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Insecticidal Activity of *Thuja occidentalis* Essential Oil and Monoterpenoids with Surfactants against the *Myzus persicae*

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Introduction

Essential oils possess acute contact and fumigant toxicity to insects, repellent activity, antifeedant activity, as well development and growth inhibitory activity.

Thuja occidentalis, commonly known as arbor vitae or white cedar, is indigenous to eastern North America and is growing in Europe as an ornamental tree(Fig. 1). In folk medicine, *T. occidentalis* has been used to treat bronchial catarrh, enuresis, cystitis, psoriasis, uterine carcinomas, amenorrhea and rheumatism and chronic infections of the upper respiratory tract, and antibiotics.

Fig. 1. Thuja occidentalis

Essential oils are made up of numerous compounds. Although several studies have proven that essential oils have insecticidal effects, studies on specific ingredients are very scarce. In our previous study, it was confirmed that terpinyl acetate and bornyl acetate have antimicrobial activities in three coniferous species, including *T. occidentalis*, through chemical profiling (Song et al., 2021). Therefore, in this study, the insecticidal activity of *Myzus persicae* of the essential oil of *T. occidentalis* and the insecticidal activity of terpinyl acetate and bornyl acetate, which are major mono terpenes of coniferous species, were tested against *M. persicae*.

Materials and Methods

Plant material

The healthy and mature leaves from 30-40 old *T. occidentalis* were collected in May, 2018 from Gyeongsang National University in Korea. The collected leaves were dried naturally on laboratory benches at room temperature (24±1° C) for 3 days prior to the extraction of essential oil.

Extraction of essential oil

Apparatus used for the extraction of essential oil from *T. occidentalis* leaves showed (Seo *et al*., 2003). *T. occidentalis* collected leaves were cut (1-2 cm) 50 g and crushed 50 g into pieces. The essential oil of *T. occidentalis* was extracted by steam distillation. The extracted oil was stored in a refrigerator at 4° C. The resulting oil solution was used for compositional analysis and aphid experiments.

Each essential oil of conifers was analyzed by a gas chromatograph (HP5890 SERIES Ⅱ) – mass spectrometer (GC-MS, HP 5971 SERIES MSD) equipped with an HP-1 Fused Silica Capillary Column for metabolite profiling. Compounds were identified by comparison with retention time and mass spectra obtained by comparison with retention time and mass spectra obtained with a standard standard on the GC-MS system used for the analysis. When an authentic sample was not available, identification was performed by comparing the mass spectrum of the mass spectrum library (The Wiley Registry of Mass Spectral Data, 6th ed). The content of terpenoid compounds was determined through the relative area (%) of the analyzed peak decided.

The peach aphid (*Myzus persicae* Sulzer) was distributed from Korea Biochemical Co(Jinju, Korea) and raised cabbage as a host(Fig. 2). Aphids were reared in acrylic insect breeding cages (L40 × W40 × H50 cm) containing host seedlings, and reared under conditions of 16 hours in the dark: 8 hours in the dark, 25±1° C, and 60±10% relative humidity.

Fig. 2. The peach aphid (*Myzus persicae* Sulzer)

Insecticidal activity against *M. periscae*

The bioassays were carried out using newborn nymphs (1–7 days old) of the aphid. The aphid were reared on plants leaf under standard conditions in a controlled environmental growth chamber under 12 : 12 h of light : dark photoperiod at 25 °C and 70-80% RH. A single monoterpene component including *T. occidentalis* essential oil, terpinyl acetate, and bornyl acetate was tested for insecticidal activity by *M. persicae*. The insecticidal assays of these compounds were verified by fumigation and spray treatment.

Fumigant bioassay was conducted by fumigating the essential oil of T. occidentalis and its components (bonyl acetate, terpinyl acetate, careen, cymene, decene, limonene) on filter paper (Advantec No. 2, cut into 70 mm diameter and 2 cm width) to obtain an insecticidal effect. Tested. insecticidal activity. Discs 4 cm long were impregnated with equal concentrations of 0.125, 0.25, 0.5, 1, 2 and 5 μL/L. The impregnated filter paper was attached to a plastic 500 mL glass bottle cap. The plastic cap was then screwed tightly onto the vial. Each bottle contains 20 adults (1-7 days old). Each concentration and control was repeated 3 times. Mortality rates were determined 3, 6, 9, 12 and 60 hours after initiation of exposure.

For the spray bioassay, 20 aphid-attached leaves were placed on filter paper (Advantec No. 2, diameter 90mm) at the bottom of a plastic container (diameter 15cm x height 10cm), and various concentrations of essential oil and monoterpene were sprayed(Fig. 3). The spray distance was sprayed at intervals of 15 minutes over 50 cm from the bottom of the plastic container, and the insecticidal effect was observed by performing this for 60 minutes

Fig. 3. Fumigant bioassay

Statistical analysis

All above experiments were conducted in five replications. One-way analysis of variance (ANOVA) followed by Duncan's multiple-range test (DMRT) was applied to evaluate differences among the treatments.

Result & Discussion

Extraction of essential oil

Extraction of essential oil from *T. occidentalis* leaves was affected according to pretreatment method. Yield of essential oil in crushing leaves 0.35% was higher than that of cutting 0.21% (Fig. 4).

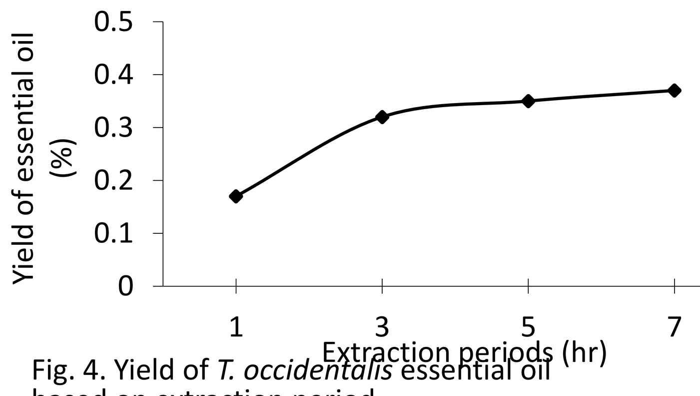


Fig. 4. Yield of *T. occidentalis* essential oil based on extraction period.

Chemical profiling of essential oils through GC-MS

The constituent substances of essential oil were identified by GC-MS in the leaves. (Table 1). The analysis led to identification of 20 volatiles from essential oil in *T. occidentalis* leaves(Fig. 5). The main constituents were thujone (16.58%), b-myrcene (14.62%), bornyl acetate (9.31%) and terpinyl acetate (3.29%) .

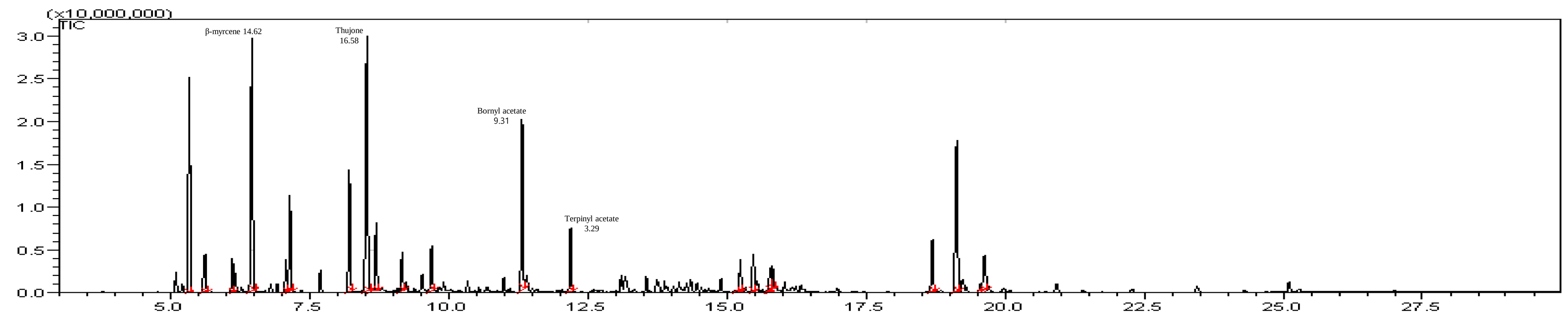


Fig. 5. Capillary gas chromatogram of *T. occidentalis* leaves volatiles obtained by steam distillation.
The GC column was a 60 mm × 0.25 mm × 0.25 ㎛ i. d. HP-1 fused silica capillary column.

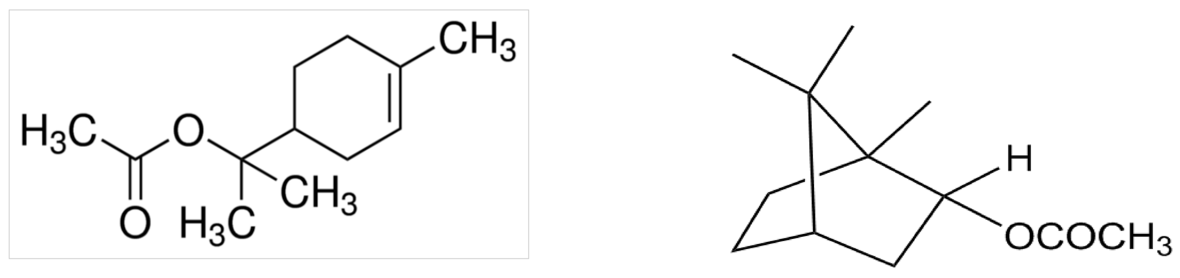
Selection of putative insecticidal compounds from the essential oil

Single compound with insecticidal activity were according metabolite profiling by GC-MS. For determination of these bio active compound, three term are designed as follow, first compounds were contained in all three essential oils. Second, a large quantity of compounds in three essential oils. Third, compounds having biological active chemical structure. Metabolic profiling of essential oils was powerful method for selection of high valuable bio-active compounds. As a results, selected terpinyl acetate and bonyl acetate (Fig. 6).

As a preliminary experiment, the chemical compositions of essential oils extracted from the leaves of three types of conifers, including *C. obtusa* and *C. pisifera*, were profiled using GC-MS and their antifungal activity was investigated (Data not shown). As a result, it has been reported that terpinyl acetate and bonyl acetate are antifungal substances.

Table 1. Volatile compounds in steam-distilled essential oil in the leaves from *T. occidentalis* identified by GC-MS.

No		Compounds	Ret. Time	Essential oil composition (%)
1	acyclic monoterpene	α-Pinene	5.33	9.21
2	bicyclic monoterpene	Camphene	5.62	2.52
3	terpene hydrocarbons	β-Phellandrene	6.11	1.64
4	monoterpene	β-myrcene	6.46	14.62
6	monoterpene	Limonene	7.14	5.05
7		Bicyclo	8.21	6.56
8	oxygenated sesquitepene	Thujone	8.53	16.58
9	oxygenated sesquitepene	α-Thujone	8.69	3.56
10	monoterpene	Camphor	9.16	1.97
11		3-Cyclohexen-1	9.69	2.28
12	oxygenated monoterpene	bornyl acetate	11.31	9.31
13	oxygenated monoterpene	Terpinyl acetate	12.18	3.29
14	oxygenated sesquitepene	Caryophyllene oxide	15.23	1.53
15	sesquitepene	α-cedrol	15.47	1.93
16	sesquitepene	7-Methanoazulen	15.77	1.07
17	sesquitepene	α-Cedrane	15.82	1.06
19	diterpene hydrocarbons	Norkaur	19.13	10.82



Terpinyl acetate Bornyl acetate

Fig. 6. Putative bioactive compound profiled by GC-MS analysis.

Insecticide activity of essential oils and terpinyl acetate and bornyl acetate

Insecticide activity of essential oils and mono terpenes by Fumigation

Using the fumigant activity, the insecticidal activity of *T. occidentalis* against *M. persicae* adults was higher in closed glass vials (500 mL)(Fig. 7 & 8). There was a concentration-dependent increase in mortality of adult insects fumigated with pure essential oil of *T. occidentalis*. Essential oil showed strong fumigant activity against *M. persicae*. *T. occidentalis* essential oil showed insecticide in 60% for 12 hr and 100% at does of 2.0 μL after treatment of 18 hr. In the fumigant assay, the highest insecticide activity showed to 100% level at does of 5.0 μL after treatment of 15 hr. But, because essential oil 5.0 μL was more and more 2.0 μL, therefore the highest insecticide activity was *T. occidentalis* essential oil, and 100% at does of 2.0 μL after treatment of 18 hr.

The insecticidal effects of mono terpenes were different, and terpinyl acetate and bonyl acetate had the highest activity.

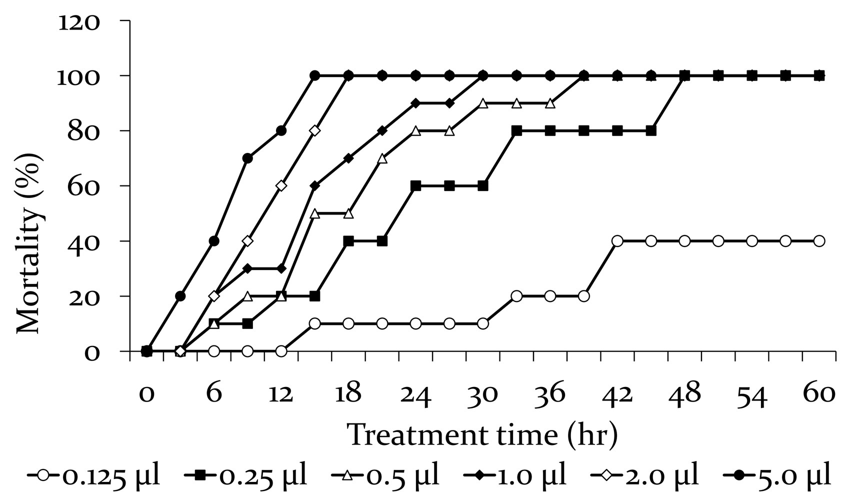


Fig. 7. Mortality of *M. periscae* on essential oil and treated various concentration by fumigation.

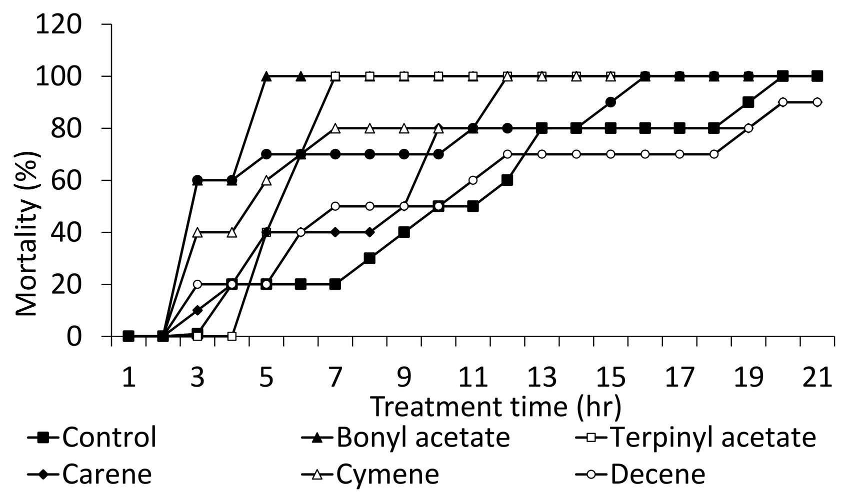


Fig. 8. Mortality of *M. periscae* on 6 monoterpene and treated 2.0 μL/L air by fumigation.

Insecticide activity of essential oils and mono terpenes by spraying

The insecticide activity based on spraying concentration (5%, 10%, 15% and 20%) of *T. occidentalis* essential oil with 8% tween #20 was determined and monitored every 15 min total 60 min (Fig. 4). As a result, each treat highest insecticide activity was *T. occidentalis* essential oil at does of 10%, leading to the 95% death for 60 min.

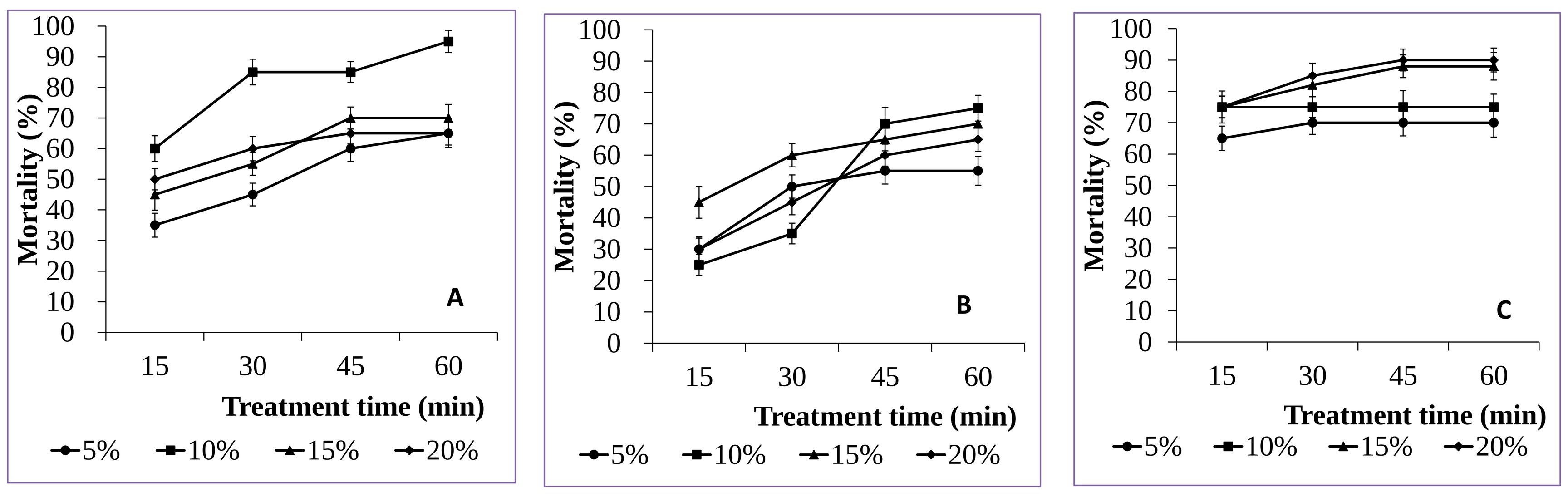


Fig. 9. Mortality of *M. periscae* of *T. occidentalis* essential oil and spectant treatment.
A:Spraying of essential oil with 8% (v/v) tween #20, B: Spraying of terpinyl acetate with 8% (v/v) tween #20, and C: Spraying of bornyl acetate with 8% (v/v) tween #20

Conclusion

Insecticidal active compounds were determined through metabolic profiling of three coniferous species including *T. occidentalis* essential oil. For optimal extraction, 50g of leaves were added to 500mL of distilled water and extracted by crushing the leaves for 7 hours at 100 °C. Essential oil showed strong fumigant activity against *Myzus periscae*. *T. occidentalis* essential oil showed insecticide in 60% for 12 hr and 100% at does of 2.0 μL after treatment of 18 hr in fumigant assay. In spraying test, *T. occidentalis* essential oil at does of 10%, leading to the 95% death for 60 min. 20 compounds in the essential oil from leaves of *T. occidentalis* were identified though GC-MS. The main constituents were terpinyl acetate, bornyl acetate. Terpinyl acetate and bonyl acetae were high insecticide activity more than other monoterpene. Terpinyl acetate was showed 100% death for 7 hr and bonyl acetae showed 100% death for 5 hr in fumigation test. Terpinyl acetate and bonyl acetae can be use for environmental-friendly insecticidal active materials

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