Oviposition deterrent activity of andrographolide against the diamondback moth (DBM), *Plutella xylostella* (Lepidoptera: Yponomeutidae)

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Abstract

Andrographolide, the main constituent of the tropical shrub *Andrographis paniculata*, was studied for its oviposition deterrence against the diamondback moth (DBM). In the no-choice experiment, andrographolide applied at 1,000 ppm on five-week-old cabbage plants significantly reduced the number of eggs laid by the moths by about 50%. However, this compound has no oviposition deterrent activity when applied at 250 and 500 ppm. On the other hand, the choice experiment showed that 250, 500 and 1,000 ppm andrographolide sprays reduced moth oviposition by 72 to 81%.

Key words: Oviposition deterrent, andrographolide, *Plutella xylostella*

INTRODUCTION

Increasing interest in developing environmentally safe pest control methods has inspired us to study the potential of natural chemicals for controlling the diamondback moth (DBM) *Plutella xylostella*, the most serious insect pest of cruciferous crops in many countries.

In Asian countries where the shrub *Andrographis paniculata* grows widely, its extract is used in traditional medicine to cure a variety of human illnesses. The main constituent has already been isolated and established as andrographolide (Charkravarti and Charkravarti, 1951). In pharmacology, andrographolide has been studied intensively, but investigations on its potential as an agent to control pests having economic importance in agriculture has just commenced recently. Results of antifeedant studies revealed that andrographolide effectively reduced the feeding of fourth stadium DBM larvae (Hermawan et al., 1997), as well as the green rice leafhopper, *Nephotettix cincticeps* (Widiarta et al., 1997).

Our preliminary study (Hermawan et al., 1994) suggested that the crude extracts from *A. paniculata* suppress oviposition by DBM females. In this study, we examined the oviposition deterrent activity of andrographolide against the DBM moth.

MATERIALS AND METHODS

Insects and andrographolide. The DBM moths used in this study were obtained from stock cultures that were established from a field population collected at a cabbage field in Okayama University on August, 1996 and 1997. The larvae were fed on young radish seedlings while adults were fed on 10% honey solution. The stock culture was maintained for 2 to 3 generations under 25°C and 16L : 8D photoperiod.

Crystalline of andrographolide was obtained from Wako Pure Chemical Industries Ltd., Japan. One milligram of andrographolide was dissolved in 0.2 ml methanol and emulsified with water containing 0.3% emulsifier (Gramin®, Sankyo Co., Japan) to prepare 100, 250, 500 and 1,000 ppm solutions.

No-choice oviposition test. One five-week-old cabbage plant (*Brassica oleraceae* L. var. Oki-na) grown in a plastic pot was put inside a screen cage (35 cm × 25 cm × 35 cm) to serve as an oviposition medium for the DBM moths. Each plant (approx. 20 cm in height with 5–6 leaves) was sprayed with ca. 15–20 ml of an andrographolide emulsion by using a hand-held applicator. Control plants were likewise established by spraying with a 1 : 5 mixture of methanol and 0.3% emulsifier in water. All treat-

ments were replicated three times. Five pairs of one-d-old male and female moths were introduced into each cage containing the test plants. A cotton pad moistened with 10% honey was provided ad-libitum to the moths. The eggs laid by the moths on the stem and leaves of cabbage within 24 h were counted. The experiment was conducted under 25°C and 16L : 8D conditions.

Choice oviposition test. Two cabbage plants, one treated with a certain andrographolide concentration and the other sprayed with the control solution, were placed together inside a screened cage. All other activities and conditions were similar to those in the no-choice experiment.

RESULTS

In the no-choice test, the effect of andrographolide on the total number of eggs laid by five DBM females on cabbage in 24 h is presented in Table 1. Andrographolide applied at 1,000 ppm significantly reduced the number of eggs laid by the moths by about 50% ($p < 0.05$, Mann-Whitney *U*-test). However, at 250 and 500 ppm, no significant decrease was observed in oviposition.

The result of the choice test (Table 2) shows that andrographolide was effective at 250, 500 and 1,000 ppm, reducing the number of eggs laid on cabbage by about 77, 72 and 81%, respectively ($p < 0.05$). DBM females laid more eggs on the plants treated with 100 ppm andrographolide solution than those with the control treatment. The difference, however, was not significant.

Table 1. Total number of eggs laid by five female adults of the DBM on cabbage plant treated with different concentrations of andrographolide (no-choice test)

Concentration (ppm)	No. of eggs laid (mean $\pm$ SE)
0 (control)	199 $\pm$ 11
250	194 $\pm$ 29
500	152 $\pm$ 20
1,000	98 $\pm$ 9*

*Significant difference between control and treatment ($p < 0.05$ by Mann-Whitney *U*-test).

The number of replications was three for each experimental plot.

DISCUSSION

The results of the two experiments indicate that andrographolide, when applied at high concentrations substantially reduced egg laying by the DBM moths. It is apparent that andrographolide was more effective in the choice experiment than in the no-choice experiment. This was reasonable because the moths have another option to lay their eggs in the choice test. On the other hand, the moths were compelled to oviposit on the leaves treated with the lower concentrations (250 and 500 ppm) in the no-choice experiment where no alternative oviposition medium was provided for the moths. The total number of eggs decreased on cabbage plant(s) treated with high concentrations of andrographolide in both no-choice and choice experiments. Some eggs were laid on the walls of the cages or pots, although we did not count them. The DBM females probably laid more eggs on the cage surfaces in the treatments which were sprayed with higher andrographolide concentrations than those that

Table 2. Total number of eggs laid by five female adults of the DBM on cabbage plant treated with different concentrations of andrographolide and on control plant (choice test)

Concentrations (ppm)	No. of eggs (mean $\pm$ SE) laid on		Mann-Whitney <i>U</i> -test
	Treated	Control	
100	137 $\pm$ 63	67 $\pm$ 22	n.s.
250	30 $\pm$ 10	100 $\pm$ 14	$p < 0.05$
500	45 $\pm$ 18	113 $\pm$ 28	$p < 0.05$
1,000	23 $\pm$ 11	101 $\pm$ 15	$p < 0.05$

The number of replications was three for each experimental plot.

were treated with low concentrations or the control. Since andrographolide is not a volatile chemical, it is unlikely that its vapor in the cage suppressed the oviposition by the DBM females.

Some studies have been conducted on the effects of phytochemicals on the feeding response of the DBM (Daido et al., 1993; Dilawari et al., 1994; Chen et al., 1996a), but only a few were done to determine their effects on the oviposition behaviour of this pest. Feeding and oviposition deterrent activities of an aqueous extract of *Tanacetum vulgare* on two cabbage pests, DBM and *Pieris rapae* was reported by Hough-Goldstein and Hahn (1992). The deterrent effect of chinaberry fruit, *Melia azedarach* on oviposition and feeding of the DBM were reported by Dilawari et al. (1994) and Chen et al. (1996b).

The results reported here indicate that natural products like andrographolide, which was earlier found as an effective larval antifeedant against the DBM (Hermawan et al., 1997), can also reduce egg laying. Therefore, andrographolide may be a possible agent for the integrated management of the DBM.

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