Vol. 3 农药学学报 No. 2
2001年6月 CHINESE JOURNAL OF PESTICIDE SCIENCE 55～59

Investigation on Antifeeding and Ovipositing-repelling Activity of the Plant *Corydalis sheareri* against Two Lepidoptera Insects

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Abstract Liquid-liquid partitioning and column chromatography were applied, respectively, to separate chloroform extract of the whole plant of *Corydalis sheareri* to find antifeeding substances for insect control in cabbage field. The benzene fraction of the liquid-liquid partitioning was found to be the most active against the larvae of *Plutella xylostella* and *Pieris rapae*, with the rate of antifeeding, at ×50 dilution, of 97.7% and 97.3%, respectively, after 12 h exposure. The fraction also showed high egg-repelling activity to the adults. More pure fractions were isolated by silica gel column chromatography. The most active one had 93.0% antifeeding rate at ×200 dilution towards *Pieris rapae*. The chemical constituents of the above-mentioned extracts were discussed based on the published data.

Key words *Corydalis sheareri*; Insect; Antifeeding; Ovipositing repelling

Use of agrochemicals such as pesticides is indispensable for crop protection. However, it has become instrumental to many environmental hazards. For ensuring crop production as well as the protection of the environment, search for alternative to these synthetic pesticides is continuing all over the world. Plants are considered to be one of the potential alternative sources for developing new pesticidal chemicals. *Corydalis sheareri*, a perennial tuberous herb growing profusely in China and many other region of the northern hemisphere, has been used in Asian folk medicine as a febrifuge and analgesic for a long time[1]. Although some alkaloids possessing insecticidal activities, separated from other species of the genus *Corydalis* were reported recently by Miyazawa *et al.*[2,3] and Popovic *et al.*[4], they seemed to be lipophobic. This paper reported the investigation of the antifeeding and ovipositivity-repelling activity of the lipophilic extracts from one species of the genus, *C. sheareri*, against two important insect pests viz. *Plutella xylostella* L. and *Pieris rapae* L.

1 Materials and Methods

1.1 Collection and processing of plant

Samples of the whole plants of *C. sheareri* (stems, leaves and roots) were collected from the *Baoshu* mountain located near West Lake, Hangzhou, Zhejiang province, P. R. China during the vegetative stage of the plant in December, 1999. Plants were air dried under shaded condition for a few days followed by drying in oven at 40 ℃ for 24 h. The dried material was powdered in a grinder to yield 2.75 kg of powdered plant.

1.2 Solvent extraction

The powdered plant material (2.5 kg) was extracted with chloroform for 12 h using Soxhlet apparatus to yield 112.5 g of concentrated crude extract designated as E_4.

1.3 Fractionation of chloroform extract (E_4)

1.3.1 Liquid-liquid partitioning A portion of the chloroform extract (E_4, 12.5 g) was dissolved in acetone and transferred to a separateory funnel. Water was added up to the maximum solubility limit and the mixture was partitioned successively with petroleum ether, benzene, chloroform and ethyl acetate. The different solvent fractions thus obtained were designated as F_1(2.4 g), F_2(1.2 g), F_3(0.3 g) and F_4(0.1 g). They were evaporated and diluted with the ratio of 1∶50 (w/v) for bioassay.

1.3.2 Column chromatography The remaining portion of the chloroform extract (E_4, 100 g) was subjected to column chromatography over silica gel (60~100 meshes). The extract dissolved in a minimum volume of chloroform was mixed with silica gel and the solvent was allowed to evaporate. The mixed material was then transferred to a glass column (length 75 cm and 4 cm in diameter) partially filled with silica gel in petroleum ether. The column was then successively eluted with solvents of increasing polarity viz. petroleum ether, petroleum ether/benzene (1∶1), benzene, benzene/chloroform (1∶1), chloroform, chloroform/methanol (9∶1), chloroform/methanol (1∶1) and methanol. Elution of 1.0 liter each was collected separately, concentrated and monitored by thin layer chromatography (TLC) for presence of chemical constituents.

1.4 Bioassay for Antifeeding Activity

The 4th instar larvae of *Plutella xylostella* L. and the 3rd instar larvae of *Pieris rapae* L. were used for bioassay. The larvae were placed in a glass beaker 3 cm in diameter and 2 cm in depth. Each container held 5 worms. Cabbage leaf was cut into a round piece 1 cm in diameter and dipped for 10 s in the extracts which were dissolved in a mixture of chloroform and methanol (9∶1). After drying the leaf was put in a beaker to feed the worm. The control group was fed with leaf treated with the solvent only. The beaker was kept at 22 ℃. The leaf was changed at suitable time when the one for the control group was almost eaten. A measuring paper was used to estimate the leaf area to be eaten.

For testing the ovipositing repelling effect, about 100 adults of *Plutella xylostella* were put in a cage of 20 cm × 20 cm × 40 cm in size. The cabbage leaves, which were spread with the extract, were placed in the center of the cage to allow the adults to oviposit on it. The control leaves were treated with the solvent only. The adults were fed with water and honey during the exposure. The leaf was changed every day to count the number of eggs oviposited on it.

1.5 Data analysis

F Test and then Duncan's SSR Test were performed to see if the fractions separated were statistically different in their activities.

2 Results and Discussion

The results of bioassay of different fractions of liquid-liquid partitioning are presented in Table 1. Among the 5 fractions tested F_2 was the most active, followed by E_4, F_1, and F_3. The last three, however, were the same from statistical point of view. Table 1 also shows the rate of antifeeding remained the same statistically at 12^{th}, 24^{th}, 36^{th}, and 48^{th}. But the rate was significantly lower at 60^{th}. This was probably because the sense of hungry in prolonged exposure partly counterweighed the antifeeding effect of the extracts. In order to confirm the impact of different fractions on development, the larvae, which had undergone the 60-hour exposure, were fed with fresh cabbage leaves.

The rates of pupation and mortality as time proceeded were recorded in Table 2. Comparing the data in Table 2 with that in Table 1, one can conclude that the rate of mortality is in directly proportional, while the rate of pupation is in inversely proportional, to the rate of antifeeding. It is also clearly indicated in Table 2 that the extracts could delay progress of the pupation.

Table 1 Time-related antifeeding activity for fractions of liquid-liquid partitioning of *Corydalis sheareri* against *Plutella xylostella** (×50)

Exposure hour	Chloroform (E_4)	Petroleum ether (F_1)	Benzene (F_2)	Chloroform (F_3)	Ethyl acetate (F_4)	Mean
12	79.2±14.3	71.7±16.4	97.7±1.9	77.8±3.8	16.7±9.1	68.6^{A}
24	81.1±5.3	69.4±20.1	92.6±4.8	71.1±9.4	30.6±21.0	69.0^{A}
36	74.9±3.8	66.9±8.9	88.8±4.9	68.7±4.9	28.4±19.3	65.5^{A}
48	68.5±10.9	73.9±20.1	83.1±13.7	75.0±15.9	12.4±35.1	62.6^{A}
60	48.3±23.8	55.0±46.2	86.8±7.9	13.2±33.5	-8.18±31.7	39.0^{B}
Mean	70.4^{B}	67.4^{B}	89.8^{A}	61.1^{B}	16.0^{C}	

Note: *Data is expressed as mean ± *SD* of rate of antifeeding (%) of four replications. Data with the same letter represents the values that are statistically the same ($P = 0.01$).

Table 2 Rate of pupation and mortality of *Plutella xylostella* after 60 h exposure to fractions of liquid-liquid partitioning of *Corydalis sheareri* (×50)

Test hour		Check	Chloroform (E_4)	Peroleum ether (F_1)	Benzene (F_2)	Chloroform (F_3)	Ethyl acetate (F_4)
48	Pupation (%)	95	42	50	16	55	70
	Mortality (%)	0	5.2	10	16	20	5
96	Pupation (%)	95	68	60	16	50	75
	Mortality (%)	5	16	10	26	35	20
120	Pupation (%)	95	68	70	58	60	75
	Mortality (%)	5	63	25	37	35	25
148	Pupation (%)	95	68	70	58	60	75
	Mortality (%)	5	32	30	42	40	25

Since F_2 proved to be the most active fraction, a further test was made to compare its antifeeding activities for the larvae of *P lutella xylostella* and *P ieris rapae*. The antifeeding potential of F_2 was almost the same at ×50 dilution for the larvae of both *P lutella xylostella* and *P ieris rapae*. With the concentration decreasing, the activity dropped quickly for the *P lutella xylostella*, while it decreased slowly for the *P ieris rapae* (Table 3). So F_2 seemed to be much more effective for the larvae of *P ieris rapae*.

F_2 had an obvious ovipositing-repelling activity for the adult of *P lutella xylostella*. The activity remained the same for at least two days and then declined slightly at 3rd day. Whether the decline was due to degradation of the residual extract on the leaves or to adaptation of the adults to the extract needs further exploration.

Table 3 Sensitivity of the larvae of *P lutella xylostella* and *P ieris rapae* to F_2

Test species	Concentration (*w*/*v*)			
	×50	×100	×200	×500
P lutella xylostella	97.7±1.9	25.6±10.9	6.40±9.2	-2.7±4.3
P ieris rapae	97.3±2.2	/	87.6±11.2	69.3±14.8

*Data is expressed as mean±*SD* of rate of antifeeding (%) of four replications after 12 h exposure.

Table 4 Ovipositing-repelling effect of F_2 (×50) for adult of *P lutella xylostella*[*]

Time of sampling	24th hour	48th hour	72nd hour
Rate of antifeeding (%)	86.6 ± 4.2^{A}	86.5 ± 5.4^{A}	76.9 ± 4.5^{B}

[*] Data is expressed as mean ±*SD*. Data with the same letter represents the values are the same statistically ($P = 0.01$).

Partition of E_4 by column chromatography gave 5 groups of relatively purified materials. With increasing polarity, they were C_1 (4.5 g), C_2 (5.0 g), C_3 (8.5 g), C_4 (3.5 g) and C_5 (2.5 g). Antifeeding activity of C_1 to C_5 is shown in Table 5. C_1, C_3 and C_4 were the same statistically, and C_2 was significantly lower than C_1～C_3. A relatively oily fraction (C_1) and one or two relatively polar fractions (C_3, C_4) were separated by the column chromatography.

Table 5 Antifeeding activity of the different column fractions of chloroform extract of *Corydalis sheareri* against *P ieris rapae*[*]

Column fractions	Dilution (*w*/*v*)	Test hour	Rate of antifeeding (%)
C_1	1∶200	18	74.6 ± 13.6^{A}
C_2	1∶200	18	17.5 ± 47.3^{B}
C_3	1∶200	18	90.8 ± 15.5^{A}
C_4	1∶200	18	93.0 ± 11.3^{A}
C_5	1∶200	18	55.3 ± 18.7^{AB}

[*] Data is expressed as mean±*SD* of rate of antifeeding (%) of four replications. Data with the same letter represent the values are the same statistically ($P = 0.01$).

A comparison of the TLC behavior of F_2, C_3 and C_4 revealed a common chemical constituent occurring in all these fractions, while C_1 is a different compound. Miyazawa *et al.* [3] separated four protoberberine alkaloids from *C. bulbosa*, which were proved to be active against *Drosophila melanogaster*. They were (-)-tetrahydroberberine (**1**), (-)-tetrahydrocoptic (**2**), (+)-corydaline (**3**), (±)-tetrahydropalmatine (**4**) and (±)-dehydrocorydaline (**5**) as its iodide tetrahydropalmatine was also found to be active against larvae of *Spodoptera littoralis*[4]. All the above-mentioned compounds were extracted by methanol, while our previous study (unpublished) indicated that the methanol extract from *Corydalis sheareri* was inactive for *Plutella xylostella* and *Pieris rapae*. This suggests that the fractions separated in the present study might be different from those got from other researches. Further purification and identification of the active compound will be continued. Attempt will also be made to develop a suitable method for extraction of this plant for use as a potent pesticidal resource.

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珠芽尖距紫堇 Corydalis sheareri 对两种鳞翅目昆虫拒食和拒产卵活性的初步研究

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摘　要　分别用液—液分配法和柱层析法对珠芽尖距紫堇 *Corydalis sheareri* 全株的氯仿粗提物进行了分离, 以求获得对甘蓝害虫有拒食活性的物质。液—液分配法中, 苯提取物的拒食活性最高, 在 50 倍稀释浓度下, 小菜蛾 *Plutella xylostella* 和菜粉蝶 *Pieris rapae* 幼虫对其拒食率分别为 97.7% 和 97.3%。苯提取物还显示了很高的拒产卵活性。通过硅胶柱层析获得了相对较纯的提取物, 其中活性最高的成分在 200 倍的稀释浓度下对菜粉蝶的拒食率为 93.0%。根据现有资料对上述分离物的化学本质进行了探讨。

关键词　珠芽尖距紫堇; 昆虫; 拒食; 拒产卵