15.1 × and 14.1 ×, respectively, which were somewhat lower than at 20℃, but not significantly different.

The nematode mobility was not significantly different among 15, 20 and 25℃, moving 13.7~11.9 mm a day (Fig. 4). The nematode was even mobile at 5℃, moving 5.1 mm away from the center of the medium.

Effect of organic matter on population change of *D. destructor* in soil. Fungal mycelia were abundantly formed on the soils treated with plant materials. PRN populations were little changed and the population growth was somewhat inhibited by the organic amendments (except for sesame leaves), compared with that of the control check which had a slight population increase (Table 1). However, significant population changes of *A. avenae* and saprophytic nematodes were noted in soil amended with organic matters, increasing from 6 to 406~939 nematodes/50 ml soil (31.2~72.2 ×) and from 110 to 2081~6569 nematodes/50 ml soil (18.9~59.7 ×) in 20 days, respectively (Table 1). The populations of *A. avenae* and the saprophytic nematodes were not significantly increased in soil with no plant materials amended. The nematode populations after 6 months were similar to those of the 20-day incubation with some variations, regardless of organic amendment.

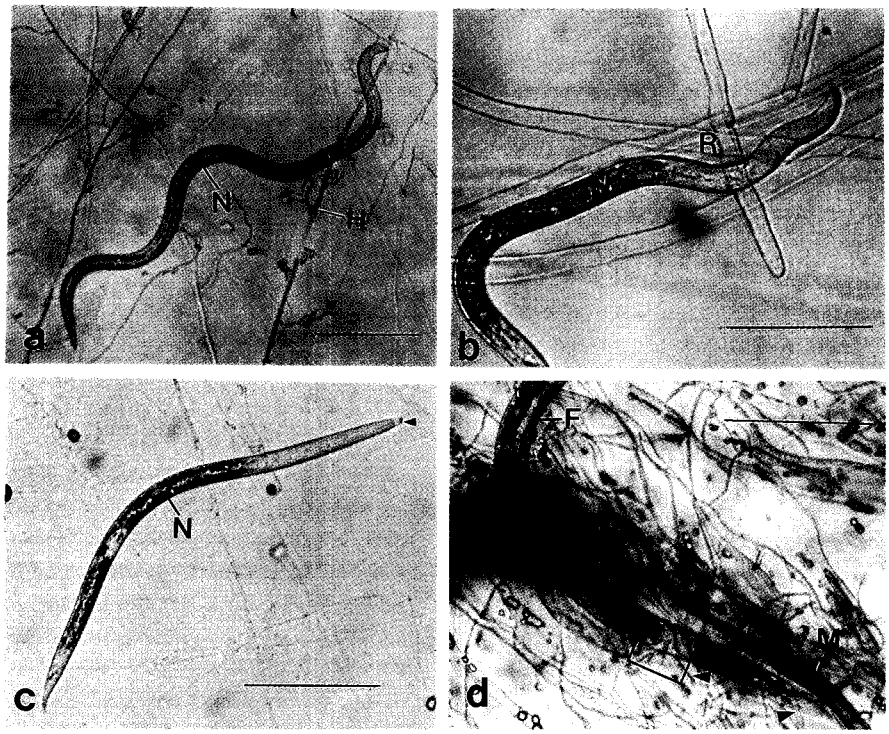

Fig. 3. Light micrographs of various behaviors of *Ditylenchus destructor*. N=nematode, H=fungal hypha, R=root hair, F=female nematode, M=male nematode. a) A juvenile of *D. destructor* feeding on *Fusarium solani*, and b) a female nematode feeding on radish. The nematode stylet was inserted into the hyphal cell and the root hair cell. c) Molting of a juvenile in the fungal culture medium, showing the conspicuous detached old cuticle at the anterior portion (arrow). d) Mating of a female and a male, showing that the nematodes pose oppositely, but the tails (arrows) arranged parallel. Bars=100 μ m.

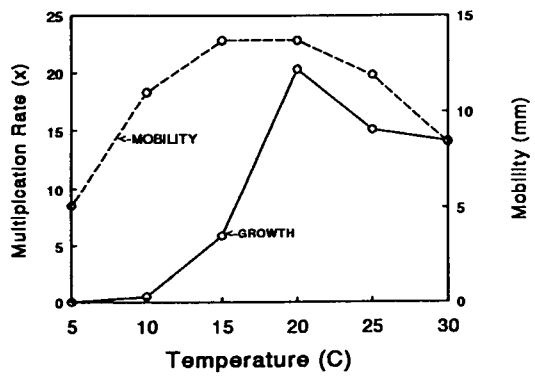

Fig. 4. Influences of temperature on the multiplication rate and mobility of *Ditylenchus destructor*. Multiplication rate is the ratio of the nematode numbers from *Fusarium solani* culture media after 45 days of incubation relative to the initial numbers of inocula. Each data point represents a mean of 6 replicates. Mobility is the nematode migration distance on water agar medium in 24 hours. Each data point represents a mean of 4 replicates.

Effect of water saturation on population change of *D. destructor* in soil. There were fewer nematodes in saturated soils, regardless of the nematode species examined, than in unsaturated soil (Fig. 5). As the temperature increased, nematode populations decreased more greatly in the saturated soil. No PRN was detected in the saturated soil at above 15℃.

DISCUSSION

Unlike *D. dipsaci* which is known to be an obligate parasite of higher plants, *D. destructor* thrives both in higher plants and fungi (22). PRN was massively cultured on groundnut callus tissue (23), and peanut embryo explants (6). Faulkner and Darling (7) demonstrated that PRN fed and reproduced on a wide range of fungi isolated from soil and infested potato tubers. In our study, PRN from ginseng root rots reproduced readily on *F. solani* mycelium, ginseng callus and carrot disc, of which the *F. solani*

Table 1. Nematode population changes in soil amended with organic matter

Organic matter[a] (Treatment)	No. nematodes/100 ml soil[b] after treatment								
	Ditylenchus destructor			*Aphelenchus avenae*			Saprophytic nematodes		
	0	20 days	6 months	0	20 days	6 months	0	20 days	6 months
Control	6	15 Z*[c,d]	25 Y*	13	4 X NS	23 X NS	110	1085 X NS	661 X NS
Perilla leaves		0 X*	5 X NS		939 Z**	711 Y**		3116 X*	6303 Y*
Soybean leaves		2 XY NS	8 X NS		406 Y*	458 Y**		2946 X*	1509 X NS
Sesame leaves		7 Y NS	23 Y*		572 Y*	1496 Z**		2081 X*	2532 X NS
Ginseng leaves		2 XY NS	4 X NS		486 Y*	1072 Z**		6569 Y*	9211 Y*

[a] Dried leaves were ground to powder and mixed with nematode-contaminated field soil (1 g/100 ml soil) in air-tight plastic containers which were placed at room temperature.

[b] Average numbers of 5 (for organic matters 0 and 20 days later), 8 (for perilla, sesami and ginseng 6 months later), 5 (for soybean 6 months later), and 7 replications (for the control 6 months later).

[c] The same letters in a column are not significantly different at p=0.05 by LSD.

[d] Significantly different at p=0.05 (*) and at p=0.01 (**), and not significantly different (NS) from the initial nematode populations.

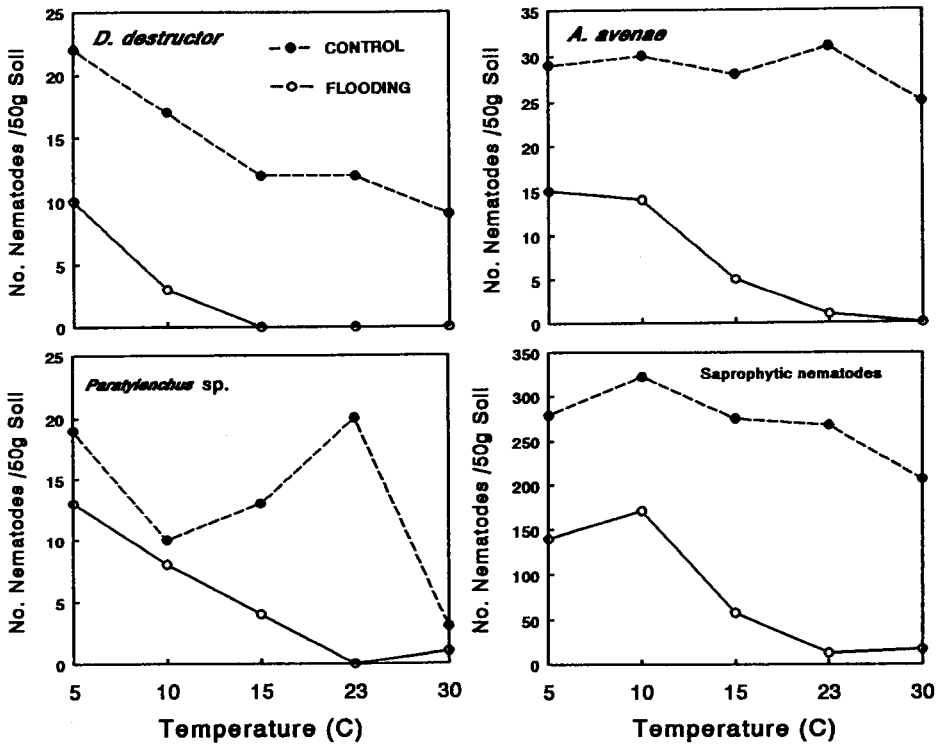

Fig. 5. Numbers of *Ditylenchus destructor*, *Aphelenchus avenae*, *Paratylenchus* sp. and saprophytic nematodes extracted by Baermann funnel from soil saturated with water (Flooding) for 20 days or of untreated control (Control) at different temperatures. Each data point is a mean of 2 replicates.

mycelium supported the largest nematode population. The fungal culture medium can be used to prepare the nematode inoculum for other experiments. The fungal mycelium can be removed during the nematode extraction by the Baermann funnel, and fungal spores by sieving through a proper-mesh

sieve. The fungal culture agar blocks were transferred into fresh fungal culture media to subculture the nematode, but the build-up of the nematode population in such conditions was greatly variable and mostly unsuccessful. This suggests that nematodes should be extracted from the fungal culture media and reinoculated into fresh fungal culture media for the safe preparation of the nematode inoculum.

The different degrees in supporting the nematode population on the different media may be explained by the difference in available biomass as reported by Jaffee (9). The ginseng callus was relatively small (ca. 3.5 cm^3), and the carrot tissue may be too compact for PRN to move readily through the tissues for feeding and mating. In another study of ours (unpublished), *Alternaria alternata* and an unknown fungal species were tested for the PRN reproduction, and supported much less and as much PRN population growths, respectively, compared with *F. solani*. However, more work is required to determine whether the *in vitro* growth of PRN from ginseng is specific or not to fungal species.

Most of the organic amendments used in our study inhibited the PRN population growth, while initial populations of *A. avenae* and saprophytic nematodes increased greatly in the soil amended with every organic matter. In another test in which the *F. solani* culture media were inoculated with PRN and covered with field soil, the population of saprophytic nematodes became more than 100 × higher than the PRN population after 45 days (unpublished data), indicating that the saprophytic nematodes multiplied much more rapidly than PRN. In the *F. solani* culture, *A. avenae* multiplied 673 × in 15 days (12), while PRN reproduced 25 × in 30 days in our study, which also suggests the *A. avenae* has much higher reproduction rates in the same culture conditions. Formation of abundant fungal mycelia were visually observed on the soils treated with the plant materials in our study, indicating that microorganisms including fungi and bacteria (food sources for *A. avenae* and saprophytic nematodes, respectively) increased by the organic amendments. Soil amendments are commonly used to control plant parasitic nematodes (15, 19). Mechanisms related to the control of nematodes by organic amendments may be variable, but one of them is shown in chitin amendment which can stimulate the build-up of chitin decomposing microorganisms antagonistic against some nematodes (20). Based on our study, organic amendments stimulate the growth of fungivorous and saprophytic nematodes which may compete with PRN for food and space, and thus may inhibit the PRN population growth.

In our study, the optimum temperature for the nematode population growth for 45 days *in vitro* was 20℃. The nematode population of the 45-day-culture at 25℃ somewhat decreased compared with that of the 30-day-culture, and the nematode populations in soil decreased more with the increase of temperature. These results suggest that the nematode population change *in vitro* may be not only dependent upon the reproduction but also mortality at a given temperature. In potato, the nematodes are killed and thus fail to repeat the life cycle under conditions of wet rots (22). Especially when the tubers are exposed to secondary invaders, temperature increase may bring much more adverse effects to the nematode population growth by accelerating tissue rot. In this respect, the nematode population growth in ginseng fields may mainly occur during the early infection stage before ginseng root rots become severe. The PRN was still active at low temperatures, suggesting that the nematode may invade the root in spring, starting a disease cycle early.

PRN fed on root hairs of a nonhost plant, radish, and survived in soil without host plants. The nematodes survive on fungi and weeds until host plants are planted (1). These aspects suggest that PRN may not be controlled only by fallowing or crop rotation. Additional control measures may be needed for the effective nematode control. The PRN of ginseng can be controlled by nematicides (18). As for nonchemical control besides fallowing and rotation, flooding has been used as a method of nematode management. Flooding is used as a control measure of root-knot nematode of vegetables (21), and recommended for nematode control in banana (14). Reductions of nematode populations in water-saturated soils occur either by a lack of oxygen or by the toxic by-products of anaerobic organisms such as *Clostridium* spp. which may increase under such conditions (10). No study has been reported on the control of PRN in ginseng by flooding; however, our study showed that flooding may be an efficient way of the PRN control because the nematodes

were eliminated by flooding in 20 days at above 15℃. However, field experiments should be conducted to confirm the control efficacy of flooding. Also its adverse effect on ginseng growth due to changes of soil structure and chemical composition in flooded conditions should be considered before the practical use of the control measure.

요 약

인삼에서 분리된 감자썩이선충(*Ditylenchus destructor*)을 인삼 callus, 곰팡이(*Fusarium solani*) 배지, 당근 절편 및 무 유묘 배지에서 배양하였다. 감자썩이선충의 밀도 변화에 대한 온도, 유기물처리 및 담수의 영향을 조사하였다. 감자썩이선충은 무 유묘 배지를 제외하고는 모든 인공배지에서 증식하였으며, 곰팡이 배지에 20℃에서 선충의 증식이 가장 잘 되었다. 인공 배지상에서 곰팡이의 균사 흡즙, 무 뿌리털 흡즙, 탈피, 짝짓기 등 선충의 생장과 관련된 행위가 관찰되었다. 소엽, 참깨잎, 콩잎, 인삼잎 등 유기물을 토양에 첨가하였을 때, 식균선충(*Aphelenchus avenae*)과 부생선충의 밀도는 증가한 반면, 감자썩이선충의 밀도는 거의 변하지 않았으며, 참깨잎을 제외하고는 유기물-무처리에 비해 밀도 증식이 억제되었다. 담수에 의해 선충의 밀도가 감소하였다. 위의 결과로 볼 때, 감자썩이선충은 기주식물이 없는 토양에서 생존은 가능하나 증식은 잘 되지 않으며, 담수에 의해 효과적으로 방제할 수 있을 것으로 생각된다.

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