Agric. Chem. Biotechnol. **48**(1), 26-28 (2005)

Article

Larvicidal Activity of *Chamaecyparis obtusa* and *Thuja orientalis* Leaf Oils against Two Mosquito Species

Ju-Hyun Jeon, Sang-Hyun Lee, Moo-Key Kim and Hoi-Seon Lee*

Faculty of Biotechnology and Research Center for Industrial Development of Biofood Materials, College of Agriculture & Life Science, Chonbuk National University, Chonju 561-756, Korea

Received January 19, 2005; Accepted February 18, 2005

Evaluation of larvicidal activities of *Chamaecyparis obtusa* and *Thuja orientalis* oils against 4th-instar larvae of *Aedes aegypti* and *Culex pipiens pallens* revealed larvicidal activities of leaf oils extracted from *C. obtusa* and *T. orientalis* were significantly higher than those of stem, fruit, and seed oils. Strong mortality was observed in age class II of *C. obtusa* and *T. orientalis* against *Ae. aegypti* and *Cx. pipiens pallens* larvae. These results show both leaf part and age class II of *C. obtusa* and *T. orientalis* have strong larvicidal activity against *Ae. aegypti* and *Cx. pipiens pallens*. Leaf oils of *C. obtusa* and *T. orientalis* leaves show promise as natural larvicides against *Ae. aegypti* and *Cx. pipiens pallens*.

Key words: Aedes aegypti, Culex pipiens pallens, Chamaecyparis obtusa, Thuja orientalis, *mosquito larvicidal activity.*

Mosquitoes are well known vectors of several disease-causing pathogens, among which *Aedes aegypti* (L.) is a vector of dengue fever, a disease endemic to South-East Asia, Africa, and the Americas,[1] and *Culex pipiens pallens* (Coquillett) is a vector of the Western Nile virus, and distributed throughout Africa, the Middle East, and tropical Eurasia.[2] Control of these mosquito larvae is frequently dependent on continued applications of organophosphates such as temephos and fenthion, and insect growth regulators such as diflubenzuron and methoprene.[3] However, although effective, their repeated use disrupted natural biological control systems and led to the outbreaks of insect species, sometimes resulting in the widespread development of resistance, had undesirable effects on non-target organisms, and fostered environmental and human health concerns.[4-8] These problems have thus highlighted the need for the development of new strategies for selective mosquito larval control.

In recent years, essential oils have received much attention as potentially useful bioactive compounds against mosquito larvae.[9] Essential oils composed by isoprenoid compounds, mainly mono- and sesquiterpenes are the carriers of smell in the aromatic plants,[10] such as sesquiterpene alcohols identified from *Chamaecyparis obtusa* leaf oil,[11] and various monoterpene components derived from *T. orientalis*.[12] Particular emphasis has been placed on their antibiotic,[13] acaricidal,[14] antimicrobial,[15,16] antipathogenic,[17] and insecticidal activities.[18,19] However, in spite of these biological activities, relatively little work has been carried out on the larvicidal effects of *C. obtusa* and *Thuja orientalis* leaf-derived materials against mosquito larvae. In this study, we, therefore, examined the lavicidal activity of the oils extracted from *C. obtusa* and *T. orientalis* leaves against two mosquito species.

Materials and Methods

Insects. Laboratory F21 strain of *Aedes aegypti* was obtained in 2004 from National Institute of Health, Seoul, Korea. *Culex pipiens pallens* were collected at Seoho stream, Suwon (Kyunggi Province), South Korea. Adult mosquitoes were maintained on a 10% aqueous sucrose solution and blood from a live mouse, while larvae were reared in plastic butt (24×35×5 cm) and fed a sterilized diet (80/20 mix of chick chow powder: yeast). Mosquitoes were held at 28 ± 2ºC and 70 ± 5% relative humidity under a photoregime of 16 : 8 h (L : D).

Plants and sample preparation. Fruits, leaves, stems, and branches (5-8 cm; with leaves) of *C. obtusa* and *T. orientalis* were collected during spring 2003 in Chonbuk province (Korea). Age classes I, II, and III of *C. obtusa* and *T. orientalis* were also collected. The essential oils tested were extracted by steam distillation as previously described by Jang *et al.*.[20]

Bioassay. Concentrations of the test samples were prepared by serial dilution of a stock solution of the sample in ethanol. Each sample in ethanol was suspended in distilled water with Triton X-100 added at 10 mg · l^{-1}. Groups of 25 early 4th-instar larvae of *Ae. aegypti* and *Cx. pipiens pallens* were put into the paper cups (270 m*l*) containing each test solution (250 m*l*). The toxicity of each sample was determined at four to seven concentrations ranging from 25 to 400 ppm. Controls received ethanol/Triton X-100 solution. Treated and control larvae were held under the same conditions mentioned earlier.

*Corresponding author
Phone; +82-63-270-2544; Fax: +82-63-270-2550
Email: hoiseon@chonbuk.ac.kr

Each part and age class of the plants were evaluated for their lavicidal activities 24 h after treatment. All treatments were replicated four times. No mortality was obtained from each control.

Statistical analyses. The percentage of mortality was determined and transformed into arcsine square-root values for analyses of variance (ANOVA). Treatment means were compared and separated by Scheffes test at $p = 0.05$.[21] Means (±SE) of untransformed data are reported.

Results and Discussion

During the first experiment, we observed that the essential oils extracted from *C. obtusa* and *T. orientalis* possess mosquito larvicidal activity against *Ae. aegypti* and *Cx. pipiens pallens*, producing 100% mortality at 500 ppm (data not shown). Therefore, bioassays were performed on various parts of *C. obtusa* and *T. orientalis* to determine plant parts having strong activities (Table 1). Essential oils extracted from both leaves and stems of *C. obtusa* gave 100% mortality against *Ae. aegypti* and *Cx. pipiens pallens* at 400 ppm. At 200 ppm *C. obtusa* leaves and stems, 100 and 68.2% larvicidal activities were observed against *Ae. Aegypti*, and 100 and 34.5% against *Cx. pipiens pallens*, respectively. In addition, the essential oils extracted from leaves and fruits of *T. orientalis* at 400 ppm exhibited 100 and 71.6% mortalities against *Ae. Aegypti*, and 100 and 53.1% against *Cx. pipiens pallens*. However, the essential oils of *T. orientalis* stems and seeds showed no mortality at 400 ppm. The essential oils of *T. orientalis* leaves and fruits exhibited 100 and 18.7% mortalities against *Ae. Aegypt,i* and 92.1 and 18.7% against *Cx. pipiens pallens* at 200 ppm. Furthermore, the larvicidal activity of the leaf oils was significantly higher than those of stem, fruit, and seed oils against *Ae. aegypti* and *Cx. pipiens pallens*. These results suggest that the leaf oils may have higher concentrations of active components than stem, fruit, and seed oils.

The larvicidal activities of the leaf oils derived from various age classes (I-III) of *C. obtusa* and *T. orientalis* were examined at various concentrations (Table 2). In the case of age class I, the leaf oils of *C. obtusa* exhibited 91.3, 72.8, and 42.2% mortalities against *Ae. Aegypti*, and 84.2, 59.6, and 31.2% against *Cx. pipiens pallens* at 100, 50, and 25 ppm, respectively. In the age class II, the leaf oils of *C. obtusa*

Table 1. Mosquito larvicidal activities of oils extracted from various parts of *C. obtusa* and *T. orientalis* against 4th-instar larvae of *Aedes aegypti* and *Culex pipiens pallens*

Sample[b]		Mortality (mean ± SE, %)[a]			
		Ae. aegypti		*Cx. pipiens pallens*	
		400 ppm	200 ppm	400 ppm	200 ppm
C. obtusa	leaf	100	100	100	100
	stem	100	68.2 ± 3.7	100	34.5 ± 3.4
	fruit	0	0	0	0
	seed	0	0	0	0
T. orientalis	leaf	100	100	100	92.1 ± 4.1
	stem	0	0	0	0
	fruit	71.6 ± 2.9	18.7 ± 5.2	53.1 ± 1.1	18.7 ± 5.2
	seed	0	0	0	0

($p = 0.05$, Scheffe's test[SAS Institute])
[a]Values are percentages (mean ± SE). [b]Exposed for 24 h.

Table 2. Mosquito larvicidal activities of various age classes of *C. obtusa* and *T. orientalis* leaf oils against 4th-instar larvae of *Aedes aegypti* and *Culex pipiens pallens*

Sample[b]		Mortality (mean ± SE, %)[a]					
		Ae. aegypti			*Cx. pipiens pallens*		
		100 ppm	50 ppm	25 ppm	100 ppm	50 ppm	25 ppm
C. obtusa	Age class I[c]	91.3 ± 3.2	72.8 ± 2.8	42.2 ± 2.6	84.2 ± 3.2	59.6 ± 2.8	31.2 ± 4.1
	Age class II	100	87.4 ± 3.3	62.7 ± 3.8	100	78.6 ± 2.7	51.5 ± 3.3
	Age class III	68.5 ± 2.5	43.1 ± 1.9	18.7 ± 3.1	54.7 ± 4.3	33.6 ± 3.1	0
T. orientalis	Age class I	79.6 ± 3.4	48.6 ± 2.7	22.5 ± 3.7	56.5 ± 2.7	31.2 ± 2.9	0
	Age class II	91.5v2.9	74.5 ± 4.1	37.8 ± 3.7	83.9 ± 1.8	57.9 ± 1.9	29.6 ± 4.5
	Age class III	34.1 ± 4.2	0	0	20.5 ± 2.5	0	0

($p = 0.05$, Scheffe's test[SAS Institute])
[a]Values are percentages (mean ± SE). [b]Exposed for 24 h. [c]Age class I: 0-10 years, Age class II: 11-20 years, Age class III: 21-30 years.

exhibited 100, 87.4, and 62.7% mortalities against *Ae. Aegypti*, and 100, 78.6, and 51.5% against *Cx. pipiens pallens* at 100, 50, and 25 ppm, respectively. However, weak larvicidal activity was observed with the age class III against *Ae. aegypti* and *Cx. pipiens pallens*. Furthermore, the leaf oils of the age class I from *T. orientalis* exhibited 79.6, 48.6, and 22.5% mortalities against *Ae. Aegypti*, and 56.5, 31.2, and 0% against *Cx. pipiens pallens* at 100, 50, and 25 ppm, respectively. In the age class II, the leaf oils of *T. orientalis* exhibited 91.5, 74.5, and 37.8% mortalities against *Ae. Aegypti*, and 83.9, 57.9, and 29.6% against *Cx. pipiens pallens* at 100, 50, and 25 ppm, respectively. However, weak larvicidal activity was observed with the age class III against *Ae. aegypti* and *Cx. pipiens pallens*. These results suggest that the age class II of *C. obtusa* and *T. orientalis* may control *Ae. aegypti* and *Cx. pipiens pallens* more effectively than the age classes I and III. Furthermore, the active components of the age class II of *C. obtusa* and *T. orientalis* might be different from those of age classes I and III.

The exact roles of the essential oils of *C. obtusa* and *T. orientalis* or their modes of action have not yet been clarified. However, our study shows the great opportunity for the control of *Ae. aegypti* and *Cx. pipiens pallens* using the leaf oils of *C. obtusa* and *T. orientalis*. Further research to identify the biologically active substances as well as the larvicidal modes of action of *C. obtusa* and *T. orientalis*, which showed the most potent larvicidal activity, is in progress.

Acknowledgments. This work was supported by Agricultural R & D Promotion Center through Chonbuk National University to Hoi-Seon Lee (203098-02-1-SB010).

References

1. Maillard, M., Marston, A. and Hostettmann, K. (1993) Search for molluscicidal and larvicidal agents from plants. In *Human medicinal agents from plants*, Balandrin, M. (ed.) American Chemical Society, Washington DC.
2. Peiris, J. S. M. and Amerasinghe, F. P. (1994) West Nile fever. In *Handbook of zoonoses*, Beran, G. W., Stelle, J. H. (ed.) CRC Press, Florida.
3. Rozendaal, J. A. (1990) Mosquitoes and other biting Diptera. In *Vector Control*. pp. 5-177, World Health Organization, Geneva, Switzerland.
4. Brown, A. W. A. (1983) Insecticide resistance as a factor in the integrated control of Culicidae. In *Integrated mosquito control methodologies*; Laird, M. and Miles, J. W. (ed.) pp. 161-235, Academic Press, New York.
5. Hayes, W. J. Jr. and Laws, E. R. Jr. (1991) In *Handbook of Pesticide Toxicology*, Vol. 1, Academic Press, New York.
6. Chandre, F., Darriet, F., Darder, M., Cuany, A., Doannio, J. M. C., Pasteur, N. and Guillet, P. (1998) Pyrethroid resistance in *Culex quinquefasciatus* from West Africa. *Med. Vet. Entomol.* **12**, 359-366.
7. Casida, J. E. and Quistad, G. B. (2000) Insecticide targets: learning to keep up with resistance and changing concepts of safety. *Agric. Chem. Biotechnol.* **43**, 185-191.
8. Lee, S. E., Kim, J. E. and Lee, H. S. (2001) Insecticide resistance in increasing interest. *Agric. Chem. Biotechnol.* **44**, 105-112.
9. Kim, M. J., Ahn, Y. J., Kim, M. K., Kim, H. Y. and Lee, H. S. (2001) Rat intestinal α-glucosidase inhibitory activities of leguminous seed extracts. *Agric. Chem. Biotechnol.* **44**, 1-5.
10. Cheng, S. S., Chang, H. T., Chang, S. T., Tsai, K. H. and Chen, W. J. (2003) Bioactivity of selected plant essential oils against the yellow fever mosquito *Aedes aegypti* larvae. *Biores. Tech.* **89**, 99-102.
11. Hieda, T., Tazaki, M., Morishita, Y., Aoki, T. and Nagahama, S. (1996) Sesquiterpene alcohols from *Chamaecyparis obtusa* leaf oil. *Phytochemistry.* **42**, 159-162
12. Chizzola, R., Hochsteiner, W. and Hajek, S. (2004) GC analysis of essential oils in the rumen fluid after incubation of *Thuja orientalis* twigs in the Rusitec system. *Res. Vet. Sci.* **76**, 77-82.
13. Koyama, S., Yamaguchi, Y., Tanaka, S. and Motoyoshiya, J. (1997) A new substance (Yoshixol) with an interesting antibiotic mechanism from wood oil of Japanese traditional tree (Kiso-hinoki), *Chamaecyparis obtusa*. *Gen. Pharmac.* **28**, 797-804.
14. Sung, B. K., Kim, M. K., Lee, S. H., Son, J. G. and Lee, H. S. (2004) Acaricidal activity of essential oils derived from 10 cupressaceae species against stored food and house dust mites. *Food Sci. Biotechnol,* **13**, 376-380.
15. Oh, D. H. (2000) Antimicrobial activities of *Thuja orientalis* L. and *Acoru graminens* scoland on the harmful microorganisms. *J. Agr. Sci.* **11**, 47-55.
16. Lee, K. K. (1999) Antimicrobial activity of *Thuja orientalis* and *Chamaecyparis obtusa* essential oil. *Kor. Soc. Cosmetology.* **5**, 567~577.
17. Yu, J. Q. and Komada, H. (1999) Hinoki (*Chamaecyparis obtusa*) bark, a substrate with anti-pathogen properties that suppress some root diseases of tomato. *Sci. Horticulturae.* **81**, 13-24.
18. Park, I. K., Lee, S. G., Choi, D. H., Park, J. D. and Ahn, Y. J. (2003) Insecticidal activities of constituents identified in the essential oil from leaves of *Chamaecyparis obtusa* against *Callosobruchus chinensis* (L.) and *Sitophilus oryzae* (L.). *J. Stored Prod. Res.* **39**, 375-384.
19. Park, I. K., Lee, S. G., Choi, D. H., Park, J. D. and Ahn, Y. J. (2003) Insecticidal activities of constituents identified in the essential oil from leaves of *Chamaecyparis obtusa* against *Callosobruchus chinensis* (L.) and *Sitophilus oryzae* (L.). *J. Stored Prod. Res.* **39**, 375-384.
20. Jang, Y. S., Baek, B. R., Yang, Y. C., Kim, M. K. and Lee, H. S. (2002) Larvicidal activity of leguminous seeds and grains against *Aedes aegypti* and *Culex pipiens pallens* (Diptera: Culicidae). *J. Am. Mosq. Control Assoc.* **18**, 210-213.
21. SAS Institute. (1990) *SAS/STAT User's Guide*, *Version 6*; SAS Institute, Cary, NC.