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Preparation of Plant-based Insecticide Chamaejasmin Soluble Liquid and Its Biological Activity against *Aphis craccivora* and *Pieris rapae*

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Chamaejasmin is the mixture of many natural insecticidal ingredients from the ethanol extract of *Stellera chamaejasme* root. 1,5-Diphenyl-1-pentanone is one of the major insecticidal components. Chamaejasmin soluble liquid was prepared and its insecticidal activity against *Aphis craccivora* and *Pieris rapae* and its stability were evaluated. Results showed that it had good insecticidal activity and good stability. Its median lethal concentration (LC_{50}) values (24 h after treatment) against *Aphis craccivora* and *Pieris rapae* were 26.9 and 38.6 mg L^{-1}, respectively. The results of analysis of variance (ANOVA) indicated that after the prepared soluble liquid was stored at 54 ± 2 °C for 14 d or at room temperature for 12 months, its insecticidal activity had no significant change. The content of 1,5-diphenyl-1-pentanone in the soluble liquid was 0.39 %.

Key Words: Insecticidal activity, Preparation, Soluble liquid, *Stellera chamaejasme*.

INTRODUCTION

Pieris rapae is a vegetable pest with serious harm. Over the past decades, synthetic insecticides have been used for its control. However, the development of resistance to these synthetic insecticides has reduced the efficacy of insecticide treatment[1]. Similarly, aphids, including *Aphis craccivora* and *Aphis gossypii*, are important pests in agriculture. In recent years, these aphids have also developed resistance to conventional synthetic insecticides including pyrethroids[2,3]. Furthermore, their scope of resistance continues to expand and has included many new organophosphorate and carbamate insecticides. For these reasons, many studies have focused on the possibility of using biopesticides of microbial and botanical origin to control these pests because they usually have special and various modes of action so that it is not easy for pests to develop resistance to them[4]. Moreover, they have low mammalian toxicity and little impact on non-target organisms and the environment[5,6]. Therefore, they may become substitutes of synthetic insecticides in the near future.

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Stellera chamaejasme L. (family Thymelaeaceae) is a good insecticidal plant. Various insecticidal components, including 1,5-diphenyl-1-pentanone, an aphicide[7], have been isolated from it[8-10]. These active ingredients may exert their toxicity by several different modes of action[11-13]. Besides, they may degrade in the natural environment, so their use will not bring about pollution and residues. The aim of the present study is to develop a new insecticidal preparation based on chamaejasmin so that the insecticidal advantages of *Stellera chamaejasme* may be fully utilized.

EXPERIMENTAL

Ethanol, glycol, dimethyl sulfoxide, acetophenone, cinnamaldehyde and NaOH were of analytical grade. Methanol was of chromatography grade. Tween-80 was of chemical pure grade. Omethoate was 400 g L^{-1} emulsifiable concentrate (EC). β-Cypermethrin was 25 g L^{-1} emulsifiable concentrate.

Stellera chamaejasme was collected from the Ruoergai grassland in Sichuan Province (China) and its identity was confirmed by related experts. *Aphis craccivora* was reared for generations in the laboratory. It was maintained at 22-24 °C, 60 % relative humidity (RH) and 14:10 h light:dark photoperiod. Vigorous and apterous adult aphids were used in the experiments. *Pieris rapae* was reared for generations in the laboratory. It was held at 24-26 °C, 75-95 % relative humidity and 12:12 h light:dark photoperiod. Third-instar larvae were used in the experiments.

Synthesis of 1,5-diphenyl-1-pentanone: 1,5-Diphenyl-1-pentanone was synthesized according to the reactions shown in Fig. 1. Acetophenone (A) was mixed with cinnamaldehyde (B). NaOH (40 %) was added to the mixture. The reaction mixture was stirred at room temperature for 12 h and then filtered. The solid product obtained was washed with distilled water and recrystallized with anhydrous EtOH to obtain a yellow crystal (C). It was dissolved in anhydrous EtOH. Catalyst Pd-C was added to the solution. Hydrogen was blown into the solution for 8 h at room temperature. The mixture was filtered. The filtered liquid was concentrated under reduced pressure to obtain a solid product. The product was chromatographed in a silica gel column (30 cm × 3 cm) and was eluted with methanol + water (80 + 20 by volume) to obtain a white solid (D) (1,5-diphenyl-1-pentanone).

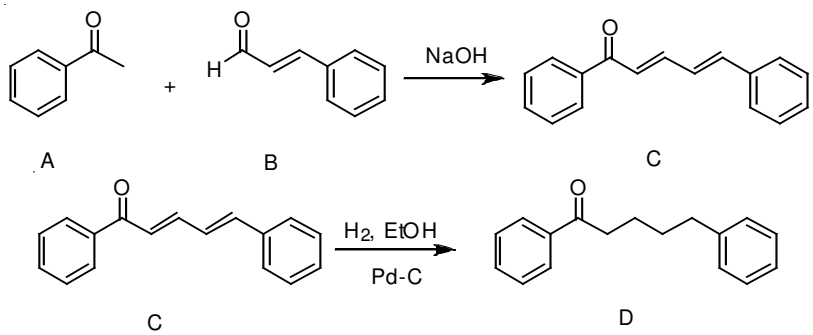

Fig. 1. Synthesis of 1,5-diphenyl-1-pentanone

High performance liquid chromatography (HPLC): A Shimadzu LC-10A liquid chromatograph (Shimadzu, Japan) with a Tianhe Kromasil C_{18} column (200 mm × 4.6 mm, 5 μm) and a SPD-10A UV detector at 239 nm was used to determine the content of 1,5-diphenyl-1-pentanone in the soluble liquid, with methanol + water (79 + 21 by volume) as the eluting solvent, at a constant flow of 1 mL min^{-1} and an injection volume of 10 μL. Quantification was performed with the standard curve method, using the synthesized 1,5-diphenyl-1-pentanone as the standard.

Extraction of chamajasmin: The root powder of *Stellera chamaejasme* was extracted with anhydrous ethanol at 50 °C for 10 h. The extracting liquid was concentrated under reduced pressure. The extract obtained was placed in a vacuum drier to remove the remaining solvent and to obtain chamajasmin extract (brown solid). Its yield was about 11 % from the dry root powder.

Preparation of chamaejasmin soluble liquid: Since the crude extract is somewhat soluble in water, it can be formulated as a microemulsin[14] or a soluble liquid. According to the hydrophilic-lipophilic balance value of surfactants and the formulating principle of soluble liquid[15,16], preliminary experiments were done to select suitable solvents, cosolvents and surfactants. Then, orthogonal tests were carried out to obtain the best combination of the crude extract, solvents, cosolvents and surfactants. Preliminary experiments and orthogonal tests were done according to the following procedures. The crude extract was mixed with solvents and cosolvents and then stirred at 50 °C until the solid particles completely disappeared and the solution became turbid. Surfactants were slowly added to the solution until the solution became clear and transparent. The soluble liquid obtained was stored at room temperature for 60 d and its appearance was observed. The concentration of 1,5-diphenyl-1-pentanone in the prepared soluble liquid was determined by HPLC.

Determination of pH value of soluble liquid: A PHS-25 acidometer was used to measure the pH value of the prepared soluble liquid.

Test of emulsion stability: The prepared soluble liquid (1 mL) was placed in a graduated glass cup. Water (99 mL) with standard hardness (342 mg L^{-1}) was added to the solution[17]. The mixture was stirred. The graduated glass cup containing the solution was placed in a water bath at 30 ± 2 °C for 1 h. The appearance of the solution was observed.

Test of thermal stability: The prepared soluble liquid was sealed in a glass bottle and left in an electric oven at 54 ± 2 °C for 14 d[17]. Its insecticidal activity and pH value as well as the content of 1,5-diphenyl-1-pentanone were determined.

Test of storing stability: The prepared soluble liquid was sealed in a glass bottle and left at room temperature for 12 months[17]. Its insecticidal activity and pH value as well as the content of 1,5-diphenyl-1-pentanone were determined.

Bioassay of *Aphis craccivora*: The prepared soluble liquid and omethoate were diluted into five different concentrations with distilled water, respectively. Broad bean leaves with *Aphis craccivora* were picked. Vigorous and apterous adult aphids were left on the leaves by selection. The leaves with aphids were dipped in the

solutions for 5 s[18]. The leaves were allowed to air-dry. The aphids on them were transferred into Petri dishes with fresh broad bean leaves. The Petri dishes were placed in the laboratory held at 22-24 °C, 60 % relative humidity and 14:10 h light:dark photoperiod. Mortality was recorded 24 h after treatment. Control groups were treated only with distilled water. The experiments for each concentration were replicated three times. Percentage mortality was corrected according to the formula of Abbott[19] and LC_{50} values were calculated with the statistics package for the social sciences (SPSS) based on probit analysis[20]. Means were separated using the least significance difference (LSD) test at $p = 0.05$.

Bioassay of *Pieris rapae*: The prepared soluble liquid and β-cypermethrin were diluted into five different concentrations with distilled water, respectively. Twenty third-instar larvae of *Pieris rapae* were selected from cabbage leaves. They were dipped in the solutions for 5 s within a small cage[18]. They were placed on filter paper to absorb the surplus solutions. Then, they were transferred into Petri dishes with fresh cabbage leaves. The Petri dishes were placed in the laboratory held at 24-26 °C, 75-95 % relative humidity and 12:12 h light:dark photoperiod. Mortality was recorded 24 h after treatment. Control groups were treated only with distilled water. The experiments for each concentration were replicated three times. The following procedures were the same as for the bioassay of *Aphis craccivora*.

RESULTS AND DISCUSSION

Preparation of chamaejasmin soluble liquid and its stability: The result of preliminary experiments was that DMSO (solvent), glycol (cosolvent) and Tween-80 could be used to formulate the soluble liquid. Through the orthogonal tests, the best formulation of the soluble liquid was obtained: crude extract (15 %), glycol (10 %), Tween-80 (15 %) and DMSO (60 %) (Table-1). The soluble liquid obtained was homogenous and transparent. Its emulsion stability was qualified[17]. It had good thermal and storing stability (Table-2). The content of 1,5-diphenyl-1-pentanone was 0.39 %.

TABLE-1
ORTHOGONAL TEST RESULTS OF PREPARING CHAMAEJASMIN SOLUBLE LIQUID [$L_9(3^4)$]

Extract (%)	Glycol (%)	Tween-80 (%)	DMSO (%)	Total (%)	Storage at room temperature for 60 d
12	12	15	61	100	Qualified*
15	12	12	61	100	Sediment
18	12	18	52	100	Sediment
12	10	18	60	100	Qualified*
15	10	15	60	100	Qualified*
18	10	12	60	100	Turbidity
12	8	12	68	100	Qualified*
15	8	18	59	100	Turbidity
18	8	15	59	100	Phase separation

*The prepared soluble liquid was transparent, devoid of sediment, floating oil and phase separation.

TABLE-2
THERMAL AND STORING STABILITY OF CHAMAEJASMIN SOLUBLE LIQUID

Tests		pH Value of soluble liquid	Content of 1,5-diphenyl-1-pentanone in soluble liquid (%)
Storage 14 d at 54 ± 2 °C	Before storage	6.13	0.39
	After storage	6.09	0.37
	Decomposition rate	–	5.10
Storage 12 months at room temperature	Before storage	6.13	0.39
	After storage	6.10	0.38
	Decomposition rate	–	2.56

Insecticidal activity against *Aphis craccivora*: The prepared soluble liquid was subjected to laboratory bioassay. The results are shown in Table-3. It showed good insecticidal activity against *Aphis craccivora*. Its LC_{50} value was 26.9 mg L^{-1}. The results indicated that the correlation was significant between concentration and efficacy. The correlative coefficient was 0.9715. ANOVA results revealed that after the prepared soluble liquid was stored at 54 ± 2 °C for 14 d or at room temperature for 12 months, its insecticidal activity showed no significant difference compared with the activity before storage. Chi square test (χ^2) demonstrated that the results were reliable.

TABLE-3
INSECTICIDAL ACTIVITY OF CHAMAEJASMIN SOLUBLE LIQUID AGAINST *Aphis craccivora*

	Omethoate (40 % EC)						Chamaejasmin soluble liquid				
Concentration* (mg L^{-1})	1000	500	250	125	62.5	(1)‡	250	125	62.5	31.3	15.6
						(2)¶	250	125	62.5	31.3	15.6
						(3)§	250	125	62.5	31.3	15.6
Mortality† (%)	87.4	64.8	50.1	35.7	22.5	(1)	93.7A	83.3B	65.1C	52.2D	39.9E
						(2)	92.4A	81.7B	63.9C	52.1D	39.3E
						(3)	92.3A	81.2B	64.0C	5.20D	39.7E
Regression equation (Y = aX + b)	Y = 1.4778X + 1.5191					(1)	Y = 1.4259X + 2.9608				
						(2)	Y = 1.3753X + 3.0155				
						(3)	Y = 1.3555X + 3.0429				
LC_{50} (mg L^{-1}) (95 % CL)	226.7 (194.7 – 262.8)					(1)	26.9 (20.1 – 33.8)				
						(2)	27.7 (22.6 – 32.9)				
						(3)	27.8 (22.7 – 32.9)				
Correlation coefficient (r)	0.9772					(1)	0.9715				
						(2)	0.9698				
						(3)	0.9711				
χ^2	4.547					(1)	1.864				
						(2)	3.107				
						(3)	3.404				

Means within a column followed by the same capital letter are not significantly different ($p > 0.05$; LSD) between treatments. *The content of crude extract for chamaejasmin soluble liquid. †Based on the mean of triplicates corrected according to Abbott formula. ‡Before storage. ¶After 14 d storage at 54 ± 2 °C. §After 12 months storage at room temperature.

Insecticidal activity against *Pieris rapae*: As shown in Table-4, the prepared soluble liquid had good insecticidal activity against *Pieris rapae*. Its LC_{50} value was 38.6 mg L^{-1}. The results of regression and correlation analyses indicated that the correlation were significant between concentration and efficacy. The correlative coefficient was 0.9667. As for the results of *Aphis craccivora*, ANOVA results showed that after the prepared soluble liquid was stored at 54 ± 2 °C for 14 d or at room temperature for 12 months, its insecticidal activity had no significant difference compared with the activity before storage. χ^2 test also showed that the results were reliable.

TABLE-4
INSECTICIDAL ACTIVITY OF CHAMAEJASMIN SOLUBLE LIQUID AGAINST *Pieris rapae*

	β-Cypermethrin (2.5 % EC)						Chamaejasmin soluble liquid				
Concentration* (mg L^{-1})	80	40	20	10	5.0	(1)‡	250	125	62.5	31.3	15.6
						(2)¶	250	125	62.5	31.3	15.6
						(3)§	250	125	62.5	31.3	15.6
Mortality† (%)	98.3	88.3	70.0	50.0	35.0	(1)	93.3A	76.7B	60.0C	46.7D	26.7E
						(2)	91.7A	75.0B	60.0C	45.0D	26.7E
						(3)	91.7A	76.7B	58.3C	45.0D	26.7E
Regression equation (Y = aX + b)	Y = 1.8887X + 3.1815					(1)	Y = 1.6216X + 2.4268				
						(2)	Y = 1.5588X + 2.5021				
						(3)	Y = 1.5798X + 2.4663				
LC_{50} (mg L^{-1}) (95 % CL)	9.2 (7.1 – 11.3)					(1)	38.6 (29.6 – 48.3)				
						(2)	40.0 (30.5 – 50.4)				
						(3)	40.2 (30.8 – 50.4)				
Correlation coefficient (r)	9.0803					(1)	0.9667				
						(2)	0.9627				
						(3)	0.9623				
χ^2	1.865					(1)	1.296				
						(2)	0.831				
						(3)	0.808				

Means within a column followed by the same capital letter are not significantly different ($p > 0.05$; LSD) between treatments. *The content of crude extract for chamaejasmin soluble liquid. †Based on the mean of triplicates corrected according to Abbott formula. ‡Before storage. After 14 d storage at 54 ± 2 °C. §After 12 months storage at room temperature.

Chamaejasmin soluble liquid was a newly developed insecticidal preparation. The above results have clearly demonstrated that it had good insecticidal activity and good thermal and storing stability. Although its insecticidal activity against *Pieris rapae* is lower than β-cypermethrin, it has no unpleasant odor and its adjuvant is devoid of volatility, poison and harm. Moreover, its insecticidal matrix may degrade by itself in the environment without pollution and residues.

On the other hand, *Stellera chamaejasme* is very abundant in China and its active components are easily extracted. Therefore, chamaejasmin soluble liquid is

a pest control agent with potential value. It may represent a new type of bio-rational insecticides with high efficacy, low toxicity and safety to non-target organisms. It can be expected that it may substitute some existing synthetic insecticides and will be used in some areas in the future so that many side effects caused by synthetic insecticides may be reduced or eliminated.

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