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Nematocidal Screening of Essential Oils and Herbal Extracts against *Bursaphelenchus xylophilus*

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Five essential oils and 15 herbal extracts were evaluated to control *Bursaphelenchus xylophillus* in laboratory. The essential oils from clove plant (*Syzygium aromaticum*), mustard (*Brassica integrefolia*), thyme (*Thymus vulgaris*), and *Pelargonium inquinans* were found to be highly promising and gave excellent control of the nematodes at all the time of exposure. Among them, the least one gave 91.3% mean mortality rate at 24 hours of exposure time, which is highly significant from the control. While in the second study, most of the methanol (*Desmodium caudatum*, *Paulownia coreana*, *Auckulandia lappa*, *Sophota flavescens*, *Aloe* sp., *Rheum palmatum*, *Zingiber officinale*, *Magnolia officinalis*, and *Eugenia caryophyllata*), hexane (*Torreya nucifera*, *Pharbitis nil*, *Prunus mume*, *Melia azedarach*, and *Xanthium strumarium*), and hot water (*Cinnamomum cassia*) herbal extracts killed the nematodes, but in varying degrees compared to the control. Only one extract was found to be promising *viz Magnolia officinalis* which found to be statistically different from the control and gave mean mortality of 72, 82.3, and 85.3% for 24, 48, and 72 hours exposure, respectively. Further screening was conducted for *M. officinalis* with concentrations of 1,000, 100, and 10 ppm against the same species of nematode with the same time of exposure. However, it gave an excellent result for 1,000 ppm for all time of exposure, whereas for the 100 and 10 ppm it gave mean mortality of 39.5 and 25.8% for the time 72 hrs, respectively that were statistically different from the control.

Keywords **:** biocontrol, *Bursaphelenchus xylophilus*, essential oil, herbal plant, pine wilt disease

The pine wood nematode, *Bursaphelenchus xylophilus* (Steiner and Buhrer, 1934) Nickle 1970, is associated with the pine wilt disease. Ultimately the nematode leads to the death of conifers, especially pines. The disease in Korea was firstly reported in Busan, 1988 (Choi and Moon, 1989; Yi et al., 1989). Pine wilt disease consequently became more distributed to the southern and middle areas of Korea (Chung, 2002; Moon et al., 1995).

Although chemical nematicides hold major promise in nematode control, the high costs, non-availability at the time of need, and the hazards they pose as environmental pollutants discourage most potential users. These matters have stimulated research on alternative nematode management practices for plant parasitic nematodes (Araya and Caswell-Chen, 1994; Ferris and Masuda, 1992; McKerny, 1987).

Several plants have accelerated the search for not only more environmentally and toxicologically safe but also more selective and efficacious pesticides. Most commercially successful pesticides have been discovered by screening compounds synthesized in the laboratory for pesticidal properties.

Many plant species are known to be highly resistant to nematodes. The well documented plants of these include marigolds (*Tagetes* spp.), rattleboxes (*Crotalaria* spp.), chrysanthemums (*Chrysanthemum* spp.), caster bean (*Ricinus communis*), neem (*Azardiracta indica*), and velvet bean (*Mucuna pruriens*) (Duke, 1990; Jourand et al., 2004; Kong et al., 2006, 2007; Park et al., 2005; Wang et al., 2002; Winder and Datalto, 1991; Zasada et al., 2006). However, plant oils are relatively available compared to plant herbal extracts (Duke, 1990).

The objectives of these studies are to describe laboratory studies aiming to screen 5 oils and 15 herbal extracts against *Bursaphelenchus xylophilus.*

Materials and Methods

Essential oil and herbal extract. In the first experiment

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Table 1. List of used plant, source, and yield

Plant	Used part	Source	Yield (%)[a]
Eugenia caryophyllata	Flower	G. market in Seoul[b]	22.3
Inula helenium	Root	G. market in Seoul	20.2
Torreya nucifera	Fruit	G. market in Seoul	32.2
Pharbitis nil	Fruit	G. market in Seoul	11.4
Aloe sp.	Leaf	G. market in Seoul	28.1
Sophora flavescens	Root	G. market in Seoul	5.3
Desmodium caudatum	Leaf/stem	Seogwipo, Jeju	17.2
Rheum palmatum	Rhizome	G. market in Seoul	16.3
Zingiber officinale	Rhizome	G. market in Seoul	7.0
Prunus mune	Fruit	G. market in Seoul	15.1
Melia azedarach	Fruit	G. market in Seoul	11.8
Xanthium strumarium	Fruit	G. market in Seoul	9.8
Paulownia coreana	Leaf	Gapyeong, Gyeonggi	8.2
Magnolia officinalis	Bark	G. market in Seoul	2.5

[a](Dried weight of methanol or hexane extract/dried weight of the sample plant)×100.
[b]G=Gyeongdong.

5 commercial plant oils (*Pimpinella anisum*, *Syzygium aromaticum*, *Brassica integrefolia*, *Thymus vulgaris*, and *Pelargonium inquinans*) were bought from a company (Samyoung Food and Chemical Co. LDT) in Korea. Concerning the source of herbal plants, used portions and their yields after extraction were listed in (Table 1). The herbal plants were dried in an oven at 60°C for 3 days and finely powdered using a home blender. Each 100 g sample was extracted twice with 500 ml methanol (leaves, rhizomes, stems or bark, and flower buds) or hexane (fruits) at room temperature and filtered (Toyo filter paper # 2, Toyo Roshi, Kaisha, Japan). The combined filtrate was concentrated in a vacuum at 35°C using a rotary vacuum evaporator (Eyela, N-12, Japan).

A hundred gram of *Cinnamomum cassia* bark was cut into small slices using lopping shears with 100 ml water in 500 ml flask. The flask was autoclaved (120°C, 1.5 kgf cm^{-2}) for 1 h and left for another 2 h. And squeezed with gauze, and finally the solid matter was removed.

Pine wood nematode. Branches of *Pinus densiflora* infested with *B. xylophilus* at Jinju, Gyeongnam Province were collected and brought to the laboratory. They were cut into small pieces and then nematodes were separated by means of Baermann funnel technique (Schlinder, 1961). Mass production of *B. xylophilus* was obtained by growing transferring-sterilized nematodes and put them on PDA (Potato Dextrose Agar) containing the fungus *Botrytis cinerea* that prepared in advance (Wingfield et al., 1983). The Petri dishes containing nematodes and fungus were kept in the incubator at 25±1°C in the dark. After 7 days the nematodes were multiplied and seen congregating on the lid of the Petri dish. They were extracted from the agar by blending the agar for 5 seconds in a home blender and the suspension was put in the Baermann funnel technique for overnight (Schlinder, 1961). The next day the nematodes were collected from funnel in a 500 ml beaker. The standard nematode suspension was prepared by appropriate dilution with distilled water.

Screening of nematicidal activity. The collected nematode suspension was automatically stirred, and 4 ml were taken separately while stirring and counted the number of nematodes as each ml. Each oil extract of 500 ppm was prepared in each Petri dish and then the nematodes were added. Test samples were suspended in distilled water with Triton X-100 added at the rate of 0.1 ml/L. All the oil extracts containing the nematodes were left for 24, 48, and 72 hrs of exposure time. Control was set using only distilled water with Triton X-100 added at the rate of 0.1 ml/L. Each treatment was replicated 4 times. All Petri dishes sealed by parafilm were kept under the room temperature, 25±1°C. Dead nematodes were counted using normal binocular microscope, a nematode considered dead when completely motionless and when in doubt it was poked with a needle (Cayrol et al., 1989).

In the second experiment 15 herbal extracts as well were screened against the same nematode species and the same procedures as mentioned above were followed. However, the further screening was conducted for *Magnolia officinalis* using 1,000, 500, 100, and 10 ppm as well using the same time of exposure of 24, 48, and 72 h.

Data analysis. Data were analyzed by using SAS program (2004); then ANOVA and means separation were set by Tukey's Studentized Range test.

Table 2. Mean mortality of *Bursaphelenchus xylophilus* in different oil extracts of concentration 500 ppm after 24, 48, and 72 hrs of exposure

Treatment	Mean % mortality ± SD		
	24 hr after exposure	48 hr after exposure	72 hr after exposure
Pimpinella anisum	9.3 ± 2.7 z[a]	9.3 ± 2.7 y	10 ± 2.2 y
Syzygium aromaticum	100 ± 0 x	100 ± 0 x	100 ± 0 x
Brassica integrefolia	91.3 ± 1.1 x	96.5 ± 0.3 x	99 ± 0.7 x
Thymus vulgaris	99.3 ± 0.5 x	99.5 ± 0.3 x	100 ± 0 x
Pelargonium inquinans	64.5 ± 8.5 y	94.3 ± 3.5 x	100 ± 0 x
Control	3.8 ± 0.7 z	9.8 ± 1.3 y	14.3 ± 1.8 y

[a]Means with the same letter are not significantly different according to Tukey's Studentized Range Test at $P<0.0001$.

Table 3. Mean mortality of *Bursaphelenchus xylophilus* in different herbal extracts concentration of 500 ppm after 24, 48, and 72 hrs of exposure

Treatment	Mean % mortality ± SD		
	24 hr after treatment	48 hr after treatment	72 hr after treatment
Eugenia caryophyllata	27.8 ± 5.9 y[a]	28.1 ± 6.2 xy	43.0 ± 0.8 y
Inula helenium	21.3 ± 4.5 xy	21.8 ± 4.3 vwx	23.0 ± 5.0 r-w
Torreya nucifera	15.3 ± 2.8 v-y	16.0 ± 3.2 t-w	18.0 ± 2.9 r-u
Pharbitis nil	15.5 ± 5.2 v-y	15.8 ± 5.6 tuv	18.8 ± 3.3 r-v
Aloe sp.	5.3 ± 1.9 v	27.8 ± 2.2 wxy	31.0 ± 6.4 t-y
Sophora flavescens	4.5 ± 1.7 v	14.3 ± 4.6 tuv	16.0 ± 3.7 rst
Cinnamomum cassia	18.5 ± 3.3 wxy	19.8 ± 3.0 u-x	34.3 ± 12.1 v-y
Desmodium caudatum	6.0 ± 8.3 vw	8.0 ± 1.6 tu	9.0 ± 1.8 r
Rheum palmatum	5.3 ± 2.0 v	30.5 ± 4.2 xy	32.0 ± 5.2 u-y
Zingiber officinale	7.3 ± 1.5 vw	34.5 ± 10.6 y	40.8 ± 11.4 xy
Prunus mune	6.5 ± 1.9 vw	16.5 ± 3.5 t-w	27.3 ± 7.4 s-x
Melia azedarach	10.5 ± 3.1 vwx	15.3 ± 1.6 tuv	34.0 ± 5.5 v-y
Xanthium strumarium	10.8 ± 2.9 vwx	21.8 ± 6.2 vwx	36.8 ± 6.1 wxy
Paulownia coreana	10.0 ± 3.6 vwx	13.8 ± 1.0 tuv	22.3 ± 4.6 r-w
Magnolia officinalis	71.8 ± 1.4 z	82.3 ± 5.1 z	85.3 ± 3.7 z
Control	7.0 ± 16.1 vw	7.3 ± 0.5 t	14.0 ± 5.1 rs

[a]Means with the same letter are not significantly different according to Tukey's Studentized Range Test at $P<0.0001$.

Results and Discussion

In the first experiment of using oil extracts we found that all the extracts controlled the nematodes in varying degrees (Table 2).

The oil extracts from clove, mustard, and thyme exerted rapid nematicidal activity with mean mortality of over 99% compared to the control at three days after exposure (df=5, 18, F=1436.12, $P<0.0001$). Whereas the extract from *Pelargonium inquinans* gave mean mortality of 64.5% at 24 hours of exposure (df=5, 18, F=147.08, $P<0.0001$), however, gave mean mortality of 94.5 and 100% at 48 (df= 5, 18, F=631.58, $P<0.0001$) and 72 hours respectively. Kong et al. (2006) found similar results when used clove bud oil and thyme (LC_{50}=0.57-0.88) against *B. xylophilus*.

Out of 15 plant extracts in the second experiment, one plant extract was found to be promising. That is, *M. officinalis* exerted potent nematicidal activity and gave mean mortality of 71.8, 82.3, and 85.3% at 24 (df=15, 48, F=41.83, $P<0.0001$), 48 (df=15, 48, F=56.79, $P<0.0001$), and 72 hrs (df=15, 48, F=34.04, $P<0.0001$), respectively (Table 3).

However, the rest of plant extracts had poor potentiality to be a good candidate for biocontrol of *B. xylophilus* although they killed the nematodes. Further screening was conducted to test *M. officinalis* with lower and higher doses of the extract using 1,000, 500, 100, and 10 ppm (Fig. 1).

The higher doses gave excellent control. However, 100 and 10 ppm gave only 39.5 and 25.8% mean mortality at 3 days after treatment although still highly significant from the control (df=4, 15, F=159.72, $P<0.0001$). LC_{50} of *B. xylophilus* was 312.2, 232.2, and 103.3 ppm at 1, 2, and 3

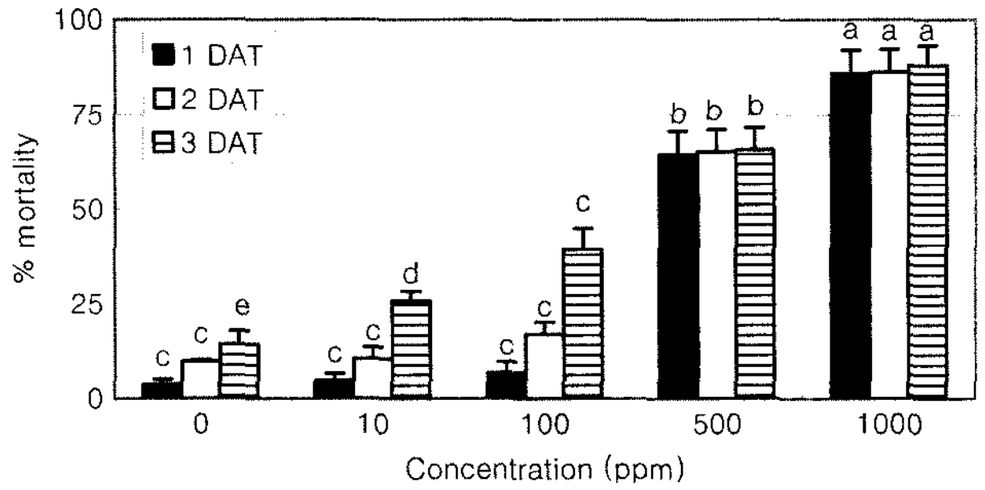

Fig. 1. Mean mortality of *Bursaphelenchus xylophilus* by extract of *Magnolia officinalis*. Vertical bars represent standard deviation of the means. The same lower case letters above the bars in each treatment day indicate no significant difference among the means (Tukey's Studentized Range Test at $P<0.0001$).

days after treatment, respectively.

Many phytochemicals are known to contain nematicidal activity. Compounds involved in plants associated with nematodes include repellants, attractants, hatching stimulants or inhibitors, and nematotoxicants, either constitutive or formed in response to nematode presence (Chitwood, 2002). Plant oils seem to be potential nematicides as biocontrol of *B. xylophilus* because they are safe and friendly to the environment (Kong et al., 2006, 2007). In addition, they may provide potential alternatives to control pine wilt disease because they contain rich sources of bioactive chemicals (Isman, 2000).

In general, many plants have yielded a broad spectrum of active compounds including polythienyls, isothoiocyanates, glucosinolates, cynogenic glycosides, polyacetalenes, alkaloids, lipids, terpenoids, sesquiterpenoids, diterpenoids, steroids, triterpenoids, simple and complex phenolics and several other classes (Chitwood, 2002). The compound responsible for the cloves' aroma is eugenol. It is the main component in the essential oil extracted from cloves comprising 72-90%. Eugenol has pronounced antiseptic and anaesthetic properties. Other important constituents include essential oils; acetyl eugenol, beta-caryophylline, and vanillin; crategolic acid; tannins, gallotannic acid, and methyl salicylate (painkiller); the flavanoids eugenin, kaempferol, rhamnetin, and eugenitin; triterpenoids like oleanolic acid, stigmasterol, and campesterol; and several sesquiterpenes (Wikipedia, 2007).

Kong et al. (2006) studied 88 plant essential oils against *B. xylophilus* by an immersion bioassay. They found cinnamon bark oil (0.12 mg/ml) was the most effective nematicide followed by coriander herb oil (0.14 mg/ml). Potent nematicidal activity was also observed by the former researchers with lemongrass, oregano, thyme red, and clove bud oils. On the other hand, Kong et al. (2007) as well studied the nematicidal activity of cassia and cinnamon oil compounds and related compounds towards *B. xylophilus* using direct contact bioassay. They found that two cassia oils (0.084-0.085 mg/ml) and 4 cinnamon oils (0.064-0.113 mg/ml) were toxic to adult *B. xylophilus*. In their further test of the compounds they found that trans-cinnamaldehyde (0.061 mg/ml) was the most effective nematicide followed by ethyl cinnamate, α-methy-trans-cinnamaldehyde, methyl cinnamate, and allyl cinnamate (0.114-0.195 mg/ml).

While Park et al. (2005) also studied plant essential oils from 43 plant species against *B. xylophilus* and good nematicidal activity against males, females, and juveniles of this pest was achieved with essential oils of *Cinnamomum verum*, *Leptospermum petersonii*, *Asiasarum siebboldi*, *Boswellia carterii*, *Pimenta racemosa*, *Cymbopogon citratus*, *Mentha spicata*, *Syzygium aromaticum*, and *Allium sativum*. However, Choi et al. (2006) tested extracts from 40 medicinal plant species for their nematicidal activity against *B. xylophilus*; they found responses varied with plant materials and concentrations. Extracts of *Acorus gramineus*, *Asiasarum sieboldi*, *Illicium verum*, and *Kaemferia galanga* showed the nematicidal activity against males, females, and juveniles of this pest at 2000 μgml^{-1}.

Because use of agricultural phytochemicals offers a tremendous potential for nematode control although currently uneconomic in many situations (Chitwood, 2002), more researches need to include as many plant taxa as possible to explore many phytochemicals that having potentiality of being alternatives to chemical nematicides. Therefore, further research of these nematicidal activities of different plant flora need to be conducted to achieve the maximum benefit and thus they can be mass-produced for large-scale applications against pine wood nematodes.

This is a useful research area where the observations of many authors' research of organisms can be utilized for the benefit of phytonematode control. Moreover, the health hazard aspects may be somewhat limited since most of these compounds have therapeutic use. By using these compounds from herbal or oils extracts will lead to decrease the use of chemical pesticides. Thus healthier environment will be available in nature.

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