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Survey of Nematodes in Coniferous Bonsai in Korea

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As preventive measures for bonsai exports, nematodes were isolated from 55 bonsai samples of five coniferous species (*Chamaecyparis pisifera*, *Juniperus chinensis*, *Pinus densiflora*, *Pinus thunbergii*, and *Taxus cuspidate*) from all 15 bonsai gardens in Korea. Nematodes belonging to 21 genera in 7 orders were isolated from the 55 bonsai samples. Among plant-parasitic nematodes, *Tylenchus* spp. was the most frequently isolated (14.9%), followed by *Ditylenchus* spp. (10.5%), *Aphelenchoides* spp. (9.5%), *Aphelenchus* sp. (5.5%), *Criconemoides* sp. (4.0%), *Helicotylenchus* sp. (0.7%), *Hemicycliophora* sp. (0.7%), *Mesocriconema* sp. (0.7%), *Tylenchorhynchus* sp. (0.7%), and *Paratylenchus* sp. (0.4%). Among non-parasitic nematodes, Cephalobina was the most frequently isolated nematodes (26.5%), followed by Rhabditida (19.3%), Dorylaimida (17.8%), Pangrolaimida (14.5%), Plectida (6.5%), Tryphylida (6.2%), Mononchida (3.3%), Alaimida (2.9%), Monhysterida (2.5%), and Triplonchida (0.4%). Based on these results, we conclude that there is no problematic plant-parasitic nematode in bonsai gardens of Korea.

Keywords: Bonsai, Coniferous, Plant-parasitic nematodes

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Introduction

Bonsai refers to trees growing in pots for people who enjoy watching them. It is considered as an elegant hobby for generations. Today, bonsai is an export commodity. It has become a high-value product. Japanese bonsai is renowned worldwide. However, Japanese bonsai is suffering due to aged technicians, high-wages, and avoidance of labor-intensive growing for bonsai by young people. China is supplying a huge amount of low quality bonsai to the world market based on large acreage and low wages. If Korean bonsai gardeners could supply high quality bonsai at affordable prices, they could have a competitive edge in the export market of bonsai.

For bonsai exports, EU and the United States require that bonsai should be grown in certified gardens that meet their specific standards. Recently, EU and other countries have strengthened nematode inspection for bonsai during the quarantine process. To promote bonsai exports, bonsai should be free of plant-parasitic nematodes. There are 1,500 plant-parasitic nematodes. They cause crop losses with estimated economic value of about $1,180 billion worldwide. In Korea, 132 species in 42 genera and 12 families of plant-parasitic nematodes have been reported (Choi, 2001). However, bonsai nematodes have not been reported yet. Therefore, the objective of this study was to survey nematodes in bonsai gardens of Korea.

Materials and Methods

Bonsai collections. The study is focused on coniferous bonsai because they are the main export commodities. We

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collected 55 bonsai samples in five coniferous species from 15 bonsai gardens (Table 1). The five species were: *Juniperus chinensis*, *Pinus densiflora*, *Pinus thunbergii*, *Taxus cuspidata*, and *Chamaecyparis pisifera*. To investigate the source of nematode contamination, bonsai culture medium and water sources were also examined (Fig. 1, Table 1). Samples were collected from July to September when nematode populations were abundant.

Nematode isolation and slide preparation. Root, leaf, stem, and soil samples were prepared. Roots, leaves, and stems were cut into size of 1 cm^2 and placed in tap water for 24 hours. After filtering through 200 and 500 mesh sieves, debris were collected from 500 mesh sieves and further processed using Baermann's funnel method (Southey, 1986). Criconematidae are slow moving nematodes. They could not be isolated by Baermann's funnel. Therefore, centrifugal flotation method was used to isolate Criconematid nematodes (Whitehead and Hemming, 1965).

Table 1. Sampling area, Bonsai species, and number of bonsai samples used in this study

Province	City	Bonsai garden*	Bonsai species	No. of bonsai sampled
Gyeonggi	Goyang	SY	Juniper (*Juniperus chinensis*)	3
Gyeongnam	Busan	SR	Juniper (*J. chinensis*)	3
			Black pine (*Pinus thunbergii*)	2
		SB	Juniper (*J. chinensis*)	3
			Black pine (*P. thunbergii*)	1
	Changwon	HR	Juniper (*J. chinensis*)	2
			Black pine (*P. thunbergii*)	2
		GA	Juniper (*J. chinensis*)	1
			Black pine (*P. thunbergii*)	1
Gyeongbuk	Gyeongsan	CD	Juniper (*J. chinensis*)	3
			Black pine (*P. thunbergii*)	3
	Pohang	GR	Juniper (*J. chinensis*)	1
	Daegu	SW	Black pine (*P. thunbergii*)	2
			Juniper (*J. chinensis*)	2
Jeonnam	Yeosu	BJ	Juniper (*J. chinensis*)	1
			Black pine (*P. thunbergii*)	1
			Yew (*Taxus cuspidata*)	1
	Muan	GS	Black pine (*P. thunbergii*)	4
Chungnam	Seosan	UJ	Sawara cypress (*Chamaecyparis pisifera*)	1
		CE	Black pine (*P. thunbergii*)	2
			Juniper (*J. chinensis*)	2
			Black pine (*P. thunbergii*)	2
	Daejeon	HG	Juniper (*J. chinensis*)	2
			Pine (*Pinus densiflora*)	2
			Juniper (*J. chinensis*)	2
Chungbuk	Chungwon	OS	Black pine (*P. thunbergii*)	3
	Okcheon	SS	Juniper (*J. chinensis*)	2
			Black pine (*P. thunbergii*)	1
Total	12	15	28	55

*Initials of bonsai garden.

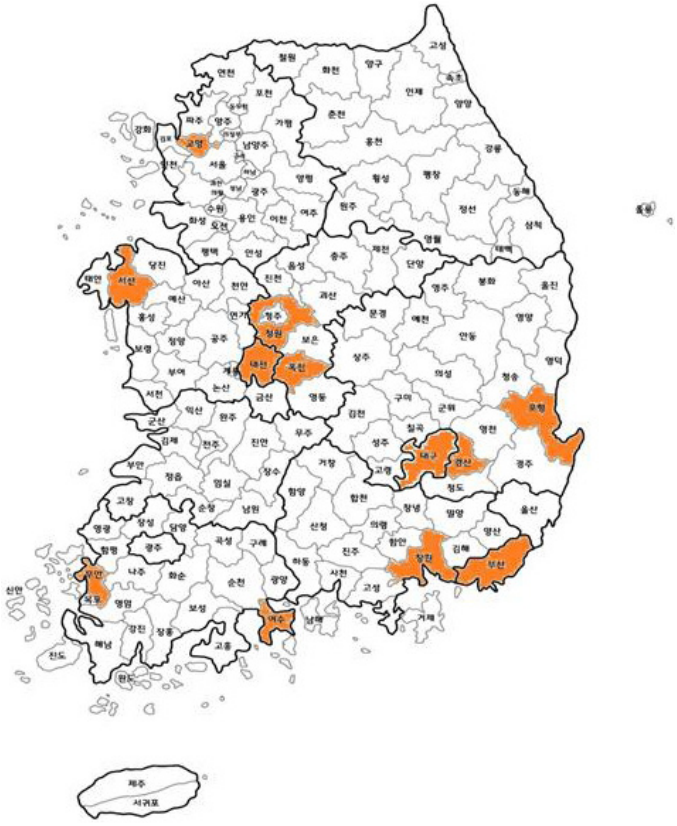

Fig. 1. Location of bonsai gardens for nematode samplings (Goyang, Seosan, Chungwon, Daejeon, Okcheon, Muan, Yeosu, Changwon, Busan, Daegu, Gyeongsan, Pohang).

To detect nematodes from water sources used in bonsai gardens, 20 l water was collected from each bonsai garden and filter with 500 mesh sieves. Nematodes were collected from 500 mesh sieves and examined under a microscope.

To detect nematodes from growing medium used in bonsai gardens, 300 g of growing medium collected from each bonsai garden was placed in water for 24 hours. Nematodes were then isolated by using 200 and 500 mesh sieves.

Isolated nematodes were fixed with hot formaldehyde-glycerol 4:1 solution and then transferred to glycerin according to Seinhorst's rapid method (Seinhorst, 1959). Slides were examined under an Olympus BX53 microscope (Olympus, Tokyo, Japan). Plant-parasitic nematodes are important in quarantine. Therefore, they were identified to the species level. Non-plant-parasitic nematodes were identified to the order level. Recently nematode classification system, especially in higher ranks, has been changed significantly due to the progress of molecular research (Nemaplex, 1999). Nematode taxonomy has been strongly based on morphological characters (De Ley et al., 2005) as the traditional classification system (Winvertebrates, 2015). Such traditional classification system is more appropriate for nematodes found in bonsai compared to molecular approaches. Therefore, we used the traditional classification system for this study.

Results and Discussion

Isolated nematodes. Nematodes were isolated from all 15 bonsai gardens and 55 coniferous bonsai species. They belonged to 21 genera in 7 orders (Table 2).

Among Tylechida, *Tylenchus* was the most frequently identified (14.9%), followed by *Ditylenchus* (10.5%), *Criconemoides* (4.0%), *Helicotylenchus* (0.7%), *Hemicycliophora* (0.7%), *Mesocriconema* (0.7%), *Tylenchorhynchus* (0.7%), and *Paratylenchus* (0.4%). Most *Tylenchus* and *Ditylenchus* are known as fungivorous.

Among Aphelenchida, *Aphelenchoides* (9.5%) and *Aphelenchus* (5.5%) were isolated. Except for few species of *Aphelenchoides*, most *Aphelenchoides* were fungivorous or insect-parasitic nematodes. *Aphelenchus* is a well-known fungivorous nematode. They are not problematic nematodes for exports.

Non-parasitic nematodes included Cephalobina (26.5%), Rhabditida (19.3%), Dorylaimida (17.8%), Pangrolaimida (14.5%), Plectida (6.5%), Tryphylida (6.2%), Mononchida (3.3%), Alaimida (2.9%), Monhysterida (2.5%), and Triplonchida (0.4%) (Table 2). These non-plant-parasitic nematodes are abundant in all natural environments in the world.

Nematode from different parts of bonsai. When roots, stems, leaves, and soil samples (Baermann's funnel method and Centrifugal flotation method) were examined, nematodes were more frequently found from roots (10.3%). They were less frequently found in stems (2.2%) or leaves (2.2%).

Among plant-parasitic nematodes isolated from roots, *Tylenchus* (30.9%) and *Ditylenchus* (29.1%) were the more frequently found ones, followed by *Aphelenchoides parasaprophilus* (16.4%), *Aphelenchus avenae* (14.5%), *Criconemoides annulatus* (1.8%), and *Helicotylenchus pseudorobustus* (1.8%). *A. parasaprophilus*, *A. avenae*, and *Ditylenchus* were also isolated from leaves and stems.

Non-parasitic nematodes isolated from roots were Cephalobina (41.8%), Dorylaimida (30.9%), Rhabditida (20.0%), Pangrolaimida (18.2%), Alaimida (1.8%), Monhysterida (3.6%), and

Mononchida (3.6%) (Table 2).

For soil isolation, Baermann's funnel method (11.7%) isolated more nematodes than the centrifugal flotation method (9.2%). The following four new nematode genera were isolated from soil only: *Hemicycliophora* (3.6%), *Mesocriconema* (3.6%), *Paratylenchus* (1.8%), and *Tylenchorhynchus crassicadatus* (1.8%). In soil isolation, *Tylenchus* and *Ditylenchus* were the most frequently isolated nematodes (25.5% and 10.9%, respectively). *Mesocriconema* and *Paratylenchus* were only isolated by the centrifugal flotation method. *C. annulatus* was recovered four times more in numbers compared to that by the Baermann's funnel method (Table 2). Non-parasitic nematodes isolated from soil samples were Cephalobina (40.0%–43.6%), Dorylaimida (14.5%–38.2%), Rhabditida (27.3%–38.2%), Tryphylida (5.5%–23.6%), Plectida (9.1%–18.1%), Mononchida (12.7%), and Pangrolaimida (10.9%–14.5%) (Table 2).

Nematodes from different bonsai garden. There was no significant difference in nematode numbers among the six provinces (data not shown). Among plant-parasitic nematodes, *Criconemoides* (5.0%) and *Helicotylenchus* (5.0%) were isolated from Chungbuk province while *Criconemoides* (9.1%), *Hemicy-*

Table 2. Nematodes isolated from coniferous bonsai in 2015*

Nematodes	Frequency (%)†					Average
	Plant			Soil		
	Root	Leaf	Stem	Sieve	Centrifugal	
Plant-parasitic nematodes						
Aphelenchida						
Aphelenchus avenae	14.5	1.8	1.8	3.6	5.5	5.5
Aphelenchoides parasaprophilus	16.4	12.7	5.5	7.3	5.5	9.5
Tylenchida						
Criconemoides annulatus	1.8	-	-	3.6	14.5	4.0
Ditylenchus spp.	29.1	3.6	3.6	10.9	5.5	10.5
Helicotylenchus pseudorobustus	1.8	-	-	1.8	-	0.7
Hemicycliophora sp.	-	-	-	3.6	-	0.7
Mesocriconema rusticum	-	-	-	-	3.6	0.7
Paratylenchus sp.	-	-	-	-	1.8	0.4
Tylenchorhynchus crassicaudatus	-	-	-	1.8	1.8	0.7
Tylenchus spp.	30.9	-	-	25.5	18.2	14.9
Non-parasitic nematodes						
Alaimida	1.8	-	-	5.5	7.3	2.9
Cephalobina	41.8	1.8	5.5	40.0	43.6	26.5
Dorylaimida	30.9	-	5.5	38.2	14.5	17.8
Monhysterida	3.6	-	-	3.6	5.5	2.5
Mononchida	3.6	-	-	12.7	-	3.3
Pangrolaimida	18.2	10.9	18.2	14.5	10.9	14.5
Plectida	-	3.6	1.8	18.2	9.1	6.5
Rhabditida	20.0	9.1	1.8	27.3	38.2	19.3
Triplonchida	-	-	-	1.8	-	0.4
Tryphylida	-	-	1.8	23.6	5.5	6.2
Average	10.3	2.2	2.2	11.7	9.2	7.1

*Total number of samples: 275.
†Frequency (%)=number of samples with nematodes/total number of samples examined×100.

cliophora (3.6%), *Mesocriconema* (1.8%), *Paratylenchus* (1.8%), *Tylenchorhynchus* (3.6%), and *Tylenchus* (34.5%) were isolated from Gyeongbuk province. Important plant-parasitic nematode was isolated from only one bonsai garden. Its soil was used as growing medium.

Nematodes from different bonsai species. The five bonsai species had different nematode species (data not shown). Since the number of samples ranged from 1 to 27, it was inappropriate to distinguish nematodes by coniferous species.

Nematodes in water source and growing medium used for bonsai gardens. When the growing media collected from bonsai gardens were examined, nematodes were detected from four bonsai gardens (SB garden, CD garden, HR garden, and BJ garden) (Table 3). Various species of non-parasitic nematodes were found from growing media. However, there was no plant-parasitic nematode (Table 3). During the survey, we found that growing medium was left opened in the yard. This could cause nematode contamination. Therefore, growing media should be kept in separate storage facilities after being opened.

When water sources used for bonsai garden were examined, only *Aphelenchoides* (5 nematodes per 2 liters of water) was detected from SR bonsai garden which used nearby riverlet as water source. Other bonsai gardens that used tap water or underground water as water source did not have nematodes.

During the survey of nematodes in bonsai gardens of Korea, nematodes were mostly isolated from roots and soil samples. Most nematodes were non-parasitic nematodes. Only one bonsai garden was infested with plant-parasitic nematodes. It used soil as growing medium. Therefore, bonsai gardens for exports must use certified growing medium. They should store growing media in separate storage facilities after the bag is opened. When bonsai gardens used underground water or tap water as water supply and bonsai roots are washed to be free of soil before exports, there should be no nematode problem for bonsai to be exported.

Table 3. Nematode detected from bonsai culture media

Province	City	Bonsai garden†	No. of nematode*						
			Aphelenchoides	*Tylenchus*	Rhabditida	*Panagrolaimida*	Dorylaimida	Mononchda	Tripylida
Gyeonggi	Goyang	SY	-	-	-	-	-	-	-
Gyeongnam	Busan	SR	-	-	-	-	-	-	-
		SB	-	-	-	10	-	-	-
	Changwon	HR	-	-	20	8	-	-	-
		GA	-	-	-	-	-	-	-
Gyeongbuk	Gyeongsan	CD	-	-	32	-	-	5	-
	Pohang	GR	-	-	-	-	-	-	-
	Daegu	SW	-	-	-	-	-	-	-
Jeonnam	Yeosu	BJ	2	10	80	-	4	-	1
	Muan	GS	-	-	-	-	-	-	-
Chungnam	Seosan	UJ	-	-	-	-	-	-	-
		CE	-	-	-	-	-	-	-
	Daejeon	HG	-	-	-	-	-	-	-
Chungbuk	Chungwon	OS	-	-	-	-	-	-	-
	Okcheon	SS	-	-	-	-	-	-	-
Total 6	12	15	2	10	132	18	4	5	1

*Number of nematode per bonsai culture medium 300 cm^3.
†Initials of bonsai garden.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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