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Larvicidal Activities of Extracts from Domestic and Japanese Plants against the Pine Needle Gall Midge (Diptera: Cecidomyiidae)

국내산 및 일본산 식물체 추출물의 솔잎혹파리 유충에 대한 살충활성

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ABSTRACT Methanol extracts from 79 domestic plant species in 41 families and the crude oil of one Japanese plant species were tested for their larvicidal activities against *Thecodiplosis japonensis* Uchida et Inouye, using filter paper-impregnated and insect-dipping methods. In a test with 10 mg/paper, strong larvicidal activity (>90% mortality) was obtained from the extracts of roots from three domestic plants belonging to Ranunculaceae (*Aconitum pseudo-leave* var. *eretum, A. carmichaeli* and *Clematis florida*) and the crude oil of sawdust from a Japanese plant, *Thujopsis dolabrata* var. *hondai* (Cupressaceae). However, only the crude oil from the *Thujopsis* sawdust revealed strong larvicidal activity when tested with 5,000 ppm by insect-dipping method. It is concluded that the *Thujopsis* sawdust-derived materials could be useful as a new control agent against *T. japonensis*.

KEY WORDS *Thecodiplosis japonensis*, larvicidal activity, plant extract, Ranunculaceae, *Thujopsis dolabrata* var. *hondai*

초　　록 국내산 41과 79종 식물체 메탄올 추출물 및 일본산 1종 식물체 정유의 솔잎혹파리(*Thecodiplosis japonensis* Uchida et Inouye) 유충에 대한 살충활성을 여지접촉법과 충체침지법으로 조사한 결과 살충활성은 식물체 종류에 따라 달리 나타났다. 여지접촉법으로 여지당 시료 10 mg을 처리하였을 때, 국내산 미나리아재비과의 진범, 부자 및 위령선 뿌리 추출물과 일본산 측백나무과의 *Thujopsis dolabrata* var. *hondai* 톱밥의 정유가 90% 이상의 강한 살충활성을 보였다. 그러나 충체침지법(5,000 ppm)을 이용한 생물검정에서는 *T. dolabrata* var. *hondai* 톱밥의 정유만이 강한 살충활성을 나타내었다. 따라서 *T. dolabrata* var. hondai 톱밥의 추출물은 솔잎혹파리에 의한 소나무림의 피해를 줄일 수 있을 것으로 생각된다.

검색어 솔잎혹파리, 살충활성, 식물체 추출물, 미나리아재비과, *Thujopsis dolabrata* var. *hondai*

Among 159 species of arthropod pests of pines in Korea (Anonymous 1986, Anonymous 1995b), the most important is the pine needle gall midge (*Thecodiplosis japonensis* Uchida et Inouye). In the spring (May to June), just as the leaf buds swell, adults deposit eggs between developing needles. After hatching, young larvae crawl down to the leaf sheath and feed by sucking sap, resulting in formation of galls. Each gall encloses several larvae. Large numbers of galls on trees cause premature defoliation which results in simultaneous retardation in both terminal and cambial growth of the tree (Park 1982, Ko & Morimoto 1985). Since this species was first recorded in Muan in 1929, it has spread rapidly every year and has been found in most pine forests by the mid-1990s (Anonymous 1995a, b). In 1995, approximately 212,220 ha of red pines (*Pinus densiflora*) and black pines (*P. thunbergii*) has been infested by this insect species. The ecology of this species has been well described by Ko (1982), Ko & Morimoto (1985), Park & Hyun (1977), Lee *et al.* (1985) and Lee (1992).

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Control of this gall midge populations is primarily dependent upon trunk implantation of a systemic insecticides such as phosphamidon (Anonymous 1995a, b). In 1994, nearly 432,745 (AI) kg of the insecticide for controlling this midge species was used in Korea (Anonymous 1995a, b). Although they have effectively controlled this species, their continued use on pines for the past decades has disrupted biological control by natural enemies and has led to resurgences in this midge populations. Besides these problems, factors such as labour cost, safety, and adverse effects on non-target organisms and environments should also be considered for integrated pest management. This economic consideration and increasing concern over adverse effects of the earlier types of insecticides have brought about the need for the development of new types of selective alternatives or alternative control methods without, or with reduced, use of organic insecticides.

Plants may be an alternative to insect control agents currently being used, because they are virtually rich source of bioactive organic chemicals (Harborne, 1988). Therefore, we assessed the larvicidal activity of methanol extracts from 79 domestic plant species and crude oil of one Japanese plant species against *T. japonensis* to search plant-derived materials for potentially useful products as commercial insecticides or as lead compounds.

MATERIALS AND METHODS

Insects tested

Twigs of red pine trees severely infested with the pine needle gall midge (*Thecodiplosis japonensis* Uchida et Inouye) were collected from damaged sites in Mikum-Si, Kyunggi Province, in September 13, 1994. Only the gall-formed needles were selected and cut by a razor, and then larvae in the gall were carefully collected with a camel's hair brush in Petri dish containing a small volume of water. In a preliminary test, larvae lived in water for 2 days without any adverse effect.

Plants and Sample Preparation

The 79 Oriental medicinal plant species in 41 families were purchased from a market in Seoul. The details along with economic importance of these plants are provided by Namba (1986). These plant materials were dried in a blower at 60°C for 2 days, finely powdered using a blender, extracted twice with methanol (1/2, v/v) at room temperature for 2 days, and filtered (Toyo filter paper No. 2). The combined filtrate was concentrated *in vacuo* by using rotatory vacuum evaporator. The sawdust of *Thujopsis dolabrata* var. *hondai* was obtained from Taiyo Kagaku Central Laboratories, Yokkaichi, Mie, Japan and subjected to steam distillation as previously described by Ahn *et al.* (1995). The plant species tested, plant parts and yield of each extraction are shown in Table 1.

Table 1. Plant species tested

Plant species	Family name	Part collected[a]	Yield[b] (%)
Angelica reticulata	Umbellifera	R	12
Angelica dahurica	Umbellifera	R	12
Torilis japonica	Umbellifera	Se	3
Bupleurum falcatum	Umbellifera	R	15
Anthriscus sylvestris	Umbellifera	R	21
Ledebourella seseliodes	Umbellifera	R	10
Syringa reticulata	Oleaceae	L	8
Fraxinus rhynchiphylla	Oleaceae	L	35
Aconitum carmichaeli	Ranuculaceae	R	16
Aconitum erectum	Ranunculaceae	R	15
Clematis florida	Ranuculaceae	R	19
Paeonia japonica	Ranuculaceae	R	10
Pueraria thunbergiana	Lebuminosae	R	21
Astagalas membranaceus	Lebuminosae	R	10
Indigofera kirilowi	Lebuminosae	R	12
Gleditsia sinensis	Lebuminosae	St	10
Rheum undulatum	Polygonale	R	50
Pleuropterus tripliana	Polygonale	R	14
Prunus persicae	Rosaceae	Se	8
Crategus maximowiczii	Rosaceae	Fl	39
Kerria japonica	Rosaceae	Se	20
Cotoneaster wilsonii	Rosaceae	Se	10
Spiraea fritschiana	Rosaceae	L	15
Spiraea prunifolia var. *simplociflora*	Rosaceae	L	13
Aralia continentalis	Araliaceae	R	12
Kalopanox pictum	Araliaceae	R	10
Aralia elata	Araliaceae	R	14

Table 1. (Continued)

Plant species	Family name	Part collected[a]	Yield[b] (%)
Eucommia ulmoides	Eucommiaceae	L	11
Vitex rotundifolia	Verbenaceae	Se	5
Liriope platyphylla	Liliaceae	R	14
Inula helenium	Composidae	R	11
Arctium lappa	Composidae	R	15
Artemisia messerschmidtiana	Composidae	St	10
Atractylodes japonica	Composidae	R	8
Carthamus tinctorius	Composidae	Fl	19
Oenanthe decumbens	Apiaceae	St	10
Imperata cylindrica	Gramineae	R	22
Beckmannia syzigachne	Gramineae	R	13
Cynanchum carmichaeli	Asdepiadaceae	R	15
Belamcanda chinensis	Iridaceae	R	20
Epimedium koreanum	Berberidaceae	R	9
Coptis japonica	Berberidaceae	R	21
Asarum sieboldii	Aristolchiaceae	L	13
Megnolia kobus	Megnoliaceae	R	7
Schizandra migra	Megnoliaceae	R	10
Polygala tatarinow	Polygalaceae	R	24
Leonurus sibiricus	Labiata	L	20
Schizonepta tenuifolia var. *kaponica*	Labiata	L	6
Lithospermum erythrorhizon	Borraginaceae	St	28
Citrus aurantium	Rutaceae	Fr	15
Poncitrus trifoliata	Rutaceae	Fr	32
Phellodendron	Rutaceae	St	15
amurense	Rutaceae	R	20
Citrus aurantium	Campanulaceae	R	12
Platycodon frandiflorum	Campanulaceae	R	10
Condonopsis lanceolata	Caprifoliaceae	St	13
Lonicera subsessilis	Caprifoliaceae	Fl	19
Lonica japonica	Convolvulaceae	Fr	6
Cuscuta japonica	Cyperaceae	Fr	5
Cyperus rotundus	Cyperaceae	R	9
Scirpus fluviatilis	Scrophulariaceae	R	27
Scrophylaria buergeriana	Equisetaceae	St	5
Eqisetum hyemale	Ladizabalaceae	St	9
Akebia quinata	Rhamnaceae	R	9
Zizyphus jujuba	Gentianaceae	R	29
Gentiana scabra	Typaceae	Wp	10
Typha oriantalis	Cucurbitaceae	R	6
Trichosantes kirilowii	Celastraceae	L	10
Euonymus oxyphyllus	Celastraceae	L	8
Euonymus macroptera	Aquifoliaceae	L	12
Ilex macropoda	Saxifragaceae	Fl	20
Hydrangea macrophylla	Saxifragaceae	L	18
Ribes fasciculatum	Saxifragaceae	R	11

Table 1. (Continued)

Plant species	Family name	Part collected[a]	Yield[b] (%)
Deutzia parviflora	Saxifragaceae	St	13
Deutzia glabrata	Saxifragaceae	R	10
Corydalis turrschaninovii	Fumariaceae	R	3
Rhododendron schlippenbachii	Ericaceae	Fl	2
Eleuterococcus senticosus	Araliaceae	St	8
Anemarrhem asphodeloides	Liliaceae	R	12
Thujopsis dolabrata var. *hondai*[c]	Cuppressaceae	Sd	1

[a]R, root; Se, seed; L, leaf; St, stem; Fl, flower; Fr, fruit; Wp, Whole plant; and Sd, sawdust.
b(Weight/dry weight of test material) × 100.
[c]The sawdust was obtained from Taiyo Kagaku Central Laboratories, Japan. The crude oil of the sawdust was obtained by steam distillation.

Toxicity Test

The larvicidal activities of test samples were determined by filter paper-impregnated and insect-dipping methods. In tests with filter paper-impregnated method, 10 mg of each sample dissolved in methanol was applied to a filter paper (ϕ 6 cm) by micro syringe. After evaporation, larvae was placed onto the papers in Petri dishes (ϕ 6 cm). Controls received methanol.

For insect-dipping method, the plant samples were tested at a concentration of 5,000 ppm for larvicidal activity, as previously described by Ahn & Cho (1992) and Ahn *et al.* (1992). Test samples suspended in distilled water with Triton X-100 spreader (Coseal Co., Seoul) added at the rate of 1 ml/liter were used. Larvae were dipped into 1 ml of test solution in Effendorf tube for 15 sec. The test solution containing larvae were poured onto filter paper (ϕ 6 cm) in Petri dishes. Controls received stock solution.

All treatments were conducted in triplicate, and 20 larvae were used in each assay. Treated larvae were held in a room at 25±1°C, 50~60% relative humidity and a photoperiod of 16 : 8 (L : D) hr. Mortalities were determined 48 hr after treatment. Data from all bioassays were corrected for control mortality using Abbott's (1925) formula. Larvicidal activities were classified as previously described by Kwon *et al.* (1994): strong activity +++, mortality≥90%; moderate ++, mortality 89~61%; weak +, mortality 60~40%; and little or no activity -, mortality<40%.

RESULTS AND DISCUSSION

Methanol extracts and crude oil were subjected to a primary screening test for their larvicidal activities against *T. japonensis* using filter paper-impregnated and insect-dipping methods, and the data are presented in Table 2. Considering the life history of the pine gall midge with short life span of adults (1~2 days) and long duration of larva in the gall and soil, these methods are suitable for a screening against this insect species.

In a test with 10 mg/paper, strong larvicidal activity (+++) was obtained from the extracts of roots from three domestic plant species belonging to Ranunculaceae (*A. pseudo-leave var.* erectum, *A. carmichaeli* and *C. florida*) and the crude oil of sawdust from a japanese plant, *T. dolabrata* var. *hondai* (Cupressaceae). Extract of root from *L. seseloides* (Umbellifera) revealed moderate activity (++), whereas little or no activity was produced from the other plant extracts.

Out of the plant samples determined by insect-dipping method, only the crude oil of the *Thujopsis* sawdust exhibited the strong lavicidal activity (+++) against *T. japonensis*.

Table 2. Larvicidal activity of plant extracts against the pine needle gall midge determined by filter paper-impregnated and insect-dipping methods

Plant species[a]	Treatment[b]	
	Filter paper-impregnated[c]	Insect dipping[d]
L. seseloides	++	+
A. pseudo-laeve var. *erectum*	+++	+
A. jaluens	+++	+
C. florida	+++	+
T. dolabrata var. *hondai*	+++	+++

[a]Plants showing insecticidal activity with >70% are presented.
[b]Mortality>90%, +++; 90-61%, ++; 40-60%, +; and <40%, -.
[c]Treated with 10 mg/filter paper (ϕ 6cm).
[d]Treated with 5,000 ppm.

Certain plant-derived extracts and phytochemicals can be useful as insecticides. Since these have selectivity towards the natural enemies of pests, act in many ways on various types of pest complex, and may be applied to the plant in the same way as other agricultural chemicals, they are being considered as potential alternatives for synthetic insecticides (Jacobson & Crosby 1971, Elliott 1977, Arnason *et al.* 1989). Additionally, plant-derived materials are found to be highly effective on insecticide-resistant insect pests (Arnason *et al.* 1989, Verkerk & Wright 1993, Kwon *et al.* 1996). Jacobson (1989) pointed out that the most promising botanicals as sources of novel plant-based insecticides for use at the present time and in the future are species of the families, Meliaceae, Rutaceae, Asteraceae, Annonaceae, Labiatae, and Canellaceae. For example, derivatives of neem (*Azadirachta indica* A. Juss) belonging to the family Meliaceae are found to have a variety of biological activities against nearly 200 species of insects without any adverse effects on non-target organisms (Saxena 1989). Although plant-derived materials are found to be effective against insects only for a relatively short period of time, neem-derived materials gave good protection of crops against *Plutella xylostella* for 6 days (Schmutterer 1992), and against swarms of *Schistocerca gregaria* for up to 2 weeks if they were not washed off by rain (Wakisaka *et al.* 1992).

In our study, extracts of roots from *A. pseudo-leave* var. *eretum, A. carmichaeli* and *C. florida* and crude oil from *Thujopsis* sawdust revealed strong insecticidal activity when tested by filter paper-impregnated method. This is the first report on insecticidal activity of these plants against the pine needle gall midge. Especially, the crude oil of the sawdust revealed 100% larvicidal activity, regardless of treatment methods. Toxicological and pharmacological investigations have demonstrated that *Thujopsis* species have antimicrobial (Ito *et al.*, 1980), antitermitic (Chaboussou 1978, Ikeda *et al.* 1978, Nakashima & Shimizu 1972), and rodent repellent activities (Ahn *et al.* 1995).

Based upon our results and these earlier findings, the crude oil from *T. dolabrata* var. *hondai* sawdust could be a useful plant material for developing new types of biorational management agents against *T. japonensis*, although its effects on non-target organisms and environments have not been fully investigated.

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