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Insecticidal Activity of Pyridalyl: Acute and Sub-Acute Symptoms in *Spodoptera litura* Larvae

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The insecticidal action of pyridalyl at various dosages against *S. litura* larva was observed. Larvae treated with 100 ng/larva and higher dosages were killed within 6 hr without any conspicuous symptoms. In contrast, larvae treated with 25 ng/larva and lower dosages showed unique symptoms similar to scar burns at the site treated with pyridalyl after molting. Such symptoms caused interference with metamorphosis, suggesting that pyridalyl would suppress populations of *S. litura* even at lower dose rates. Taking such unique insecticidal symptoms into consideration, it is suspected that pyridalyl has a novel biochemical mode of action. © Pesticide Science Society of Japan

Keywords: pyridalyl, insecticidal activity, *Spodoptera litura*, Lepidoptera.

INTRODUCTION

Pyridalyl (experimental code: S-1812) is a novel synthetic insecticide discovered at the Agricultural Chemicals Research Laboratory of Sumitomo Chemical Co. Ltd.¹⁻³⁾ The compound has a novel chemical structure not related to any other existing insecticides and shows high insecticidal activity against various lepidopterous larvae.⁴⁾ No cross-resistance was found in *Plutella xylostella* or *Heliothis virescens* between pyridalyl and synthetic pyrethroids, organic phosphates or benzoylphenylureas. Pyridalyl showed excellent control of a *P. xylostella* population resistant to various insecticides in field tests. While pyridalyl is highly active against target pests, its impact on various important beneficial arthropods is minimal.^{4,5)} These characteristics suggest that pyridalyl would be a useful new compound for managing insecticide resistance and for integrated pest management in crop

protection.

While insecticidal activities of pyridalyl against various lepidopterous pests and beneficial arthropods have been reported, details of the insecticidal action of the compound in target insects have not been adequately described. Observation of the insecticidal action of and symptoms caused by pyridalyl could provide for the efficient use of the compound in insect control and elucidation of its mode of action. In this paper, we describe symptoms produced by the compound at various dosages in larvae of *S. litura*, the major target pest of pyridalyl, and discuss the insecticidal mechanism.

MATERIALS AND METHODS

1. Chemicals

A technical grade sample (98%) of pyridalyl was synthesized by the Agricultural Chemicals Research Laboratory of Sumitomo Chemical Co., Ltd. (Fig. 1).

2. Insects

The Sumitomo Chemical colony of a susceptible strain of *Spodoptera litura* larvae were reared on an artificial diet (Nihon Nosan K.K.) at 25–27°C and 60% RH with a photoperiod of 16 : 8 (L : D). Fifth-instar larvae (40–50 mg body weight) within 20 hr after ecdysis were used in the experiments.

3. Bioassays

A topical application method was used to evaluate the toxicity of pyridalyl. The technical grade pyridalyl was applied directly to the dorsum of the larva in a 1 µl drop of acetone. The treated larvae were reared on an artificial diet. In the assay, five to six different dosages were used on 30 larvae. Larvae were maintained under conditions of 25°C, 60% RH and 14 : 10 (L : D) after treatment. The control group was treated with the solvent alone. Mortality was determined daily until 3 days after treatment. Larvae that lost elasticity and showed only weak responses when their tails were pinched with tweezers were regarded as dead. The dose-response data were subjected to a probit analysis,⁶⁾ and the toxicity was assessed with LD₅₀ values (95% F.L.).

Acute and sub-acute effects at selected dosages were determined in separate tests. In both tests, 30 larvae were treated in the manner described above. In the acute test, the mortality was determined every hour until 6 hr after treatment. In the sub-acute test, each treated larva was reared individually on an artificial diet and symptoms were recorded daily until 5 days after treatment. In addition, percentages of individuals turning into sixth-instar

Fig. 1. Structure of pyridalyl.

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Table 1. Insecticidal activity of pyridalyl against larvae of *Spodoptera litura*

Days after treatment	LD ₅₀ (95% FL ^a) ^b	Slope
1	57.3 (50.5–65.2)	7.75
2	50.1 (41.9–60.9)	3.79
3	24.4 (18.9–32.4)	1.87

^a 95% fiducial limits. ^b LD₅₀ values are expressed in ng/larva.

larvae, pupae and adults were recorded at 4, 14 and 28 days after treatment, respectively.

RESULTS

1. Insecticidal Activity of Pyridalyl

The LD₅₀ value and slope of the regression line decreased gradually until 3 days after treatment (Table 1). Mortality was not determined later than 4 days after treatment because most larvae in the control group molted and turned into sixth-instars at 4 days after treatment, while some larvae in the treatment with 25 ng/larva and lower doses were apparently killed by the sub-acute effects of pyridalyl as described later. Based on the LD₅₀ value (24.4 ng/larva) calculated from the mortality at 3 days after treatment, we used ≥ 50 ng/larva and ≤ 25 ng/larva as the dosages to determine acute and sub-acute symptoms, respectively.

2. Acute Symptoms of Pyridalyl at Higher Dosages in Larvae of *Spodoptera litura*

In the treatment at 100 ng/larva, dead larvae first appeared at 2 hr and the mortality reached 100% at 6 hr after treatment (Fig. 2). In the treatments at 200 and 400 ng/larva, dead larvae first appeared at 1 hr and the mortality reached 100% at 3 and 2 hr after treatment, respectively. Although no mortality was seen at 50 ng/larva at 6 hr after treatment in this experiment, more than half of the larvae tended to die within 3 days after treatment as suggested in Table 1. The larvae intoxicated with pyridalyl showed typical symptoms of flaccid paralysis. No hypercontraction, convulsions or vomiting, but decreasing vigor, mobile activity and body elasticity were observed.

3. Sub-Acute Symptoms of Pyridalyl

Symptoms in the larvae treated with lower dosages of pyridalyl are shown in Fig. 3. Until 1 day after treatment, no conspicuous change occurred in larvae treated with 1.56, 6.25 and 25 ng of pyridalyl. In most larvae treated with 1.56 and 6.25 ng/larva of pyridalyl, the dorsum, which had pyridalyl applied to it, turned darker at 2–3 days after treatment and the area where pyridalyl was applied had a unique symptom similar to a burn scar after molting at 4 days after treatment (Figs. 3B, C). Symptoms in the larvae treated with 25 ng of pyridalyl are shown in Fig. 3D. The symptoms at 3 days after treatment were similar to those expressed in Figs. 3B and C before molting. Such larvae surviv-

Fig. 2. Time course of mortality in larvae treated with pyridalyl topically at 50, 100, 200 and 400 ng/larva.

Fig. 4. Effect of sub-lethal dosages of pyridalyl on metamorphoses. Percentages of individuals turning into sixth-instar larvae (L6), pupae and adults were recorded at 4, 14 and 28 days after treatment, respectively.

ing at 3 days after treatment did not turn into sixth-instars, but rather, turned black and died within 5 days after treatment.

The percentage of individuals that reached the sixth-instar stage at 4 days after treatment, pupated at 14 days after treatment, and emerged at 28 days after treatment was lower at higher dosages (Fig. 4). With pyridalyl treatment at 1.56, 6.25 and 25 ng/larva, some larvae failed to achieve ecdysis and died at 5 days after treatment and later (Fig. 5). Some pupae at 1.56, 6.25 and 25 ng/larva developed abnormal shapes and failed to emerge as adults (Fig. 6).

DISCUSSION

Pyridalyl is an insecticide with a novel chemical structure that is different from any other existing insecticide class. Its insecticidal actions observed in *S. litura* larvae were unique, as described above. As suggested by the LD₅₀ values and slopes which decreased with days after treatment, the speed of the insecticidal action apparently depended on the treatment dosage. At dosages between 100 and 400 ng/larva, the treated larvae died within several hours with no conspicuous symptoms such as vomiting or convulsions. In contrast, it took more than 3 days for the larvae to die at dosages of 6.25 and 25 ng/larva and unique symptoms occurred in these insects. Most individuals treated with 6.25 and

Fig. 3. Development of *S. litura* larvae in the control group (A) and sub-lethal symptoms caused by lower dosages of pyridalyl (B, C, D). Each larva was photographed 1 to 5 days after treatment (left to right). The larvae in the control group (A) developed normally. B, C and D show symptoms (circled area) occurring in larvae treated with pyridalyl at 1.56, 6.25 and 25 ng, respectively. The larvae treated with 25 ng of pyridalyl turned black and died 5 days after treatment (D).

Fig. 5. Larva failing to achieve ecdysis at 4 days after treatment with 6.25 ng of pyridalyl. The gut appeared at a break in the epidermis caused by incomplete chitin formation at a site treated with pyridalyl.

25 ng of pyridalyl could not survive to the adult stage due to a failure to metamorphose normally as a sub-acute effect. This suggests that pyridalyl has a wide range of effective dosages against *S. litura* larvae. Such symptoms were also observed in the larvae of other lepidopterous insects such as *Helicoverpa armigera*, *Heliothis virescens* and *Spodoptera exigua*. In other words, pyridalyl may suppress populations of target pests under

Fig. 6. Normal pupae in the control (left) and pupae of abnormal shape (right) at 14 days after treatment with 6.25 ng of pyridalyl.

practical conditions even when the target insects are exposed to lower dosages of the compound.

The biochemical mechanisms of pyridalyl's insecticidal action have not been identified to date. However, the unique symptoms described above strongly suggest that pyridalyl has a novel mode of action. Similar symptoms to those described above, such as abnormalities in larvae and decreased mobility, have been

reported for validoxylamine A^{7,8)} and diafenthiuron.⁹⁾ Each of these substances is known as an inhibitor of trehalase^{7,8)} and of mitochondrial ATP synthesis,^{9–12)} respectively. In both cases, it was suggested that interference with cell function caused by a disruption of energy metabolism was the cause of such symptoms. Although the results from this study did not conclusively show that pyridalyl interferes with cell function, it would be worthwhile to investigate its effect on cell function in a future study.

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