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Nematicidal Activity of Natural Ester Compounds and Their Analogues against Pine Wood Nematode, *Bursaphelenchus xylophilus*

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ABSTRACT: In this study, we evaluated the nematicidal activity of natural ester compounds against the pine wood nematode, *Bursaphelenchus xylophilus*, to identify candidates for the development of novel, safe nematicides. We also tested the nematicidal activity of synthesized analogues of these ester compounds to determine the structure−activity relationship. Among 28 ester compounds tested, isobutyl 2-methylbutanoate, 3-methylbutyl 2-methylbutanoate, 3-methylbutyl tiglate, 3-methyl-2-butenyl 2-methylbutanoate, and pentyl 2-methylbutanoate showed strong nematicidal activity against the pine wood nematode at a 1 mg/mL concentration. The other ester compounds showed weak nematicidal activity. The LC_{50} values of 3-methylbutyl tiglate, isobutyl 2-methylbutanoate, 3-methylbutyl 2-methylbutanoate, 3-methyl-2-butenyl 2-methylbutanoate, and pentyl 2-methylbutanoate were 0.0218, 0.0284, 0.0326, 0.0402, and 0.0480 mg/mL, respectively. The ester compounds described herein merit further study as potential nematicides for pine wood nematode control.

KEYWORDS: *pine wood nematode, Bursaphelenchus xylophilus, nematicidal activity, ester compounds*

INTRODUCTION

The pine wood nematode, *Bursaphelenchus xylophilus*, is a plant-parasitic nematode that causes pine wilt disease in several Asian and European countries.[1] Pine wilt disease was first reported in Busan city in 1988.[2] Since then, it has spread to several areas of the Korean peninsula. Until 2005, *Pinus densiflora* and *Pinus thunbergii* were reported as natural hosts of the pine wood nematode. However, the Korean white pine, *Pinus koraiensis*, was also found to be affected by the pine wood nematode in 2006.[1] Because of the devastating losses of numerous pine tree species, this disease is considered to be a serious threat to Korea's pine forests. Many physical, chemical, and biological control methods have been used to manage the disease, such as the felling and fumigation of dead pine trees[3] with metham sodium, the aerial spraying of thiacloprid (to control the insect vector, *Monochamus alternatus*),[4] trunk injection with synthetic nematicides (e.g., abamectin or emarmectin benzoate),[4] and preventive silvicultural control in infected areas.[5] Among the control methods, the use of synthetic pesticides and nematicides such as metham sodium, thiacloprid, abamection, and emarmectin benzoate is often the primary method of control. However, this use is known to cause several side effects.[6−8] Thus, in order to reduce the side effects caused by synthetic insecticides and nematicides, the development of new and safe type of pesticides with low toxicity has been suggested as a promising alternative, particularly with regard to the use of essential oils or phytochemicals that are naturally synthesized by plants. Many plant solvent extracts, plant essential oils, and their constituents have demonstrated strong nematicidal activity against the pine wood nematode, *B. xylophilus*, in previous studies.[9−17]

In this study, we evaluated the nematicidal activity of natural ester compounds such as isobutyl isobutanoate, isobutyl 2-methylbutanoate, isobutyl isovalerate, 3-methylbutyl isobutanoate, 2-methylbutyl isobutanoate, 3-methylbutyl 2-methylbutanoate, 2-methylbutyl 2-methylbutanoate, 2-methylbutyl isovalerate, isobutyl angelate, 2-methylbutyl angelate, and 3-methylbutyl angelate found in some Asteraceae plant essential oils to identify candidates for the development of new and safe nematicides. We also tested the nematicidal activity of synthesized analogues of these ester compounds to determine the structure−activity relationship.

MATERIALS AND METHODS

Collection of the Pine Wood Nematode. *B. xylophilus* was isolated from the chips of infected pine wood collected in the Haman region, Gyeongsangnam-do Province, Korea and extracted by the Baermann funnel method.[18] The colony was maintained on a lawn of *Botrytis cinerea* cultured on potato dextrose agar medium (PDA) in the dark at 28 °C.

Chemicals. 4-Dimethylaminopyridine (DMAP) and dicyclohexylcarbodiimide (DCC) were purchased from Sigma-Aldrich (MO, USA). Wakogel 200 was purchased from Wako Pure Chemicals (Osaka, Japan). Dichloromethane was dried over $CaCl_2$ and distilled prior to use. The solutions were dried over anhydrous $MgSO_4$ and concentrated by a rotary evaporator. The synthesized compounds were purified by silica gel chromatography (Wakogel 200). Isobutyl angelate

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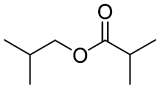
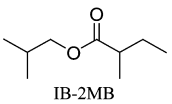
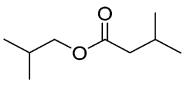
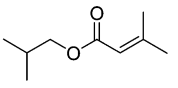
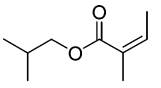
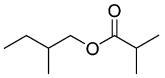
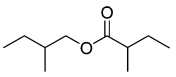
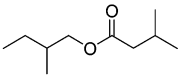
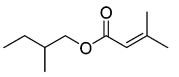
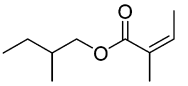
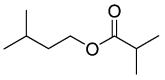
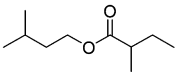
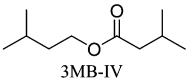
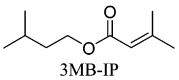
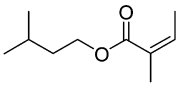
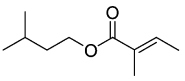
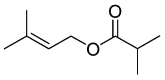
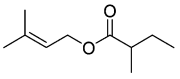
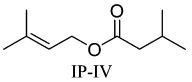
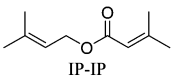
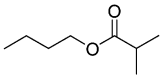
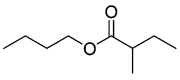
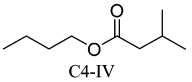
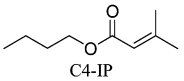
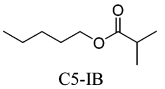
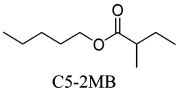
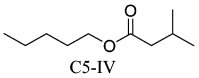
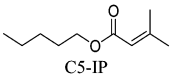
Table 1. List of Ester Compounds Tested

Alcohol moieties	Acid moieties: Isobutyric acid[a] (-IB)	2-Methylbutyric acid[b] (-2MB)	Isovaleric acid[c] (-IV)	3-Methyl-2-butenoic acid[d] (-IP)	Angelic acid[e]	Tiglic acid[f]
Isobutyl (IB-)	 IB-IB Y; 88.5%, P; 98.8%	 IB-2MB Y; 77.2%, P; 99.0%	 IB-IV Y; 79.7%, P; 98.8%	 IB-IP Y; 92.6%, P; 96.2%	 IB-A[g] TCI, P: 98%	-
2-Methylbutyl (2MB-)	 2MB-IB Y; 80.4%, P; 97.5%	 2MB-2MB Y; 91.9%, P; 99.0%	 2MB-IV Y; 99.7%, P; 97.5%	 2MB-IP Y; quant., P; 95.1%	 2MB-A Y; 54.0%, P; >99%	-
3-Methylbutyl (3MB-)	 3MB-IB Y; 87.8%, P; 98.5%	 3MB-2MB Y; 87.2%, P; 99.0%	 3MB-IV Y; 85.5%, P; 99.0%	 3MB-IP Y; quant., P; 96.3%	 3MB-A[g] TCI, P: 99%	 3MB-T Y; 78.0%, P; 99.0%;
3-Methyl-2-butenyl (IP-)	 IP-IB Y; 99.0%, P; 99.0%	 IP-2MB Y; 88.5%, P; 99.0%	 IP-IV Y; quant., P; 98.6%	 IP-IP Y; 97.9%, P; 95.9%	-	-
Butyl (C4-)	 C4-IB Y; 85.8%, P;98.2%	 C4-2MB Y; 69.6%, P; 99.0%	 C4-IV Y; quant., P; 99.9%	 C4-IP Y; 96.8%, P; 96.2%	-	-
Pentyl (C5-)	 C5-IB Y; 99.0%, P; 99.0%	 C5-2MB Y; 95.9%, P; 99.0%	 C5-IV Y; 95.6%, P; 99.9%	 C5-IP Y; 99.7%, P; 96.3%	-	-

[a,c,d]These compounds were synthesized following synthetic method A with isobutyl chloride, isovaleryl chloride, and 3,3-dimethylacryloyl chloride, respectively. [b,e,f]These compounds were synthesized following synthetic method B with the corresponding acids (see first footnote). [g]These compounds were purchased from Tokyo Chemical Industry. Y: yield (by isolation), P: purity (determined by GC).

(purity: 98%) and 3-methylbutyl angelate (i.e., = isoamyl angelate; purity, 99%) were purchased from Tokyo Chemical Industry (Tokyo, Japan).

Instrumental Analysis. Gas chromatography (GC) analysis was performed using an Agilent 6890N equipped a DB-1MS column (30 m × 0.25 mm i.d., 0.25 μm film thickness; J&W Scientific, Folsom, CA). GC-mass spectrometry (GC-MS) analysis was performed on an Agilent 7980A coupled with a 5975C mass selective detector (MSD). A DB-5MS (30 m × 0.25 mm i.d., 0.25 μm film thickness; J&W Scientific, Folsom, CA) was used for the separation of the analytes. The oven temperature was programmed as 40 °C for 1 min, then raised to 250 °C at 6 °C/min, and the temperature held for 4 min. Purities of synthesized compounds were checked by GC. NMR spectra were taken on a Varian UI500 spectrometer (^{1}H; 500 MHz, ^{13}C; 125 MHz) using TMS in $CDCl_3$ as an internal standard at the Korea Basic Science Institute (Seoul, Korea). Infrared (IR) spectra were recorded on a Nicolet FT-IR spectrometer (Thermo Fisher Scientific Inc.). The structure was confirmed by comparison of its mass spectrum with data from the NIST mass spectrum library, IR data, and the NMR spectrum.

Synthesis of Esters. The esters listed in Table 1 were synthesized using two different procedures (i.e., methods A and B). In method A, the desired esters were synthesized with their corresponding alcohols and acyl chlorides. Briefly, a solution of alcohol (1.0 equiv) and acyl chloride (1.5 equiv) in CH_2Cl_2, pyridine (1.5 equiv), and a catalytic amount of DMAP were added at 0 °C. After 30 min of stirring at 0 °C, the solution was stirred for 2 h at room temperature. The solution was diluted with ether, and the organic phase was washed with 2 N HCl, water, and brine and then dried. After the solvent was removed, the residue was distilled and subjected to silica gel chromatography to give the desired ester (5% ether in hexane fraction). In method B, the esters were synthesized with their corresponding alcohols and acids, DMAP and DCC, following the method by Neises and Steglich.[19] Alcohol (2.0 equiv), acid (1.0 equiv), and DMAP (1.6 equiv) were dissolved in CH_2Cl_2 at 0 °C. DCC (2.2 equiv) was added to the solution over a 4 min period. The solution was stirred for 30 min at 0 °C and then for 3 h at room temperature. The precipitate was removed by filtration; the filtrate was diluted with ether and then worked up. The purification procedure was the same as that in method A.

Isobutyl isobutanoate (IB-IB): GC-MS, m/z (%); 129 (0.1, M^+-CH_3), 101 (7.2), 89 (27.4), 71 (100.0), 56 (39.8).

2-Methylbutyl isobutanoate (2MB-IB): GC-MS, m/z (%); 129 (0.5, $M^+-CH_2CH_3$), 101 (5.8), 89 (8.9), 71 (100.0), 70 (63.7), 55 (17.8).

3-Methylbutyl isobutanoate (3MB-IB): GC-MS, m/z (%); 143 (0.2, M^+-CH_3), 115 (6.0), 101 (2.4), 89 (21.1), 71 (80.0), 70 (100.0), 55 (35.2).

3-Methyl-2-butenyl isobutanoate (IP-IB): GC-MS, m/z (%); 156 (2.1, M^+), 113 (1.1), 85 (3.5), 71 (46.1), 69 (100.0), 68 (78.4), 67 (74.6), 53 (38.1).

Butyl isobutanoate (C4-IB): GC-MS, m/z (%); 115 (0.6, $M^+-CH_2CH_3$), 101 (6.5), 89 (85.9), 71 (100), 57 (38.7), 56 (44.6).

Pentyl isobutanoate (C5-IB): GC-MS, m/z (%); 129 (0.4, $M^+-CH_2CH_3$), 115 (2.4), 101 (3.4), 89 (77.7), 71 (80.6), 70 (48.1), 55 (20.6), 43 (100.0).

Isobutyl 2-methylbutanoate (IB-2MB): GC-MS, m/z (%); 143 (0.2, M^+-CH_3), 130 (4.5), 115 (3.2), 103 (36.7), 85 (78.9), 74 (12.0), 57 (100.0), 56 (42.0).

2-Methylbutyl 2-methylbutanoate (2MB-2MB): GC-MS, m/z (%); 143 (0.5 $M^+-CH_2CH_3$), 103 (14.9), 85 (100.0), 71 (24.7), 70 (86.5), 57 (92.2), 55 (25.4).

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3-Methylbutyl 2-methylbutanoate (3MB-2MB): GC-MS, *m/z* (%). 157 (0.3, M^+-CH_3), 144 (1.1), 129 (2.9), 115 (2.2), 103 (20.8), 85 (42.9), 70 (100.0), 57 (62.9), 55 (31.1).

3-Methyl-2-butenyl 2-methylbutanoate (IP-2MB): GC-MS, *m/z* (%): 170 (2.1, M^+), 103 (1.4), 85 (25.0), 74 (18.0), 69 (100.0), 68 (76.7), 67 (75.1), 57 (71.7), 53 (42.1).

Butyl 2-methylbutanoate (C4-2MB): GC-MS, *m/z* (%); 143 (0.2, M^+-CH_3), 130 (5.5), 115 (1.9), 103 (78.7), 85 (61.9), 74 (20.8), 57 (100.0), 56 (41.0).

Pentyl 2-methylbutanoate (C5-2MB): GC-MS, *m/z* (%); 172 (0.1, M^+), 144 (3.5), 115 (5.1), 103 (100.0), 85 (60.6), 74 (20.1), 71 (10.4), 70 (45.0), 57 (77.6), 56 (10.0), 55 (22.5).

Isobutyl isovalerate (IB-IV): GC-MS, *m/z* (%) 158 (0.08, M^+), 143 (0.1), 128 (1.5), 115 (3.6), 103 (22.0), 85 (100.0), 73 (2.1), 60 (13.7), 57 (74.3).

2-Methylbutyl isovalerate (2MB-IV): GC-MS, *m/z* (%) 172 (0.4, M^+), 142 (0.8), 115 (4.1), 103 (8.3), 85 (100.0), 71 (23.6), 70 (66.2), 57 (44.0), 55 (16.7).

3-Methylbutyl isovalerate (3MB-IV): GC-MS, *m/z* (%) 157 (0.2, M^+-CH_3), 142 (0.1), 115 (3.2), 103 (14.9), 85 (56.8), 71 (29.7), 70 (100.0), 57 (34.1), 55 (28.2).

3-Methyl-2-butenyl isovalerate (IP-IV): GC-MS, *m/z* (%) 170 (1.6, M^+), 128 (1.0), 110 (0.5), 103 (1.8), 85 (69.6), 69 (82.5), 68 (100.0), 67 (92.1), 60 (38.0), 57 (65.1), 53 (45.6).

Butyl isovalerate (C4-IV): GC-MS, *m/z* (%) 158 (0.1, M^+), 143 (0.2), 116 (5.0), 103 (69.1), 87 (20.9), 85 (100.0), 73 (1.5), 60 (24.1), 57 (74.3), 56 (68.0)

Pentyl isovalerate (C5-IV): GC-MS, *m/z* (%) 170 (0.1, M^+), 157 (0.2), 130 (3.4), 115 (6.6), 103 (89.1), 85 (100.0), 71 (16.9), 70 (85.7), 61(14.7), 60 (18.3), 57 (55.9), 55 (26.3).

Isobutyl 3-methyl-2-butenoate (IB-IP): GC-MS, *m/z* (%) 156 (1.7, M^+), 156 (1.7), 141 (0.2), 126 (0.1), 113 (0.5), 101 (16.8), 100 (36.8), 99 (0.3), 83 (100.0), 73 (0.4), 67 (0.8), 57 (10.6), 55 (19.7).

2-Methylbutyl 3-methyl-2-butenoate (2MB-IP): GC-MS, *m/z* (%) 170 (0.7, M^+), 155 (0.1), 113 (0.5), 101 (16.4), 100 (34.7), 83 (100.0), 70 (17.2), 67 (1.0), 55 (25.6).

3-Methylbutyl 3-methyl-2-butenoate (3MB-IP): GC-MS, *m/z* (%) 170 (3.0, M^+), 155 (0.3), 113 (0.4), 101 (23.9), 100 (42.2), 83 (100.0), 82 (10.3), 71 (19.6), 70 (23.2), 67 (2.2), 55 (40.7).

3-Methyl-2-butenyl 3-methyl-2-butenoate (IP-IP): GC-MS, *m/z* (%) 168 (0.7, M^+), 153 (0.7), 109 (6.9), 100 (42.2), 95 (0.8), 83 (43.3), 77 (1.4), 69 (24.6), 68 (69.6), 67 (100.0), 55 (10.4), 53 (60.9).

Butyl 3-methyl-2-butenoate (C4-IP): GC-MS, *m/z* (%) 156 (7.5, M^+), 141 (0.9), 113 (0.6), 101 (19.3), 100 (57.2), 85 (11.1), 83 (100.0), 82 (19.6), 55 (25.6).

Pentyl 3-methyl-2-butenoate (C5-IP): GC-MS, *m/z* (%) 170 (5.4, M^+), 155 (0.4), 101 (32.1), 100 (68.9), 83 (100.0), 82 (17.3), 71 (3.2), 70 (5.6), 55 (33.8).

2-Methylbutyl angelate (2MB-A): GC-MS, *m/z* (%) 170 (1.6, M^+), 100 (100.0), 83 (90.0), 71 (23.4), 70 (21.9), 55 (85.4). IR (neat, cm^{-1}), 3005 (w), 2980 (s), 1710 (s), 1670 (m), 1220 (s), 1160 (s), 850 (s).

3-Methylbutyl tiglate (3MB-T): ^{1}H NMR (ppm), δ 0.936 (6H, d, J = 6.5), 1.567 (2H, dt, J = 7, 6.5), 1.722 (1H, nonet, J = 6.5), 1.785 (3H, dq, J = 7, 1.5), 1.829 (3H, quin., J = 1.5), 4.159 (2H, t, J = 7), 6.840 (1H, qq, J = 7, 1.5); ^{13}C NMR (ppm), δ 12.03 (CH_3), 14.13 (CH_3), 22.52 (CH_3 × 2), 25.22 (CH), 37.47 (CH_2), 63.07 (CH_2), 128.83 (=C), 136.79 (=CH), 168.22 (O=C). IR (neat, cm^{-1}), 2980 (s), 2105 (m), 1710 (s), 1670 (m), 1280 (s), 1110 (s), 730 (s).

Nematicidal Activity. To test the nematicidal activity of ester compounds, test compounds were suspended in ethanol (100 mg/mL). Test solutions (1 μL) were placed in the wells of a 96-well plate (Falcon, USA) containing 50−150 nematodes (mixture of juvenile and adult nematodes, male−female−juvenile ≈1:1:2) in 99 μL of water. Thus, the total volume of the solution in each well was 100 μL, and the concentration of the test ester compounds was 1 mg/mL. As a control, four wells were treated with ethanol (1 μL) in the same volume as the test samples. In four adjacent wells (i.e., in a column) on the plate, nematodes were treated with the ester compounds, and a set of treatment samples was placed in the wells of every second column. The entire completely experiment was repeated four times. The order of the ester compounds was randomly determined. The tested plates were stored under a 14:10 h (light−dark) regime at 25 ± 1 °C and 60% relative humidity. Nematode mortality was recorded after 48 h of treatment as follows: 10 μL of the test suspension was transferred to 100 μL of fresh water with a micropipette. Ten minutes after transfer, nematode mortality was observed under a microscope. Nematodes were classified as dead if their bodies were motionless and straightened. The mortality of termites was transformed to the arcsine square root values for analysis of variance (ANOVA). Mean values for treatment data were compared and separated by Scheffé's test.[20] Five chemicals (i.e., isobutyl 2-methylbutanoate, 3-methylbutyl 2-methylbutanoate, 3-methyl-2-butenyl 2-methylbutanoate, 3-methylbutyl tiglate, and pentyl 2-methylbutanoate) that showed strong nematicidal activity at a concentration of 1 mg/mL against the pine wood nematode were chosen for testing at a lower concentration. These chemicals were serially diluted with ethanol to obtain four different concentrations; the final concentrations were 0.250, 0.125, 0.0625, and 0.03125 mg/mL for pentyl 2-methylbutanoate; 0.125, 0.0625, 0.03125, and 0.015625 mg/mL for isobutyl 2-methylbutanoate, 3-methylbutyl 2-methylbutanoate, and 3-methyl-2-butenyl 2-methylbutanoate; and 0.0625, 0.03125, 0.015625, and 0.0078125 mg/mL for 3-methylbutyl tiglate. The LC_{50} was estimated by probit analysis.[20]

RESULTS AND DISCUSSION

The nematicidal activity of the ester compounds is shown in Table 2. Among the ester compounds tested, isobutyl 2-methylbutanoate, 3-methylbutyl 2-methylbutanoate, and 3-methylbutyl tiglate showed 100% nematicidal activity against the pine wood nematode at a concentration of 1 mg/mL. The nematicidal activities of 3-methyl-2-butenyl 2-methylbutanoate and pentyl 2-methylbutanoate were 98.73% and 97.63% against the pine wood nematode at a concentration of 1 mg/mL, respectively. The nematicidal activities of the other ester compounds were <11%. The nematicidal activities of isobutyl 2-methylbutanoate, 3-methylbutyl 2-methylbutanoate, 3-methylbutyl tiglate, 3-methyl-2-butenyl 2-methylbutanoate, and pentyl 2-methylbutanoate, which showed strong nematicidal activities at a 1 mg/mL concentration, were also tested at a lower concentration (Table 3). The LC_{50} values of 3-methylbutyl tiglate, isobutyl 2-methylbutanoate, 3-methylbutyl 2-methylbutanoate, 3-methyl-2-butenyl 2-methylbutanoate, and pentyl 2-methylbutanoate were 0.0218, 0.0284, 0.0326, 0.0402, and 0.0480 mg/mL, respectively. Nematicidal activities of many phytochemicals from essential oils have been reported.[9−17] Choi et al.[9] reported that LC_{50} values of thymol, carvacrol, citral, citronellol, and citronellal were 0.119, 0.125, 0.187, 0.245, and 0.321 mg/mL, respectively. Some natural ester compounds tested in this study have been reported as the main compounds of certain plant essential oils.[21,22] Isobutyl angelate was the most abundant compound identified in *Chamaemelum nobile* plant essential oil, followed by 2-methylbutyl angelate, isobutyl isobutyrate, methyl 2-methylbuyrate, prenyl acetate, 2-methylbutyl 2-methylbutyrate, and 2-methylbutyl acetate.[21] Mierendorff et al.[22] reported that the main compounds of *Eriocephalus punctulatus* were 2-methylbutyl isobutanoate (21.2%), 2-methylbutyl 2-methylbutanoate (5.6%), isobutyl isobutanoate (5.3%), and linalyl acetate (4.4%). Bail et al.[21] reported the antimicrobial activities of some ester compounds identified in *Chamaemelum nobile* plant essential oils. Seo et al.[23] reported the fumigant toxicities of some ester compounds derived from *E. punctulatus* and *C. mobile* plant essential oils against Japanese termites (*Reticulitermes speratus*); however, there have been no reports on the nematicidal activities of the natural ester

Table 2. Nematicidal Activity of Ester Compounds against Pine Wood Nematode, *B. xylophilus*

ester compounds	mortality (%, mean ± S.E., $N = 4$)[a]
isobutyl isobutanoate (IB-IB)	0b[b]
isobutyl 2-methylbutanoate (IB-2MB)	100a
isobutyl isovalerate (IB-IV)	0b
isobutyl 3-methyl-2-butenoate (IB-IP)	0.68 ± 1.35b
isobutyl angelate (IB-A)	9.5 ± 15.08b
2-methylbutyl isobutanoate (2MB-IB)	0.63 ± 1.25b
2-methylbutyl 2-methylbutanoate (2MB-2MB)	0b
2-methylbutyl isovalerate (2MB-IV)	1.05 ± 1.26b
2-methylbutyl 3-methyl-2-butenoate (2MB-IP)	0.65 ± 1.30b
2-methylbutyl angelate (2MB-A)	9.0 ± 5.15b
3-methylbutyl isobutanoate (3MB-IB)	0b
3-methylbutyl 2-methylbutanoate (3MB-2MB)	100a
3-methylbutyl isovalerate (3MB-IV)	0b
3-methylbutyl 3-methyl-2-butenoate (3MB-IP)	0.58 ± 1.15b
3-methylbutyl angelate (3MB-A)	10.6 ± 9.06b
3-methylbutyl tiglate (3MB-T)	100a
3-methyl-2-butenyl isobutanoate (IP-IB)	4.3 ± 2.84b
3-methyl-2-butenyl 2-methylbutanoate (IP-2MB)	98.73 ± 0.85a
3-methyl-2-butenyl isovalerate (IP-IV)	5.63 ± 1.8b
3-methyl-2-butenyl 3-methyl-2-butenoate (IP-IP)	8.08 ± 2.65b
butyl isobutanoate (C4-IB)	1.5 ± 3.0b
butyl 2-methylbutanoate (C4-2MB)	0b
butyl isovalerate (C4-IV)	1.2 ± 1.47b
butyl 3-methyl-2-butenoate (C4-IP)	1.48 ± 1.81b
pentyl isobutanoate (C5-IB)	0.5 ± 1.0b
pentyl 2-methylbutanoate (C5-2MB)	97.63 ± 2.06a
pentyl isovalerate (C5-IV)	0b
pentyl 3-methyl-2-butenoate (C5-IP)	0.55 ± 1.1b
control	0b
	$F_{28,87} = 485.72$, $p < 0.0001$

[a]1 mg/mL concentration. [b]Means within a column followed by same letters are not significantly different (Scheffe's test).

Table 3. LC_{50} Values of Five Active Ester Compounds against Pine Wood Nematode, *B. xylophilus*

compounds	LC_{50} (mg/mL)	slope	95% cl[a]	χ^2
isobutyl 2-methylbutanoate	0.0284	4.25 ± 0.37	0.0258−0.0311	1.11
3-methylbutyl 2-methylbutanoate	0.0326	3.86 ± 0.32	0.0295−0.0359	7.83
3-methylbutyl tiglate	0.0218	4.13 ± 0.33	0.0199−0.0239	2.71
3-methyl-2-butenyl 2-methylbutanoate	0.0402	4.55 ± 0.37	0.0368−0.0439	0.12
pentyl 2-methylbutanoate	0.0480	2.81 ± 0.27	0.0413−0.0545	5.66

[a]Confidence limit.

compounds utilized in the current study against the pine wood nematode.

Among the ester compounds tested in the current study, there was a significant difference in nematicidal activity against the pine wood nematode. The nematicidal activities of the ester compounds with 2-methylbutyric acid moiety, such as isobutyl 2-methylbutanoate, 3-methylbutyl 2-methylbutanoate, 3-methyl-2-butenyl 2-methylbutanoate, and pentyl 2-methylbutanoate, were stronger than those of the other ester compounds, except 3-methylbutyl tiglate. This result indicates that the 2-methylbutyric acid moiety may play an important role in nematicidal activity against the pine wood nematode. Specific functional groups in the chemical structure are closely related to the nematicidal activity against the pine wood nematode.[9,16] Choi et al.[9] investigated the nematicidal activities of monoterpenoids typically found in plant essential oils and found that chemicals belonging to the phenols, alcohols, and aldehydes were generally more toxic than the other monoterpenoid groups such as the ketones and hydrocarbons. Park et al.[16] compared the nematicidal activities of eugenol, methyl eugenol, isoeugenol, methyl isoeugenol, and acetyl eugenol and insisted that a functional group at the C1 position of the benzene ring was very important for nematicidal activity.

There was a significant difference in nematicidal activities among the ester compounds with a 2-methylbutyric acid moiety. Nematicidal activities of isobutyl 2-methylbutanoate, 3-methylbutyl 2-methylbutanoate, 3-methyl-2-butenyl 2-methylbutanoate, and pentyl 2-methylbutanoate were strong; however, 2-methylbutyl 2-methylbutanoate and butyl 2-methylbutanoate did not show nematicidal activity against the pine wood nematode. Grodnitzky and Coats[24] investigated the QSAR of monoterpenoids against the house fly, *Musca domestica*. They insisted that the optimum shape and size of monoterpenoids was necessary to fit into a site of toxic action. Seo et al.[25] studied the structure−activity relationship of aliphatic compounds for nematicidal activity against the pine wood nematode. There was a significant difference in nematicidal activity according to chain length. Among the aliphatic compounds belonging to the alkanols and 2*E*-alkenols, compounds with a C_9−C_{11} chain length showed strong nematicidal activity when compared to the other compounds with different chain lengths. In the case of the 2*E*-alkenals and alkanoic acids groups, compounds with C_8−C_9 and C_9−C_{10} chain lengths, respectively, showed strong nematicidal activity; however, compounds with C_{12}−C_{14} chain lengths showed weak nematicidal activity against the pine wood nematode. The current study is in accordance with a previous study, indicating that an optimum shape and size of chemicals are necessary for the nematicidal activity against the pine wood nematode.

α,β-Unsaturated carbonyl compounds such as citral, 2-decenal, *trans*-cinnamate, and 2*E*-alkenal were reported to be responsible for nematicidal activities against the pine wood nematode.[9,13,14,25] Ester compounds with 3-methyl-2-butenoic acid, angelic acid, and tiglic acid moiety provide *α,β*-unsaturated carbonyl structure, but only 3-methylbutyl tiglate showed strong nematicidal activity. The relationship between nematicidal activity and *α,β*-unsaturated carbonyl structure was not clarified in the current study.

The structure−activity relationships of the *cis*- and *trans*-isomers of various compounds have been well-studied. Park et al.[26] reported that the insecticidal activity of *cis*-asarone was stronger than that of *trans*-asarone against *Sitophilus oryzae*, *Callosobruchus chinensis*, and *Lasioderma serricorene*. Lee et al.[27] also reported that the insecticidal activity of *cis*- and *trans*-asarone were different against *Nilaparvata lugens* (Homoptera: Delphacidae) and *Plutella xylostella* (Lepidoptera: Yponomeutoidae). Park et al.[16] reported that the nematicidal activity of geranial was ∼4.3 times stronger than that of neral, a

geometrical isomer of geranial. In the current study, there was a difference in the nematicidal activity of two geometrical isomers—3-methylbutyl angelate and 3-methylbutyl tiglate. The nematicidal activity of 3-methylbutyl tiglate was much stronger than that of 3-methylbutyl angelate. The current study and previous studies indicate that the position of the substituent in the geometrical isomer is very important for nematicidal or insecticidal activities.

Clarifying the mode of action for active compounds is of practical importance for nematode control because it provides valuable information for developing the most appropriate methods for delivery. Lei et al.[28] reported that the nematicidal activity of thymol and carvacrol against two nematodes, *Caenorhabditis elegans* and *Ascaris suum*, might be mediated through a tyramine receptor (TyrR). Recently, Kang et al.[29] investigated the inhibition activities of phytochemicals from plant essential oils against acetylcholinesterases of the pine wood nematode. They reported that (+)-α-pinene, (−)-α-pinene, 3-carene, *o*-anisaldehyde, coniferyl alcohol, and *cis*-nerolidol showed inhibition activities against acetylcholinesterases of the pine wood nematode. Kang et al.[30] also reported the inhibition of acetylcholinesterase and glutathione *S*-transferase of the pine wood nematode by aliphatic compounds. They found that the nematicidal activity of the 2*E*-alkenal compounds is related to the inhibition of BxACE (*B. xylophilus* acetylcholinesterase); however, ester compounds tested in the current study did not show inhibition activity against BxACE (data not shown here). Moreover, the exact mode-of-action for ester compounds used in current study remains unclear.

In conclusion, natural ester compounds such as isobutyl 2-methylbutanoate, 3-methylbutyl 2-methylbutanoate, 3-methylbutyl tiglate, 3-methyl-2-butenyl 2-methylbutanoate, 3-methyl-2-butenyl 2-methylbutanoate, and pentyl 2-methylbutanoate showed very strong nematicidal activity against the pine wood nematode. We also investigated the structure−activity relationship of natural ester compounds and their analogues. For the practical use of these ester compounds as novel nematicides, further studies investigating systemic action, phytotoxicity, mode-of-action, and formulation for improving nematicidal potency and stability are necessary.

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Notes

The authors declare no competing financial interest.

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