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RESEARCH ARTICLE

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Inhibitory effects of alkaloids from *Sophora alopecuroids* on feeding, development and reproduction of *Clostera anastomosis*

Abstract Alkaloids from *Sophora alopecuroids* were bioassayed with *Clostera anastomosis* for their antifeedant and growth inhibitory effects. The antifeedant rate in choice test reached 62%–86% at the dose 2.5 mg/mL, while in non-choice bioassay the rate was only 20%–29%. In choice bioassay, the antifeedant rate increased with larval instars of *C. anastomosis* and did not in non-choice experiment. The alkaloids also imposed a strong influence on the growth of *C. anastomosis* larvae, i.e., after feeding on the leaves treated with alkaloid, the larvae lost their weight, weight gain, and relative growth rate (RGR) significantly when compared with the controls. In the second day after treatment with the dose at 10 mg/mL of the alkaloid, the RGR reduced by 39.8%, and the food intake and the feces weight were respectively 57.7% and 57.4% of the controls. The approximate digestibility (AD) increased significantly, and the efficiency in converting digested food (ECD), and the efficiency in converting ingested food (ECI) decreased greatly after feeding the treated leaves. Moreover, the eggs laid per female were also inhibited by this alkaloid. The significance and prospect of the alkaloids in controlling forest insect pests were also discussed.

Keywords *Clostera anastomosis*, alkaloids, *Sophora alopecuroids*, antifeedant effect, growth, food utilization rate

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1 Introduction

Clostera anastomosis is one of the disastrous insect pests, which feeds on plants in Salicaceae and Betulaceae. It mainly occurs in northern, northeastern and northwestern of China. The outbreak of the pest was recorded in the Inner Mongolia and other Provinces (Li, 1996; Wang et al., 1998; Li et al., 2000). Larvae feed on the leaves, and make the trees greenish looking in the morning and yellowish in evening. Only leaf stalks were left on the damaged trees. The growth rate and the timber value of the defoliated trees were seriously reduced.

C. anastomosis infected poplar forests often in company with *Micromelalopha troglodyta*, another important Salicaceae insect pest, and their unexpected outbreaks were observed in Jiangsu, China. Chemical control is the only way to deal with *C. anastomosis* (Yang et al., 2003). However, application of chemical insecticides have been restricted, because of their "3R" problems, which is resistance in target insect, resurgence of the target insect pests, and residue in environments. Thus, the most urgent task for scientists in the fields of plant protection is to find new insecticides that are safer to the environments, more effective to the target insect pests, and less toxic to non-target animals. Attention has been paid to seek plant secondary metabolites for insecticides, since they were safer to environments, human, animals, and crops and the resistance of the pest to these products developed slower. In the past years, screening new insecticides from plants has been highlighted. The special chemical structures of those natural products will provide a new model for novel insecticide.

Sophora alopecuroids is a shrub plant in Fabaceae, distributed mainly in semi-desert and desert areas, such as Ningxia, Gansu, Xinjiang in China and Tibet. It is abundantly available in the above areas and it is very useful in medicine and has been recorded in Chinese Medicinal Herb Pharmacopoeia. More than 20 alkaloids were isolated and identified from *S. alopecuroids* by Russian scientists (Zhong, 1983). Eleven ones have also been isolated in

China. In China, the pesticide activities of *S. alopecuroids* alkaloids have been screened extensively (Zhao, 1980, 1994, 1996, 1999; Luo et al., 1997a, 1997b; Zhao, 1980; 1998; Zhao and Grant, 1998; Zhao et al., 1998; Zhao and Jiang, 1999; Xia et al., 2001). For example, by using 0.21 g/tree aloperine to treat 6–7-year-old *Pintus thunbergii* infected by *Bursaphelenchus xylophilus*, the survival rate was 93.8%, and the control was only 20% (Zhao, 1996). The national patent for technique was approved. The alkaloid, aloperine is the first natural compound used in field for control pine wilt disease. Aloperine was also very toxic to the aphid, Lipaphis erysim community structure of the insect changed greatly before and after spraying the solution of the alkaloid (Chen, 2001; Liu et al., 2002). Moreover, aloperine affected the activities of enzymes, such as carboxyl esterase, and other physiological indices, for example, the respiration rate (Luo et al., 1997a, 1997b).

The toxicities of the active compounds of *S. alopecuroids* have been extensively reported, but there are only a fewer studies on antifeedant activity, repellent activity, and growth inhibitory effects. The present authors studied the antifeedant and growth inhibitory effects of *S. alopecuroids* alkaloids on *C. anastomosis* preliminarily to explore *S. alopecuroids* alkaloids as natural pesticides.

2 Materials and methods

2.1 Materials

Alkaloids were isolated from *S. alopecuroids* from Ningxia and Inner Mongolia, the isolated methods were referred to by Zhao (1980). Analysis by Waters HPLC and C_{18} column (Li et al., 2000) indicated that the contents of the primary alkaloids were: sophocarpine 22%, matrine 17%, sophoramine 5.5%, sophoridine 51%. Leaves of *Poplus* × *eur*. cv. I-7/58 and *C. anastomosi* were were collected from the campus of Nanjing Forestry University. The test did not begin until the insects were reared in laboratory to the fourth generation. The larvae used in the test were selected in their same instar and should be physiologically healthy.

2.2 Antifeedant effects

2.2.1 Choice test

Solutions of *S. alopecuroids* alkaloids were prepared as 0.625, 1.25, 2.5, 5.0, and 10 mg/mL. Fresh leaf discs (2 cm×2 cm) were immersed in solutions for 5 s, with distilled water as control. After air dried, the treated leaf squares and the control squares were placed in a Petri dish (diameter 9 cm), which was lined at the bottom with a moist filter paper. Three larvae starved for 3 h were inoculated and the dish was sealed with a piece of plastic membrane. The dish then was put in an incubator (28±1°C). For each solution replicated 10 times. After 24 h, the leaf areas fed were measured, and the antifeedant rate and AFC_{50} were calculated according the following formula:

$$AFR = (S_{CK} - S_T)/(S_{CK} + S_T) \times 100\% \quad (1)$$

where AFR is the antifeedant rate, S_{CK} is the leaf area fed in control, S_T is the leaf area treated. The abbreviations are also used in the following formulas.

2.2.2 Non-choice test

Five solutions of alkaloids were prepared as 2.5, 5.0, 10, 20, and 40 mg/mL, and the leaves were treated as in section 1.2.1, one leaf disc was put in a Petri dish (diameter 6 cm), and one 3–5 instar larva, which had been starved for 3 h was inoculated into the Petri dish and it was sealed with a piece of plastic membrane, and cultured in an incubator (28±1°C, light:dark=12:12). Each solution was replicated 30 times. After 24 h, the leaf areas fed were measured, and the antifeedant rate and AFC_{50} were calculated by the following formula:

$$AFR = (S_{CK} - S_T)/S_{CK} \times 100\% \quad (2)$$

2.3 Larval growth and food utilization

Four fresh leaf discs (3 cm×4 cm) were immersed in 2.5 and 10 mg/mL alkaloid solutions for 5 s, using distilled water as control. After air dried and weighted, the leaf discs were put into a diameter 9 cm Petri dish. Then the third instar larvae starved for 3 h was weighted and inoculated into the dish and the dish was sealed with a piece of plastic membrane. Each solution replicated 10 times. The leaves were replaced each day, and the larvae, the food left, and the feces were also weighted until the insects pupated.

Larval food intake was calculated as:

Food intake each day = (The food given each day – the food left the second day)×(1 – the water lost) (Li et al., 1998).

Food utilization was calculated as:

$$RGR = \Delta G/(G \times T),\ AD = (A - B)/A \times 100\%,\ ECI = \Delta G/A \times 100\%,\ ECD = \Delta G/(A - B) \times 100\% \quad (3)$$

where RGR is the relative growth rate, AD is the approximate digestibility, ECI is the efficiency of ingested food, ECD is the efficiency of conversion of digested food, ΔG is the body weight gain, G is the mean of body weight, T is the feeding time, A is the food intake and B is the feces quantity.

2.4 Oviposition and adult longevity

Leaves were treated as in section 1.2.1, where one leaf disc was put in a Petri dish (diameter 6 cm), one third instar larva starved for 3 h was inoculated and sealed with a piece of plastic membrane, cultured in an incubator (28±1°C, light:dark=12:12). After 24 h, the leaf was replaced by untreated one and reared to pupation. After mating, adults

were placed in a diameter 9 cm Petri dish lined at the bottom with moist filter paper, calculating the eggs laid and adult longevity.

3 Results

3.1 Antifeedant effects of *S. alopecuroids* alkaloids on the larvae of *C. anastomosis*

Both in choice and non-choice tests, great antifeedant effects of different alkaloid solutions on *C. anastomosis* larvae were observed and quite different between each solution, the higher the concentration the greater the antifeedant rate (Table 1). In choice test, the AFC_{50} for third and fourth instar larvae was 2.1 and 0.67 mg/mL, respectively, while in non-choice test, the AFC_{50} for third, fourth, and fifth was 5.0, 6.6, and 6.3 mg/mL, respectively. At the same concentration, the antifeedant rate in choice test was higher than that in the non-choice test. In choice test, the antifeedant rate was greater at larger instar larvae, and this tendency was not observed in the non-choice test.

Table 1 Antifeedant effects of different concentrations of alkaloids on larvae of *C. anastomosis*

Concentrations /(mg·mL^{-1})	Antifeedant rate in choice test /%		Antifeedant rate in non-choice test /%		
	3rd instar	4th instar	3rd instar	4th instar	5th instar
0.625	19.25±9.79 a	39.71±22.54a	-	-	-
1.25	27.49±6.28 a	72.12±8.14 b	-	-	-
2.5	62.12±10.66 b	85.97±6.11 b	29.0±2.85a	20.73±3.48a	22.17±2.42 a
5	79.25±6.31b c	89.83±5.52 b	48.31±3.82b	39.46±1.75b	41.40±2.20 b
10	89.08±3.57 c	93.30±3.34 b	70.06±2.93c	66.95±1.56c	69.21±2.04 c
20	90.59±3.99 c	98.85±1.15 b	90.90±2.53d	82.88±1.68d	83.58±1.45 d
40	-	-	96.10±1.39d	91.35±0.91e	88.25±1.42 d

The data in the table denote mean±s.d. and those followed by the same letter indicate no significant difference at the level of 0.05 by Duncan's multiple range test. The same is for the following tables.

3.2 Effect of *S. alopecuroids* alkaloids on the larval growth and food utilization of *C. anastomosis*

3.2.1 Effects on larval growth

Great inhibitory effects on larval growth of *C. anastomosis* were observed, the body weights, the net increase of body weights, and relative growth rate of the larvae feeding on treated leaves were quite lower than those of control (Table 2). Treated with 10 mg/mL alkaloid concentration, the RGR decreased by 39.8% the following day; the body weight decreased by 25.54% and 37.82% on the following and the fourth day, respectively; the net increase of body weight decreased by 60.6% on the fourth day, all compared with the control larvae. Reared with 2.5 and 10 mg/mL alkaloid solutions, the larvae mean body weight was decreased by 12.28% and 31.12%, the net increase of body weight was decreased by 23.73% and 50.17% compared with the control larvae, respectively.

Table 2 Effect of different concentrations of alkaloids on the growth of *C. anastomosis* larvae

Time after treatment /day	Concentrations /(mg·mL^{-1})	Larval weight /mg	Weight gain /mg	RGR /(mg·mg^{-1}·day^{-1})
2	2.5	69.36±4.11 a	28.36±4.18 a	0.409±0.06 ab
	10	55.74±2.89 b	15.34±2.81 b	0.275±0.05 a
	CK	74.77±2.91 a	34.17±2.84 c	0.457±0.038 b
3	2.5	95.67±2.87 a	26.31±3.02 a	0.275±0.032 a
	10	78.01±3.77 b	22.27±2.89 a	0.286±0.037 a
	CK	103.56±4.34 a	28.79±3.29 a	0.278±0.032 a
4	2.5	136.64±6.42 a	38.97±8.25 a	0.289±0.061a
	10	101.54±4.12 b	23.56±3.13 b	0.232±0.031 a
	CK	163.29±6.39 a	59.80±7.65 c	0.366±0.047 a
Mean	2.5	100.56±19.58	31.21±3.92	0.324±0.043
	10	78.43±13.22	20.39±2.55	0.264±0.017
	CK	113.87±26.07	40.92±9.57	0.367±0.052

3.2.2 Effect on food utilization

Effect on larval food utilization between various alkaloids was quite different, and food intake decreased with the increased concentrations (Table 3). The food intake the following day was only 57.7% of that of the control when the larval fed on leaves treated with the 10 mg/mL alkaloid, and at the peak of the feed activity, the food intake was only 61.4% of that of the control on the fifth day.

The feeding dynamics of larvae fed on leaves treated with lower alkaloid concentration was similar to that of the control, whereas at higher concentrations, the dynamics increased and decreased slowly (Fig. 1a).

Table 3 Effect of different concentrations of alkaloids on food intake by *C. anastomosis* larvae

Concentrations /(mg·mL^{-1})	Food intake /(mg·head^{-1})					
	2 day	3 day	4 day	5 day	6 day	Mean
2.5	68.15±8.35a	124.72±10.56a	187.40±19.71a	305.85±35.14a	122.46±16.83ab	161.72±14.55
10	44.6±5.46b	80.34±7.39b	109.81±8.21b	203.41±22.59b	91.78±8.33a	105.99±9.20
CK	77.51±5.18a	126.48±9.29a	213.68±6.59a	331.38±44.21a	162.75±32.90b	182.20±16.69

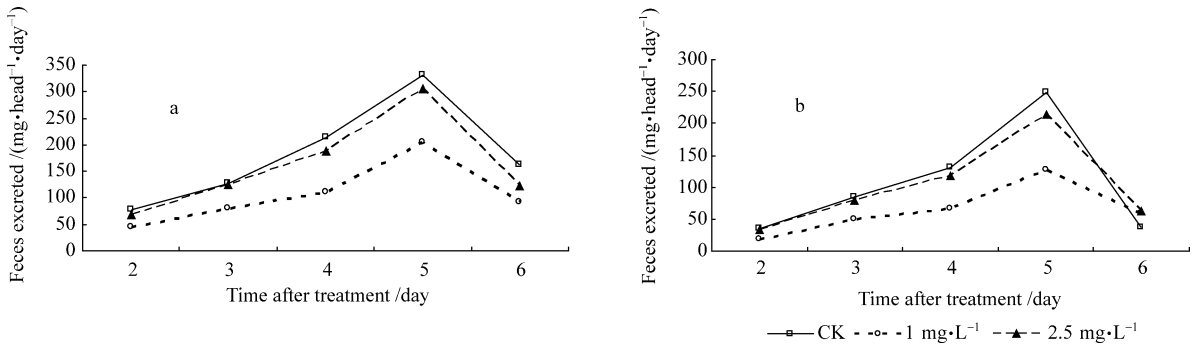

Fig. 1 Dynamics of food intake (**a**) and feces excretion (**b**) of *C. anastomosis* larvae treated with different concentrations of alkaloids

3.2.3 Effect on feces quantity

The variation of feces quantity after feeding on different alkaloid treated leaves was similar to that of the larval food intake (Table 4). After treated, with larval development, the quantity of feces increased, and on the fifth day reached the peak, and on the sixth day began to decrease rapidly. Higher concentration treated feeding dynamics increased and decreased slowly (Fig. 1b)

Table 4 Effect of different concentrations of alkaloids on feces excretion of *C. anastomosis* larvae

Concentration /(mg·mL^{-1})	Feces excreted /(mg·head^{-1})					
	2 day	3 day	4 day	5 day	6 day	Mean
2.5	32.68±4.20 a	79.63±10.48 a	117.35±12.61 a	213.05±28.65 a	61.67±16.18 a	100.88±11.40
10	17.87±2.12 b	48.95±6.57 b	66.75±6.05 b	126.93±19.65 b	58.75±11.40 a	63.85±6.82
CK	35.11±2.87 a	85.31±7.53 a	131.46±11.72 a	248.96±33.88 a	36.67±24.56 a	107.50±14.13

3.2.4 Effect on larval food consumption and utilization

Treated with alkaloids, larval food consumption and utilization varied with the time (Table 5). On the following day, treated with high concentration of alkaloids, the *AD* increased obviously, while the *ECI* and *ECD* decreased apparently; however, there was no such change on these indices when the concentration of alkaloids was low. On the fourth day after high concentration treatment, *AD* and *ECD* were not different between the treatment and the control, but there was a difference in *ECI*. On the third day treated with high concentration alkaloids, *AD* was quite higher than that of the control, but *ECI* and *ECD* were not different between treatment and the control. On the fourth day treated with lower concentration alkaloids, both *ECD* and *ECI* were lower than those of the control, but no differences were observed in *AD*.

ECD and *ECI* are important indices that indicate the food quality. After feeding on the food treated with alkaloid, both *ECD* and *ECI* decreased, and larval growth inhibited. *AD* both in treatment and control were lower, but on the second and third day after treatment, the *AD* of both the lower treatment and the control were statistically different compared with the *AD* treated with higher concentration

alkaloids, indicating larval food consumption and ingestion in lower concentration treatment were better than those in higher concentration treatments. This was also indicated by higher *ECD* and *ECI* in lower concentration treatment and control.

Table 5 Feeding by larvae of *C. anastomosis* after treatment with different concentrations of alkaloids

Time after treatment /day	Concentrations /(mg·mL^{-1})	*AD* /%	*ECI* /%	*ECD* /%
2	2.5	52.56±0.94 a	40.43±1.95 a	77.32±4.05 a
	10	59.48±1.85 b	32.01±2.58 b	54.20±4.49 b
	CK	54.98±1.14 a	43.80±1.48 a	79.90±2.98 a
3	2.5	37.75±3.75 a	22.90±3.36 a	59.58±6.79 a
	10	40.88±3.12 b	27.73±2.69 a	68.79±5.91 a
	CK	33.17±1.34 a	22.64±1.99 a	68.46±5.33 a
4	2.5	37.75±2.74 a	18.74±2.99 a	51.86±7.60 a
	10	39.63±2.63 a	22.02±2.96 ab	57.59±9.13 ab
	CK	38.54±2.24 a	27.29±2.03 b	71.24±4.13 b
Mean	2.5	42.55±2.01	27.36±2.35	69.72±4.04
	10	46.66±2.22	27.25±1.71	60.19±3.96
	CK	42.23±1.95	31.24±1.98	73.20±2.54

3.3 Effect of *S. alopecuroids* alkaloids on adult longevity and oviposition of *C. anastomosis*

The eggs laid per female treated with alkaloids were quite fewer than that of the control (Table 6). The eggs laid after feeding on 10 mg/mL-treated leaves were only 53.95% those of the control, but 83.55% of the control in higher concentration treatment (40 mg/mL), and increased by 29.6% compared with the 10 mg/mL treatment. This was largely the strong repellent effect of higher concentration alkaloid (Table 1), and little leaves were fed and little alkaloid was taken into the alimentary canal to accumulate high toxicity. After 24 h, the treated leaves were replaced with fresh untreated leaves, and the larvae rapidly recovered from treated states. The effect of *S. alopecuroids* alkaloids on adult longevity is similar to that on oviposition. The longevity was only 7.8 day at 2.5 mg/mL treatment, and there was no differences between the high concentration treatment (40 mg/mL) and the control.

Table 6 Effect of different concentrations of alkaloids on female oviposition and life-span of of *C. anastomosis* adult

Concentration /(mg·mL^{-1})	Life-span of adult /day	Number of eggs laid /femal[①e]
CK	10(30)	304(15)
2.5	7.8(30)	257(13)
5	9(28)	218(14)
10	8(29)	164(15)
20	9(27)	186(14)
40	10(29)	254(15)

①The values in brackets are the number of head tested

4 Discussions

The results of this study indicated that the alkaloids from *S. alopecuroids* imposed great inhibitory influences on the larval feeding, food utilization, larval growth, and adult oviposition of *C. anastomosis.*

Feeding leaves treated with the alkaloids resulted in decreasing food intake, and the antifeedant effects were observed in both the choice and non-choice tests. In the choice test, the older the larval instars, the stronger the repellent effects, because larger larvae had high movable ability and could find untreated food (the control leaves); while in non-choice test, the older the larvae, the weaker the repellent effect, because under the condition of no choice for food those larger larvae had more tolerance to starvation. The results of this investigation showed that regardless of the larval instars, the antifeedant effects of *S. alopecuroids* alkaloids on *C. anastomosis* were evident, indicating their great potential for insect pest management.

S. alopecuroids alkaloids inhibited the food utilization of *C. anastomosis* and limited its growth. Adequate food supply and full utilization are necessary for larval growth and development. If not, many negative effects will appear in insect life cycle, such as small body size, less competitive ability, and low reproductive rate, and so on. All those will lead to the population decline of *C. anastomosis*. Our experiments were conducted in laboratory, and further field studies are necessary, for example, to find out how long the effects of the alkaloids will last. Most natural insecticides from plants have lower stabilities and last for a short time at field circumstances. Tree trunk injection technique could be considered to apply such insecticides, for example, the patented tree trunk injector 6HZ–0503 invented by Prof.

Zhao Boguang of Nanjing Forestry University, China can inject high volumes of insecticides with high pressure.

Larvae of *C. anastomosis* fed on leaves treated with alkaloid showed reduction of egg production resulted from low food utilization, so that it affected its egg formation.

In literature, it was reported that *S. alopecuroids* alkaloids could affect the activities of several esterase and respiration (Zhao, 1994, 1996), but it is needed to do more tests in detail. Zhang and Luo (2002) showed that these alkaloids had synergistic effects with other chemical insecticides on insects. The present study indicated that after feeding on leaves treated with the alkaloids, the food intake of larvae, feces quantity, *ECI*, and *ECD* decreased. It resulted in the inhibition of larval growth and development. It is necessary to undertake a detailed study to clarify the mechanisms that alkaloids of *S. alopecuroids* affect the *ECI* and *ECD*.

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