

Insecticidal Activity of MK-139 (CME 134) Against Diamondback Moth

Yohichi Kohyama

Agricultural Products Laboratory, Research Center, Mitsubishi Chemical Industries Limited, 1000 Kamoshida-cho, Midori-ku, Yokohama-city, Kanagawa, 227 Japan

Abstract

A chitin synthesis inhibitor, MK-139 (CME 134) (1-(3,5-dichloro-2, 4-difluorophenyl)-3-(2, 6-difluorobenzoyl)-urea) was tested on various stages of diamondback moth, *Plutella xylostella*. The hatching of the eggs that were oviposited on the MK-139-treated surface, or that were dipped into an aqueous solution of MK-139 was greatly inhibited. When different instars were fed on an MK-139-treated diet until pupation, the activity of MK-139 did not differ with the instar under test. Treatment of larvae at the final instar stage with a low concentration (0.04 ppm) of MK-139 resulted in inhibited reproduction of the adults derived from treated larvae. In a field trial, MK-139 controlled a strain of *P. xylostella* against which a mixture of pyrethroid and organophosphorus insecticides was not effective.

Introduction

The insecticidal activity at CME-134(1-(3, 5-dichloro-2, 4-difluorophenyl)-3-(2, 6-difluorobenzoyl)-urea) was first discovered by scientists at Celamerck GmbH. This chemical is patented by Celamerck, and Mitsubishi Chemical Industries Limited is developing it under the code number of MK-139 in Japan and certain other Asian countries. In many laboratory tests and field trials, MK-139 exhibited exceptionally good insecticidal activity against Lepidoptera, Coleoptera and Hemiptera species (Becher et al 1983).

It is well known that benzoylphenyl urea insecticides affect the development of various stages of insect (Hammann and Sirrenberg 1980, Hajjar and Casida 1979). Some test results suggest that MK-139 influences various stages of diamondback moth (DBM), *Plutella xylostella* L (Lepidoptera: Yponomeutidae). This report describes the influence of MK-139 on the eggs, larvae, and adults of DBM.

Materials and Methods

Activity against adults

The acetone solution of MK-139 was applied on a glass petri dish to make a dry film of the active ingredient. The solution was allowed to evaporate for two hours. Male and female pupae, just before adult emergence, were placed on the treated dish separately. The insects were maintained at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH. The newly emerged moths were allowed to remain in contact with the MK-139 film for two days. One treated female moth was mated with one treated male moth. The number of eggs laid per female and the hatchability were investigated two days after the initiation of oviposition and five days after the end of oviposition, respectively.

Activity against eggs

Pre-treatment Cabbage leaves, ca 5 cm long, were dipped into an aqueous solution of MK-139 for one minute. The aqueous solution used for all tests contained different concentrations of MK-139 and 200 ppm of the spreader, Sorpol 3005X. After drying, the treated and untreated leaves were left in a plastic cage (35 cm x 27 cm x 30 cm) where 100 pairs of three day old adult moths were maintained. The insects were maintained at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH. The adult moths were allowed to oviposit on the leaves for 12 h. The hatchability of eggs laid was investigated five days after oviposition.

Post-treatment The untreated cabbage leaves were left in a plastic cage where 100 pairs of three-day old moths were kept. The adult moths were allowed to oviposit on the leaves for 12 h. The leaves with DBM eggs on them were dipped into an aqueous solution of appropriate concentration of MK-139 for one minute. After drying, the treated leaves were maintained at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH. The hatchability of the treated eggs was investigated five days after the end of oviposition.

Activity against larvae

Pieces of cabbage leaf (4 cm x 4 cm) were dipped into an aqueous solution of appropriate concentration of MK-139 for one minute. After drying, the treated leaf pieces were fed to 1st instar larvae of DBM. The MK-139-treated leaf pieces were fed successively and changed once every three days. The larvae were bred on treated leaf pieces until they became pupae. In the case of tests with 2nd, 3rd, and 4th instar larvae, insects freshly emerged from molting were used. The other procedures were similar to those of the tests with 1st instar larvae. All insects were maintained at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH. Mortality of larvae, pre-pupae, and pupae was recorded five days after treatment irrespective of the stage at which the insects had been treated. The death of each pre-pupa and pupa was confirmed by the nature of pupation or the absence of adult emergence from the pupae. LC_{50} value was calculated by the probit analysis (Bliss 1935).

Inhibition of reproduction by MK-139

Cabbage leaves were dipped into an 0.04 ppm aqueous solution of MK-139 for one minute. The 4th instar larvae, within three hours after molting, were fed with treated leaves until they became pupae. Male and female larvae were bred separately. The pupae derived from the treated larvae were graded by their appearance. Only pupae that appeared normal were used for the test, in order to give the best chance of effective mating. In each case one male and one female pupa derived from treated larvae were put into plastic cup (7 cm diam). The progress of each pair was observed until death. The male/female pairings were as follows:

Treated female x untreated male
Untreated female x treated male
Untreated female x untreated male

The oviposition period, when females did lay eggs, was two days. The number of eggs laid, and their hatchability, were investigated.

Field trial

An official field trial of MK-139 was conducted at Aira-gun, Kagoshima prefecture, Japan where a DBM strain has developed resistance to pyrethroids. Each insecticide was

sprayed twice onto the cabbage at a spray volume of 2000 liter/ha for the first application and 3000 liter/ha for the second application, respectively.

Results and Discussion

The number of eggs laid by MK-139 treated DBM adults was less than those laid by untreated adults (Figure 1). The higher the MK-139 concentration, the lower the number of eggs laid. Similarly, the total number of eggs hatched was reduced considerably by MK-139 treatment of the adult insects. The higher the MK-139 concentration, the lower the number of eggs hatched. Statistically, however, the difference between the numbers of eggs laid and eggs hatched for untreated and treated insects was not significant. It is likely that MK-139 has an adverse effect on the reproduction in adults, but the effect is rather weak. Becker (1985) reports similar effects when *Lymantria dispar* was treated with CME-134.

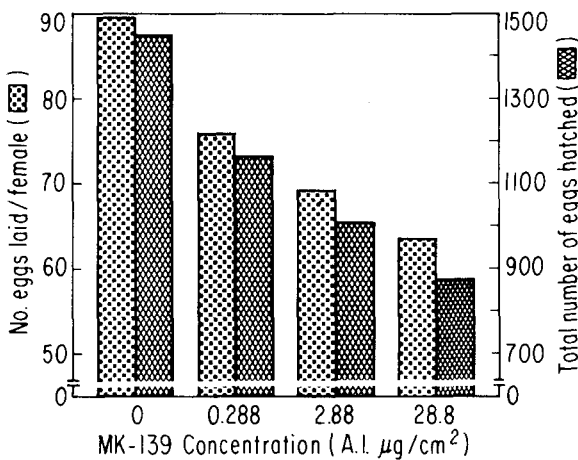


Figure 1.
Influence of adult exposure to MK-139 film on the fecundity of DBM

The hatching of DBM eggs laid on the cabbage leaves treated with MK-139 (pre-treatment) was greatly reduced (Figure 2). As described above, contact with MK-139 did not greatly reduce the fecundity of the adults. Therefore, the reduction of egg hatch is considered to be caused by the contact of eggs with the MK-139-treated surface. Although the contact area between egg and treated cabbage was quite small, most of the eggs did not hatch even at a 5 ppm concentration.

Egg hatching was markedly inhibited by the dipping of the eggs into an aqueous solution of MK-139 (Figure 2). In this test, the ovicidal activity of MK-139 was very high and most of the treated eggs did not hatch even at a 0.5 ppm treatment. The intensity of egg hatch inhibition—as reflected in EC_{50} —by treatment after the eggs were laid was six times higher than when cabbage leaves were treated prior to egg laying. This difference is considered partially due to the difference in area of eggshell that came in contact with MK-139. In both tests, the embryo of the unhatched egg when treated with MK-139 developed completely but could not hatch from the eggshell.

Since the lifecycle of DBM is relatively shorter than that of most other lepidopterous species, and because MK-139 acts quite slowly, it is difficult to evaluate the activity of MK-139 against DBM within one larval stage. For this reason, mortality at five days after treatment of larvae, irrespective of whether the insect was still larva, or had become pre-pupa or pupa, was used for the calculation of the LC_{50} value presented in Table 1. The LC_{50} values did not vary with the instar at which treatment started. This result

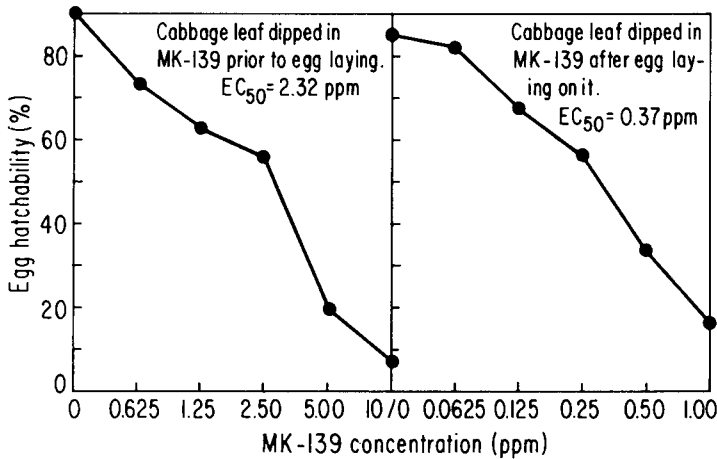


Figure 2. Hatchability of DBM eggs treated with MK-139 by two different methods

Table 1. Susceptibility of various larval stages of DBM exposed to diet containing MK-139^a

Starting stage	LC ₅₀ (ppm) ^b
1st instar	0.027
2nd instar	0.061
3rd instar	0.039
4th instar	0.051

^a Fifty larvae of each stage were used for the test.

^b At five days after treatment.

suggests that a mixed instar population can be controlled by one appropriate dosage of MK-139.

Feeding of 4th instar larvae on a diet containing MK-139 had significant adverse effect on reproduction of adults deriving from such larvae (Table 2). Hatchability of eggs from a combination of untreated female x treated male was very low. This may be attributable to sterilization and/or loss of mating ability of the male moth caused by MK-139 treatment. In this combination, although the female was untreated, fecundity was reduced considerably. This phenomenon may have been caused by the absence or reduction of oviposition stimulation by the male. In combinations of treated females x untreated males, the numbers of females that laid eggs decreased to one fourth that when untreated females mated with untreated males. Treatment with MK-139 also decreased significantly the number of eggs per treated female. The effects on the reproductive activity of both sexes are considered significant in suppression of population of succeeding generations.

The results of the field test to investigate the control of DBM by MK-139 are shown in Table 3. DBM could not be controlled by the application of a combination of fenvalerate and malathion. Application of MK-139 gave good control of DBM. MK-139 was slightly superior to IKI-7899. There was no indication of cross-resistance between pyrethroid and both benzoylphenyl urea insecticides.

In conclusion, it is obvious that MK-139 affects various stages of DBM. If MK-139 does not exhibit activity on the stage actually treated, the succeeding stage may be affected. For instance, treatment of larvae causes pupal death or inhibits the reproduction

of adults derived from treated larvae. This unique activity should contribute to the effective control of DBM.

Table 2. Effect of feeding of DBM larvae on a diet containing MK-139 on the reproduction in adults

Mating combination	No. of pairs	No. of females oviposited	No. of eggs/fertile female ^a	No. of hatched eggs/fertile female ^a	Egg hatchability (%)
Test I					
U FXT M ^b	40	32	52.9a	3.0b	5.6
T FXU M	40	10	75.3b	63.3a	84.1
U FXU M	40	40	93.5c	81.0a	86.7
Test II					
T FXT M ^c	22	12	24.1a	2.8a	11.1
U FXU M	19	19	90.3b	83.1b	92.0

^a Means in each vertical column for each test followed by the same letter are not significantly different at 5% level by Duncan's multiple range test. ^b U = pupae from untreated larvae, F = female, T = pupae from treated larvae, and M = male. ^c T = adults from treated larvae, U = adults from untreated larvae

Table 3. Field trial with MK-139 for the control of DBM on cabbage^a

Compound	Rate AI (ppm)	Control (%) at	
		2 DAT ^b	11 DAT
MK-139 5EC	25	97.2	97.8
IKI-7899 5EC	25	78.3	73.8
Hakusap ^c 40WP	400	30.0	42.7
Control ^d	—	71.5	185.0

^a Aira-gun, Kagoshima prefecture, Japan. ^b Days after treatment. ^c Combination of fenvalerate 10% and malathion 30%. ^d Number of living DBM larvae/10 plants.

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