Short Communication

A Mite Growth Regulator from *Chamaecyparis pisifera* Which Inhibits Hatching and Larval Feeding of the Two-spotted Spider Mite, *Tetranychus urticae*

Jong-Woong AHN, Kojiro WADA,
Shingo MARUMO, Hideki TANAKA*
and Yoichi OSAKA*

Department of Agricultural Chemistry,
Nagoya University,
Chikusa-ku, Nagoya 464, Japan

*Pesticide Research Laboratory of Yashima
Chemical Industry Co., Ltd.,

Tomitake, Nagano-shi, Nagano 380, Japan

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The two-spotted spider mite, *Tetranychus urticae* Koch, is a serious pest to many crops because, in the repetition of its short life cycle, it rapidly develops resistance to a wide range of miticidal chemicals. We here report the isolation of a hatching inhibitor of *T. urticae* from *Chamaecyparis pisifera* Endl (SAWARA in Japanese), which also inhibits the feeding of mite larvae.

Isolation of the active substance was guided by our egg immersion method.¹⁾ The methanol extract of leaves of *C. pisifera* caused hatching failure of *T. urticae*. Solvent fractionation of this methanol extract obtained from 1 kg of *C. pisifera* indicated that ethyl acetate-soluble neutral fraction was active. Sequential purification of this neutral fraction by partition between benzene and 2% aqueous NaOH solution, using silica gel column chromatography and HPLC (LiChrosorb RP-18, 80% aqueous MeOH), led to the isolation of an active substance as a colorless solid (yield, 40 mg), $[\alpha]_D^{31} +214^\circ$ ($c=0.13$, MeOH), $C_{20}H_{30}O_3$ (M^+ , m/z 330).

Spectroscopic data (UV, IR, MS and 1H NMR)²⁾ of this substance and its methyl ester derivative (prepared with diazomethane) show-

ed this hatching inhibitor to be *O*-methyl pisiferic acid (**1**).^{3,4)} Although it is unusual that **1** was isolated from the neutral fraction, it may be because of the very weak acidity of its hindered carboxyl group. Further purification of the ethyl acetate-soluble, weakly acidic fraction by silica gel column chromatography also led to the isolation of **1** (yield, 170 mg) and pisiferic acid (**2**) (yield, 4.4 g).

Bean leaf disks on which mite eggs had been laid were dipped in various concentrations of compound **1** or its derivatives (**2**, **3**, **4**) and tested as described in note 1. The most prominent result of this bioassay was obtained from *O*-methyl pisiferic acid (**1**) which inhibited the normal hatching of eggs (Table I). When mite eggs on the leaf disks were treated with 500 ppm of **1**, 59% failed to hatch normally, and dead larvae remained in the

FIG. 1. Abnormal Larvae of *T. urticae* Which Emerged from an Egg Treated with *O*-Methyl Pisiferic Acid.

TABLE I. GROWTH REGULATING ACTIVITY OF *O*-METHYL PISIFERIC ACID AND ITS CONGENERS ON *T. urticae* KOCH

Compounds	Conc. (ppm)	%Eggs dead	%IH ^a	%AL ^b	%Larvae dead	%Nymphs dead	%Nymphs surviving
1	500	9	59	8	10	0	14
	250	12	21	5	21	2	39
	125	13	19	3	18	0	47
	62.5	3	7	3	19	6	62
	31.3	5	0	0	8	0	87
	15.7	7	0	0	3	3	87
Control	0	7	0	0	1	0	92
2	500	6	0	0	0	0	94
	250	0	3	0	0	0	97
	125	2	0	0	0	0	98
Control	0	3	0	0	0	0	97
3	500	4	0	0	0	0	96
	250	6	0	0	0	0	94
	125	4	0	0	0	0	96
Control	0	6	0	0	0	0	94
4	500	4	0	0	3	0	93
	250	7	0	0	0	0	93
	125	3	0	0	3	0	94
Control	0	2	0	0	0	0	98

^a Incompletely hatched larvae which remained within the transparent broken egg shells and died.

^b Hatched with abnormally short bodies.

Data are averages of the results for treatment of 40~60 eggs.

transparent broken egg shells; we called this phenomenon incomplete hatching (IH). Eight percent of the eggs hatched but produced abnormal larvae with bodies shorter than normal (Fig. 1) and 10% hatched normally but the larvae died during growth. Only 14% of the eggs produced normal larvae that survived into the nymphal stage. Derivatives **2**, **3**, and **4** showed no activity in this bioassay even at 500 ppm. A commercial miticidal agent, KELTHANE, showed ovicidal activity only at concentrations of more than 300 ppm.

The activity of **1** was dose-dependent. When treated with 62.5 ppm of **1**, 10% of the eggs showed incomplete hatching (IH) or produced abnormal larvae (AL) and 19% of the larvae that hatched normally died without feeding within 5 days. These results indicate that compound **1** has dual effects on the mite: it interferes with laval development in the eggs, which results in incomplete hatching, and it

acts as a feeding inhibitor on mite larvae.

To evaluate its inhibitory activity on feeding, a leaf disk test was conducted with larvae that had been starved for 24 hr after being hatched on the cover glass of a slide. About 25 starved larvae were transferred to a bean leaf disk that had been treated with a sample solution. Antifeeding activity was estimated by whether the larvae produced two spots on their back within a period of 2 days. The bioassay results are shown in Table II. Of the four compounds tested, **1** had remarkable antifeeding activity; at 500 ppm, 95% of the larvae did not produce the two spots on their backs and finally died of starvation. The effective dose of compound **1** for 50% feeding inhibition was 62.5 ppm. None of the other three congeners tested showed antifeeding activity.

This is the first isolation, to our knowledge, of a mite growth and development regulator from plants. The results of the research re-

TABLE II. ANTIFEEDING ACTIVITY OF *O*-METHYL PISIFERIC ACID AND ITS CONGENERS ON THE LARVAE OF *T. urticae* KOCH.

Compounds	Conc. (ppm)	%Larvae dead without feeding	%Larvae dead with feeding	%Nymphs dead	%Nymphs surviving
1	500	95	0	0	5
	250	90	0	0	10
	125	80	0	0	20
	62.5	50	0	0	50
	31.3	22	13	0	65
	15.7	0	10	0	90
Control	0	0	3	0	97
2	500	0	1	0	99
	250	0	0	0	100
	125	0	0	0	100
	Control	0	0	0	100
3	500	0	0	1	99
	250	0	0	1	99
	125	0	0	0	100
	Control	0	0	1	99
4	500	11	0	0	89
	250	6	2	0	92
	125	0	2	0	98
	Control	0	0	0	100

Data are averages of the results for treatment of 20~30 larvae.

ported here show that our egg immersion method is a usefully versatile bioassay for screening mite growth and development regulators from extracts of higher plants. We are continuing our research in the hope of finding other mite metamorphosis regulators in higher plants.

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REFERENCES AND NOTES

- 1) Egg immersion method: Five adult female mites were placed on a bean (a cultivar of *Phaseolus vulgaris* L., Kisayakurosandosaito in Japanese) leaf disk ($\phi 10$ mm) on a wet filter paper. Eggs were laid on the disk within 24 hr. A leaf disk with 40 to 60 eggs was dipped for 5 sec in the sample solution dissolved in 80% aqueous acetone, and it was then air dried. As the control, a leaf disk with eggs dipped in 80% aqueous acetone only was used. The disks were incubated at 25°C for 6 days, after which the egg-hatching and growth stages of the mites were observed under stereomicroscopy.
- 2) *O*-methyl pisiferic acid (1): MS m/z 330 (M^+); UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ) 235 sh. (7700), 280 (3450), 289 (3400); IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm^{-1} 3300~2500 (br.), 1690, 1610, 1500, 1460, 1260, 1160; ^1H NMR (CDCl_3) 0.83 (3H, s), 0.96 (3H, s), 1.17 (6H, d, $J=7$ Hz), 3.21 (1H, sep, $J=7$ Hz), 3.73 (3H, s), 6.74 (1H, s), 6.90 (1H, s).
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- 4) M. Yatagai, T. Shirato, Y. Hayashi, N. Fukuhara and T. Takahashi, *Mokuzai Gakkaishi*, **24**, 267 (1978).