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Studies on the Pathogenicity of *Aphelenchoides* sp. and *Rhabditis* sp. attacking Cultivated Mushroom, *Agaricus bisporus*(Lange) Sing.

Yong Sik Han, Jung Su Park,
Dong Soo Kim, and Kwan Chul Shin

Institute of Plant Environment, O.R.D., Suwon, Korea

Aphelenchoides sp.와 *Rhabditis* sp.의 양송이에 대한 병원성에 관한 연구

한용식 · 박중수 · 김동수 · 신관철

농촌진흥청 식물환경연구소

Abstract : Four species of nematodes attacking mushroom beds were found in samples taken from 35 mushroom farms throughout Korea. These were *Rhabditis* sp., *Aphelenchoides* sp., *Ditylenchus* sp. and *Aphelenchus* sp., *Rhabditis* sp. was found from compost and casing from all mushroom farms and the frequencies of *Aphelenchoides* sp. was 31.4% in the both compost and casing.

Both *Ditylenchus* sp. and *Aphelenchus* sp. showed 2.7% of frequencies in the compost, none in casing.

Temperature and moisture content of compost affected pathogenicity of *Aphelenchoides* sp. on mushroom mycelia grown in compost.

The higher temperature and moisture content the sooner the damage became apparent, and the more rapid was subsequent destruction of mycelia. There was no mycelial destruction at the lowest temperature of 10°C.

Rhabditis sp. completely disintegrated mycelia grown in the compost, in the early stage, the numbers of *Rhabditis* sp. rose gradually and then increased suddenly to reach a peak but soon declined.

At first, the pH of *Rhabditis*-infested spawned compost declined but then rose gradually as mycelia was disintegrated by nematodes. The trend in pH of infested unspawned compost was similar to those of uninfested, unspawned compost.

Cultures inoculated with surface-disinfected dead *Rhabditis* sp. and with tap water used in the nematode extraction procedures showed no mycelial injury associated microorganisms containing within or outside the nematodes even though added by artificial wounding of the

mycelia.

Cultures artificially wounded showed no injury away from the wounds without the presence of living *Rhabditis* sp., such wounded mycelia slowly regenerated. On the other hand, artificial wounding accelerated the breakdown of mycelia in the presence of living *Rhabditis* sp.

Introduction

In the past two years, samples of compost and casing from mushroom beds suffering severe crop losses were analysed by several methods. The results showed that mushroom mycelia were completely disappeared in most cases and all samples were heavily infested with various species of nematodes.

Severe infestations of nematodes causing damage to mushroom were described by Cairns and Thomas(1950), Moreton, John, and Goody (1956), Kuk and Rempe (1953) and others (1965), (1952), (1953), (1962), (1966).

Damage to mushroom mycelium by mycephagous nematodes, *Ditylenchus* spp. and *Aphelenchoides* spp., is now comparatively well-known from intensive studies by many workers.

On the other hand, the pathogenicity of saprophagous nematodes is not clear and conflicting results have been obtained.

Kuk and Rempe(1953) reported that *Rhabditis*-like nematodes may not attack mycelium immediately and the sudden breakdown of mycelium in heavily infested beds demonstrates that there must be an accumulation of toxin or ferments excreaed by nematodes in the substratum.

Cairns and Thomas (1950) also demonstrated that the combined action of metabolic products of large populations of *Rhabditis* spp. and bacteria may cause inhibition of normal development of fruiting bodies of mushroom.

Riter (1956) and Black & Conroy (1959) suggested that the excretory products of saprophagous nematodes might be toxic to mushroom.

In contrast to previous findings of other workers mentioned earlier, studies by Moreton, John & Goody (1956), Goody (1960), Hesling (1962, 1966) and Mcleod(1968) have failed to show that saprophagous nematodes ccmpete with or harm mushroom mycelia growing on compost.

Steiner (1933) has concluded that saprophagous nematodes are not the primary pathogens of mushroom, but that they may act as a carrier of pathogenic bacteria.

The present experiments were conducted to determine the frequencies of various species of nematodes attacking mushroom beds in Korea and the pathogenicity of *Aphelenchoides* sp. and *Rhabditis* sp.

Materials and Methods

Two species of nematodes were extracted from the stock cultures in which a pure population had been preserved, and were cleaned with sterile water.

Sterile water was added to appropriate volumese of nematode suspensions to give the desired nematode concentration.

The freshly pasteurized rice straw compost was used as culture medium.

Fragments of compost were placed in a test-tube or glass bottle and exposed to 65°C in a water bath temperature for 4 hours to eradicate all nematodes.

Equal numbers of nematodes suspended in sterile water were added to compost in the container.

Check cultures were inoculated with equal amounts of this water minus the nematodes.

Throughout the experiments, the extent of the mycelial growth and appearance of mycelium damage were observed through the transparent wall of the container. The tubes were ringed with a wax pencil at the top of

the compost and measurements of the growth of mycelium from this line were made.

Nematode populations were determined at regular intervals under a microscope and nematodes were extracted from compost samples by Baermann funnel method for a period of 24 hours.

Experiment 1. Effects of temperature and moisture content in compost on pathogenicity of *Aphelenchoides* sp.

(A) Effect of temperature

About 100 *Aphelenchoides* sp. were added to test-tube (19.5cm×2.5cm) placed with 30 gr. compost, 5gr. grain spawn was then inoculated to the each test-tube of culture medium.

Incubation temperature was varied at 5°C intervals from 15°C to 30°C. A series of nematode-free cultures served as checks at each temperature.

(B) Effect of moisture content of compost

Moisture content of compost was artificially adjusted through the range of 45% to 76%, and dry weight of compost in each glass bottle was approximately 45 gr.

About 200 nematodes were introduced into compost inoculated with 10gr. grain spawn.

All treatments were replicated four times and the compost-spawn cultures were incubated at 25±1°C.

The series of nematode-free cultures at each moisture content served as checks.

Experiment 2. Investigation into the pathogenicity of the saprophagous nematode, *Rhabditis* sp.

Test-tubes (20×3.5cm) containing 20 gr. compost were immediately inoculated with about 200 nematodes, after which an additional 10 gr. grain spawn.

Test-tubes containing compost without nematodes and without both nematodes and spawn served as checks. Cultures were incubated at 25±1°C throughout the experiment, and observation were made on the harmful effects of nematodes on mycelial growth.

Nematode populations and the pH of nematode-infested and uninfested compost were determined with 3 replicates at 2 week intervals.

A Beckmann pH meter (model 96A) was used to determine the pH of a 30 gr. sample of compost. The sample was first agitated for 10 min. with 150 ml of distilled water and then allowed to stand a further 30 min. to attain room temperature.

In addition, a laboratory test was carried out to investigate factors leading to damage to mushroom mycelia. These factors were the activity of microorganisms associated with nematodes in the compost, and the contact action of nematodes moving through the mycelia.

This test comprised the following treatments:

1) inoculation with a suspension of surface-disinfected dead nematodes.
2) with tap water used in the nematode extraction procedures.
3) with a living nematode suspension.
4) with sterile water as check.

Dead nematodes were obtained by submerging nematodes in water for 8 days at room temperature. To disinfect the surface of dead nematodes, the material was first immersed in 1 : 1000 mercuric chloride solution for 5 minutes followed by 2 changes of sterile water. Air dried fragments of compost were evenly wetted with nematode suspension or water without nematodes, then packed into glass tube (1.7×9cm) and inoculated with grain spawn. These cultures were incubated at 25°C.

A portion of compost colonised with growing mycelia was vigorously agitated with a needle to induce wounding of mycelia at 6 days after spawning. These were 4 replicates in each treatment.

The extent of mycelial growth, signs of breakdown of mycelia and regeneration of wounded mycelia were observed throughout this test.

Results and Discussions

Investigation into the frequencies of various species of nematodes attacking mushrooms.

To investigate the frequencies and species of nematodes infesting mushroom farms, nematodes were extracted from samples of compost and casing materials collected from 35 mushroom farms throughout Korea.

The Baermann funnel method was employed to extract nematodes from samples.

The identification was done according to morphological characteristics described by Franklin (1957), Goodey (1958), Steiner(1933) and others (1963), (1967).

The results indicated that there are 4 species of nematodes attacking mushroom beds in Korea. They are *Aphelenchoides* sp.,*Ditylenchus* sp., *Aphelenchus* sp. and *Rhabditis* sp. *Rhabditis* sp. was extracted from all samples taken. The frequency of *Aphelenchoides* sp. was 31.4% in the both compost and casing soil.

In the case of both *Ditylenchus* sp. and *Aphelenchus* sp., the frequencies were 2.9% in the compost and none in casing.

Kuk and Rempe (1953) found *Rhabditis*-like nematodes in most samples of sawdust compost, and Cayrol (1962) reported that *Aphelenchoides composticola, A. limberi, A. winchesi* and *Ditylenchus myceliophagus* have frequently been found in mushroom beds.

Because of their high frequency of occurrence, *Aphelenchoides* sp. and *Rhabditis* sp. are clearly the most important species of nematode in Korea.

Effect of the temperature and moisture content of compost on the pathogenicity of *Aphelenchoides* sp.

Temperature and moisture content of compost affected the pathogenicity of *Aphelenchoides* sp. on mushroom mycelia grown in compost. The higher the temperature the sooner the damage to mycelia became apparent, and in general the more rapid was subsequent destruction of mycelia. (Table 1)

Table 1. Temperature effects on the destruction of mushroom mycelia in compost-spawn cultures inoculated with *Aphelinchoides* sp.

	Temperature(C)				
	10a	15	20	25	30
Days to first appearance of destruction	—	105	34	32	24
Days required for total destruction of mycelia	—	38	12	7	6
Multiples of increase at the time of complete destruction	—	305	362	368	101

(a) No mycelial destruction at 10°C

Also, mycelial growth until first appearance of destruction was severely inhibited at the higher temperatures, 25°C and 30°C. (Fig. 1)

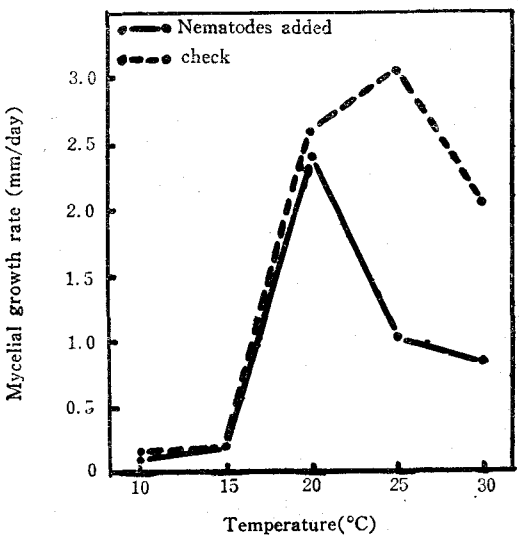

Fig. 1. Mycelial growth rates in compost with and without *Aphelenchoides* sp. until apparent appearance of mycelium destruction at each temperature.

A considerable retardation of pathogenicity was conspicous at 15°C. There was no mycelial destruction at the lowest temperature of 10°C. No mycelium destruction occurred in any of the check cultures. Mycelium grew more rapidly at 25°C than 10, 15, 20 or 30°C in the check cultures. Similarly at 25°C. (Fig. 2)

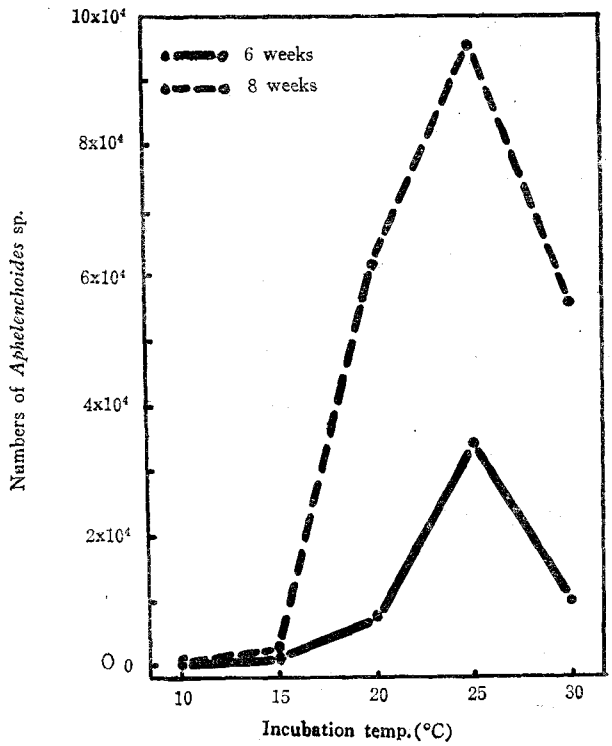

Fig.2. The effect of temperature on the multiplication of *Aphelenchoides* sp. in compost-spawn cultures.

Multiples of increase in the original number of nematodes at the time of complete mycelium destruction were found to vary at the different incubation temperatures.

Although the nematode population increase was greater at 25°C than at 30°C, and the multiples of increase at the time of complete mycelium destruction were relatively lower at 30°C, the occurrence of mycelium destruction was earlier and somewhat faster at 30°C than at 20°C and 25°C.

The trend of mycelium destruction at the various moisture contents of compost were similar to those in the temperature experiment. (Fig.3, 4)

According to Cairns and Thomas (1950) Cairns(1952) and Hesling (1962), mycephagous nematodes pierce the mycelial cell and suck out their contents.

Arrold and Blake (1966) and Cayrol (1965) indicated that 25°C was the optimal temperature for the reproduction of *Aphelenchoides composticola.*

Our experiment with *Aphelenchoides* sp. confirmed previous findings on pathogenicity and fecundity by Cayrol, Cairns and Thomas

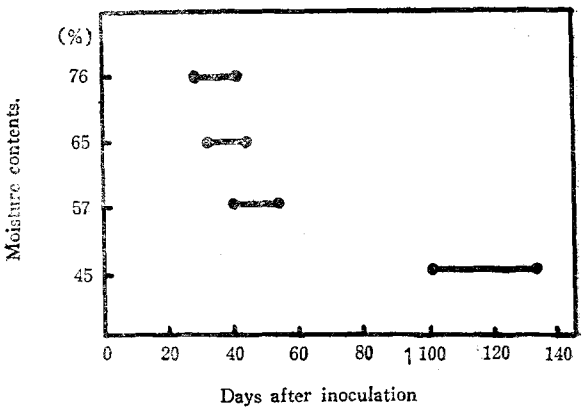

Fig. 3. The effect of moisture content of compost on the time of first appearance of mycelium destruction and the subsequent destruction(•—•)in the compost-spawn cultures inoculated with *Aphelenchoides* sp.

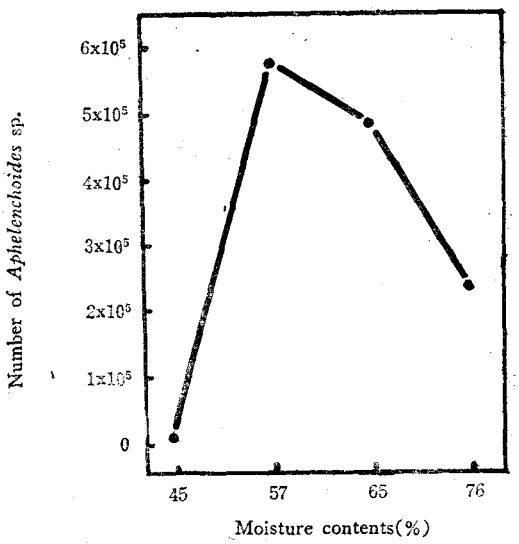

Fig.4. The effect of moisture content of compost on multiplication of *Aphelenchoides* sp. at 9 weeks after inoculation.

(1950) and Cairns (1952), Hesling (1966), Mcleod (1967), Mcleod (1968).

Cayrol (1962) reported that mycephagous nematodes can produce secondary attacks by transporting bacteria by feeding on the mycelium directly.

Studies by Cairns (1953) have shown that temperature influences the activity and the reproductive rate of *Ditylenchus* sp. and also the reproductive rate of bacteria as secondary invaders present in the compost.

From these viewpoints it is assumed that temperature and moisture play an important role not only in multiplication of *Aphelenchoides* sp. but in multiplication of bacteria and increased bacteria, as secondary invaders, accelerate destruction of mycelia at the higher temperature and moisture content of compost.

The results of these studies demonstrate that when *Aphelenchoides* sp. is present, crop loss might be diminished by lowering the temperature and avoiding an excessive moisture content in newly prepared compost.

Investigation into the pathogenicity of the saprophagous nematode, *Rhabiditis* sp.

As the compost became colonized by mycelium, much of the white colored mycelia turned to a yellowish brown, and brown colored liquld resembling the metabolic products of old spawn, collected in the bottom of the test-tube. The first mycelium damage became apparent at about 3 weeks after inoculation as brown patches in mycelial weft covering the compost. As the disintegration progressed, bubbles formed on the surface of mycelia and on the wall of test-tube, and the compost shrank, pulling away from the test-tube wall. Finally the mycelium disintegrated leaving the compost brown in color. (Fig.5)

After complete mycelial destruction, the compost developed a peculiar rotten odor, and felt greasy to the touch. Also, it seemed to be more foul, sunken and soggy than in these case of compost infesting by *Aphelenchoides* sp.

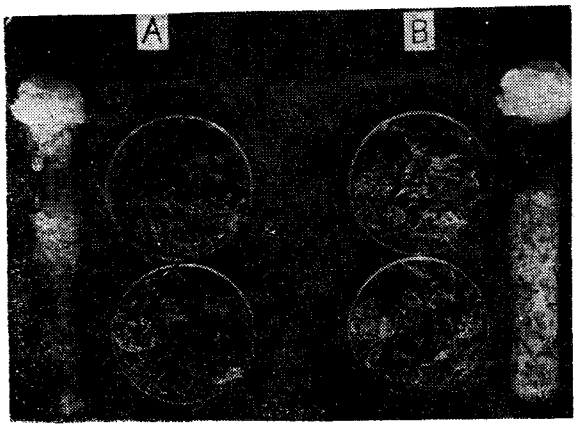

Fig.5. Breakdown of mushroom mycelium by *Rhabditis* sp. at 25°C for 47 days.
A) *Rhabditis* sp. added
B) Check

In the early stage, the numbers of *Rhabditis* sp. rose gradually in the presence of mycelia grown on the compost, increased suddenly to peak populations but soon declined with the disappearance of mycelium. (Fig.6)

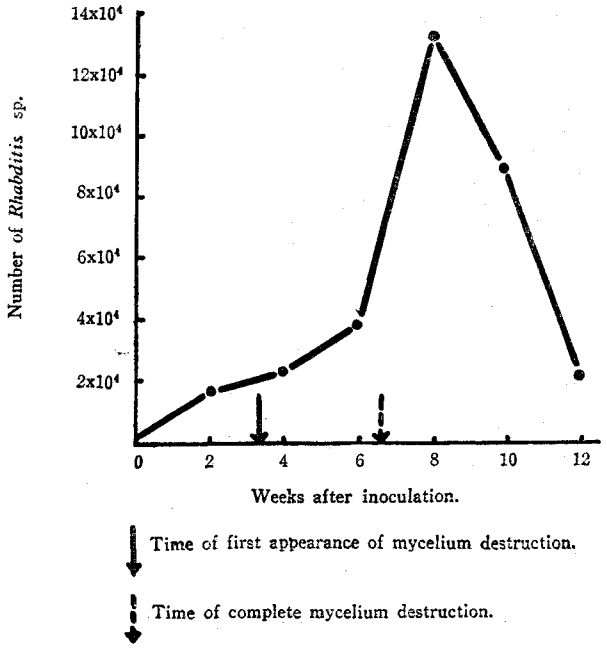

Fig. 6. Development of *Rhabditis* sp. population and damage to mycelia.

Cultures inoculated with surface-disinfected dead nematodes and with tap water used in the nematode extraction procedures failed to show the breakdown of mycelia, nor was there evidence that disease producing agents were contained within or outside the nematodes even though added by artificial wounding of the mycelia.

Such wounded mycelia slowly regenerated, sparsely recolonised the previously agitated compost and regenerated mycelia continued to grow normally on the non-agitated portion of compost.

In contrast, artificial wounding of mycelia accelerated the breakdown of mycelia in the presence of living nematodes.

The pH of uninfested spawned compost fell repidly as the compost was colonized with mycelium. However, at first the pH of infested spawned compost declined but then rose gradually in the course of mycelium disintegration.

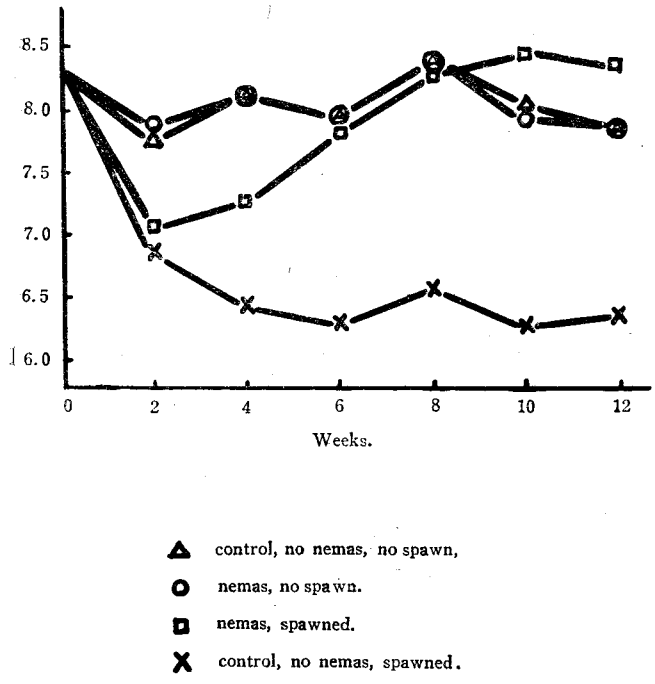

Fig. 7. Trends in the pH of *Rhabditis* sp. infested spawned and unspawned compost.

In contrast, the changes in the pH of infested, unspawned compost were similar to those in uninfested, unspawned compost; any effect on pH by nematode excretions was undetectable. (Fig.7)

The reason for the sudden decline of nematode after peak populations is not clear, but it may be associated with autolysis caused by the accumulation of nematode secretions.

Also, the results of these studies suggest that microorganisms associated with the nematodes and possible wounding of mycelia aroused by migration of nematodes, are not able to cause the breakdown of mycelia without the presence of living nematodes.

Although the evidence from the pH experiment suggests that nematodes had no effect on the pH of compost, this phenomenon probably resulted from the powerful buffer action of the compost itself.

It is a well known fact that the free-living, non-stylet nematodes of *Rhabditis* sp. do not attack mushroom mycelium immediately.

In these respects, the results of these studies demonstrate that the breakdown of mycelium in heavy infestations of *Rhabditis* sp. may be caused by an accumulation of metablic products released in a distinct concentration by large number of nematodes in the compost.

Kuk and Rempe (1953) contended that the breakdown of mycelium by *Rhabditis* sp. demonstrates an accumulation of toxic substance excreted by nematodes.

Also, Black and Conroy (1959) and Cairns (1950), Cairns (1952), Cayrol (1962), Ritter (1956) suggested that saprophagous nematode excretions might be toxic to mushroom.

On the other hand the results suggesting that *Rhabditis* sp. disintegrate the mushroom mycelium are utterly contrary to the findings of Moreton, John & Goodey (1956) Goodey (1960) Hesling (1966) and Mcleod (1968) who showed that saprorhagous nematodes are probybly harmless to mycelium growing on the compost.

The rise in compost pH in nematode-infested spawned compost during mycelium disintegration is probably caused by the breakdown of mycelium, not by excretory products of nematodes.

Observations by Hesling (1966) showed that the pH trend of *Aphelenchoides* infested, spawned compost was at first similer to that in uninfested compost, but after complete mycelial destruction, pH rose rapidly as the infested compost decomposed.

According to Allison and Kneebone (1962) compost pH fell in spawned compost from 7.5 to approximataly 6.0 during the cropping period and compost from which the mycelium had disappeared at the and of the crop showed an increase in pH.

Thus, it is considered that compost pH may play the part of an indicator for nematode damage in the early stage of cropping.

The results of this study demonstrate the importance of severely reducing the number of *Rhabditis* sp. in the newly prepared compost and in the other infestation sources.

적 요

본시험은 우리나라 양송이 재배균상에서 서식하는 선충(線蟲)의 종별(種別) 발생빈도(發生頻度)와 *Aphelenchoides* sp.의 환경적 요인의 영향에 따른 양송이균사 가해정도 및 *Rhabditis* sp.의 양송이균사 가해여부를 구명코자 실시하였다.

시험결과 전국 35개 재배농가에서 수집된 퇴비와 복토에서 *Rhabditis* sp. *Aphelechoides* sp. *Ditylenchus* sp. *Aphelenchus* sp.등 4개의 종(種)에 속하는 선충이 발견되었으며 *Rhabditis* sp.는 모든 농가의 퇴비와 복토에서 발견되었고 *Aphelechoides* sp.는 퇴비와 복토에서 각각 31.4%, *Ditylenchus* sp.와 *Aphelenchus* sp.는 퇴비에서 각각 2.7%의 빈도를 보였고 복토에서는 발견되지 않았다.

Aphelenchoides sp.는 온도 및 퇴비수분함량에 따라 양송이 균사가해정도에 차이가 있었으며 온도 혹은 퇴비수분함량이 높을수록 균사피해가 조기에 나타났으며 균사소멸기간도 단축되었다. 10°C에서는 본시험기간중 균사소멸증상이 없었다.

양송이균사는 *Rhabditis* sp.의 밀도가 증가함에 따라 완전히 소멸되었으며, *Rhabditis* sp.의 밀도는 초기에는 서서히 증가되다가 최고밀도를 보인 후 감소되었다.

Rhabditis sp.와 양송이종균이 접종된 퇴비의 pH는 균사가 퇴비에서 생장하는 초기에는 떨어지나 선충에 의한 균사피해가 진전됨에 따라 점차적으로 높아졌다. 선충을 접종하고 종균이 접종되지 않은 퇴비의 pH와 선충과 종균이 접종되지 않은 퇴비의 pH와는 큰 차이가 없었다.

충체소독된 죽은 *Rhabditis* sp. 혹은 선충분리시 사용되었던 물을 퇴비에 처리하였을 때, 퇴비에서 생장하는 균사에 인공적인 상처를 가했음에도 불구하고 아무런 균사피해증상이 나타나지 알았으며 선충의 내외부에 존재하는 미생물의 양송이 균사에 대한 병원성은 나타나지 않았다. 양송이균사의 인공적인 상처는 산선충이 없는 상태에서는 균사피해요인이 되지 않았으나 선충이 증식되고 있는 상태에서는 균사피해를 촉진시켰다.

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