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

Survey of Nematodes in Coniferous Bonsai in Korea

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Keywords: Bonsai, Coniferous, Plant-parasitic nematodes
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 cm² 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                     |
|              |              | SB             | Black pine (*Pinus thunbergii*)       | 2                     |
| Changwon     | HR           |                | Juniper (*J. chinensis*)              | 2                     |
|              |              | GA             | 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                     |
| 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        |              |                |                                       | 55                    |

*Initials of bonsai garden.
To detect nematodes from water sources used in bonsai gardens, 20 l water was collected from each bonsai garden and filtered 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 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%), *Mesocriocnema* (0.7%), *Tylencchorhynchus* (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. Nematodes isolated from coniferous bonsai in 2015*

| Nematodes           | Frequency (%) | Plant | Soil | Average |
|---------------------|---------------|-------|------|---------|
|                     | 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          |
| Hemicicliophora sp. |  -   |  -   |  -   | 3.6   |  -          | 0.7          |
| Mesocriconema rusticum |  -   |  -   |  -   |  -    | 3.6         | 0.7          |
| Paratylenchus sp.   |  -   |  -   |  -   |  -    | 1.8         | 0.4          |
| Tylenchorhynchus crassicadatus |  -   |  -   |  -   | 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.

...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-
*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 | *Aphelen­cho­ides* | *Tylenchus* | Rhabditida | Panagrolai­midia | Dorylaimida | Mononchda | Tripylida |
|--------------|----------|---------------|---------------------|-------------|------------|-------------------|-------------|----------|----------|
| Gyeonggi     | Goyang   | SY            | -                   | -           | -          | -                 | -           | -        | -        |
| Gyeongnam    | Busan    | SR            | -                   | -           | -          | -                 | -           | -        | -        |
|              |          | SB            | -                   | -           | -          | 10                | -           | -        | -        |
| Changwon     | HR       | -             | -                   | -           | 20         | 8                 | -           | -        | -        |
|              | GA       | -             | -                   | -           | -          | -                 | -           | -        | -        |
| Gyeongbuk    | Gyeongsan| CD            | -                   | -           | 32         | -                 | -           | -        | -        |
|              | Pohang   | GR            | -                   | -           | -          | -                 | -           | -        | -        |
|              | Daegu    | SW            | -                   | -           | -          | -                 | -           | -        | -        |
| Jeonnam      | Yeosu    | BJ            | 2                   | 10          | 80         | -                 | 4           | -        | 1        |
|              | Muan     | GS            | -                   | -           | -          | -                 | -           | -        | -        |
| Chungnam     | Seosan   | UJ            | -                   | -           | -          | -                 | -           | -        | -        |
|              | Seosan   | 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 cm³.
†Initials of bonsai garden.*
Conflicts of Interest

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

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