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INSECTICIDAL ACTIVITY OF CRUDE EXTRACT OF THE SEEDS OF *MILLETTIA PACHYARPA* BENTH. ON THE LARVAE OF *PIERIS RAPAE* LINNE

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Abstract

The purpose of the study was to measure the insecticidal activity of crude extract of the seeds of *Millettia pachycarpa* Benth. The method for extracting the crude extract of the seeds of *M. pachycarpa* was conventional extract approach with three kinds of organic solvents: methanol, ethanol and acetone. The method of measuring the contact activity of the crude extracts against *Pieris rapae* Linne was the leaf immersion method in a Petri dish. The method of control effect of the crude ethanol extract against the larvae of *P. rapae* was the field measurement method in a cabbage field. The results showed that 0.2 mg/ml crude extracts of the seeds of *M. pachyarpa* extracted with methanol, ethanol, acetone solvents had demonstrable contact activity against cabbage caterpillar and killed 65, 89 and 52 per cents larvae, respectively. The crude ethanol extract doses, *viz*., 10, 100, 200, 500 and 1000 mg/ml were applied in the field and the larval mortalities on the 2nd day were 32, 38, 67, 68 and 69 percents, respectively and on the 7th day were 64, 74, 93, 87 and 87 per cents, respectively.

Introduction

Millettia pachycarpa Benth. (synonym *M. taiwaniana* Hayata) is a perennial climbing shrub (Agarwal 2003, Ye *et al*. 2014). It is one of the most well known among the approximately 150 species of *Millettia*, as it is widely used in traditional practices like agricultural pesticides, blood tonics, fish poison, and treatments for cancer and infertility (Kang *et al*. 2003, Wang *et al*. 2013, Mo *et al*. 2014). The bark fiber is used for making strong ropes (Agarwal 2003, Perry and Metzger 1980). Chinese traditional medicine has widely used a suspension of the finely ground seeds as an insecticide (Eisenberget *et al*. 2009). A 5% water suspension of the seeds is as effective as 0.1% benzene hexachloride spray when used against *Oides decempunclata.* The suspension is also more potent than phenothiazine against *Pieris rapae* (Chiu 1950). The dried powder of the root is also effective against bean aphids (Prakash and Rao 1997). When the rotenoids - including rotenone from the root - are isolated, there is little doubt of their insecticidal properties (Singhal *et al*. 1982, Ye *et al*. 2008). Rotenone is valued as an easily degradable soil and plant pesticide (Yang *et al*. 2008, Zhou *et al*. 2013) .

The larvae of the cabbage butterfly, *Pieris rapae* Linne, (Lepidoptera, Pieridae), is one of the most common pests found in cruciferous vegetables. The cabbage caterpillar causes major damage to cruciferous vegetables, especially kale, cabbage, cauliflower (Yang *et al*. 2014).

The goal of the research outlined in this paper was to explore the potential uses of *M. pachycarpa* in pollution-free prevention and control of vegetable pests, *P. rapae*.

Materials and Methods

Fresh seeds of *M. pachycarpa* were collected from Mount Emei, Sichuan province, China in July, 2014. The seeds were dried in a draught drying cabinet at 50°C, crushed with a pulverizer,

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sieved through a 40 mesh sieve. 50 g of the resulting seed powder was added to 100 ml each of the three kinds of organic solvents: methanol, ethanol and acetone. This seed powder was soaked for 24 hrs, then extracted for half an hour in a tissue blender. Then, 100 ml of corresponding solvent were added to the crude extract. Extraction was made twice for half an hour each. All the extracts were combined, filtered, and concentrated by evaporation under reduced pressure. A yellow thick crude extract was formed; it was weighed and diluted into 0.02 g/ml as the mother liquor using acetone as solvent dilution. The crude extracts of *M. pachycarpa* with the solvents were named methanol-, ethanol-and acetone extracts, respectively.

The cabbage caterpillars of *P. rapae* were collected from the field. They were fed in with cabbage leaves as food the laboratory. The healthy 4^{th} instar larvae developed from rearing were used for the test.

The contact toxic effects of the crude extracts of the seeds of *M. pachyarpa* on the larvae of *P. rapae* were measured by the leaf immersion method in a Petri dish. Each crude extracts was diluted with sterile water into 0.2 mg/ml solution. The cabbage leaves with larvae of *P. rapae* were dipped into the prepared crude extract dilutions for five seconds, then removed. Excess liquid on the cabbage leaves with larvae of *P. rapae* was removed with a piece of absorbent paper, then the cabbage leaves were put on a moisturizing dish to feed the larvae. A sterile water replication was tested along with the crude extract as a control. Each treatment was repeated five times including the control, using 20 cabbage caterpillars in each replication. The numbers of larvae survived were recorded after the samples were left for 24 hrs in a moisturizing dish. Mortality and corrected mortality of the larvae were calculated as follows.

Mortality (per cent) = (The numbers of larvae tested – the numbers of larvae lived)/the numbers of larvae tested × 100.

Corrected mortality (per cent) = (Treatment mortality – control mortality)/(1 – control mortality) × 100.

The insecticidal experiment of the crude extracts was carried out in a cabbage field in a vegetable base in Emei Mount City. The test soil on which cabbages were grown was loamy, with medium fertility, uniform cultivation conditions, and evenly growing cabbage. The crude extracts with the acute contact toxic effects obtained in the laboratory test were selected for control effects testing in the field. Five concentrations (including 1000, 500, 200, 100, and 10 μg/ml) of the crude ethanol extract was used. Sterile water was also tested as a control treatment. Each dose was repeated three times. Each experimental plot area was 20 m^2. The crude extracts were sprayed uniformly on the surface of cabbage leaves. Then, fixed-point line inspection was carried out. The number of larvae survived was counted on the 2nd and 7th days of extracts spraying. Death rate and corrected mortality were calculated following the above equations for both testing days.

Results and Discussion

The crude extract of the seeds of M. *pachyarpa* (0.2 mg/ml) extracted with methanol, ethanol and acetone were bioassayed as contact activeity against the cabbage caterpillar *P. rapae* (Table 1). The cabbage caterpillars did not die in the control treatment of sterile water. The mortality rates of methanol-, dry ethanol-, and acetone extracts were 65, 89, and 52%, respectively after 24 hrs in a moisturizing dish. The contact action effect of the ethanol crude extract against the larvae was higher than the other two crude extracts tested.

Various concentrations of the ethanol crude extracts of the seeds of *M. pachyarpa* had good control effects against the larvae of *P. rapae* in cabbage fields (Table 2). The average correction control effects of 200, 500, and 1000 μg/ml concentrations of ethanol crude extracts against the larvae of *P. rapae* were 66.87, 68.05, and 69.43 per cent, respectively after 2 days of crude extract

spraying. The average correction control effect of the three concentrations (200, 500, 1000 μg/ml) of the ethanol crude extract was significantly higher than that of the other two concentrations (10 and 100 μg/ml). After 7 days of spraying crude extracts, the average correction control effect against the larvae of *P. rapae* in all concentrations of the ethanol crude extracts had greatly improved compared to 2 day spraying. The average correction control effect of 200 μg/ml

Table 1. The contact toxic effects of 0.2 mg/ml crude extracts from seeds of *M. pachyarpa* on the larvae of *P. rapae.*

Treatments		Replications					Mean ± Sd
		1	2	3	4	5	
The number of larvae tested		20	20	20	20	20	20.0 ± 0.0
Methanol extracts	No. of larvae died	12	13	14	14	12	13.0 ± 1.0
	Death rate (%)	60	65	70	70	60	65.0 ± 5.0 bB
Ethanol extracts	No. of larvae died	17	17	19	18	18	17.6 ± 0.8
	Death rate (%)	85	85	95	90	90	89 ± 4.2 aA
Acetone extracts	No. of larvae died	8	11	12	11	10	10.4 ± 1.5
	Death rate (%)	40	55	60	55	50	52 ± 7.6 cC
CK	No. of larvae died	0	0	0	0	0	0 ± 0.0
	Death rate (%)	0	0	0	0	0	0 ± 0.0 dD

In the same column, subscripts denote a significance level of 5%, while superscripts denote a significance level of 1%.

Table 2. The effects of the ethanol extracts of the seeds of *M. pachyarpa* on the larvae of *P. rapae* in fields (n = 3 for each dose concentrateion).

Dose μg/ml	Number of larvae tested (Mean ± SD)	2 day			7 day		
		No. of larvae survived (Mean ± SD)	No. of larvae died (%) (Mean ± SD)	Correction control effect (%) (Mean ± SD)	No. of larvae survived (Mean ± SD)	No. of larvae died (%) (Mean ± SD)	Correction control effect (%) (Mean ± SD)
10	26.67 ± 1.53	21.00 ± 1.00	27.02 ± 5.58	31.99 ± 6.37 bB	12.33 ± 0.57	53.57 ± 4.88	63.92 ± 3.79 dC
100	26.33 ± 1.52	17.33 ± 0.58	34.11 ± 1.91	38.01 ± 1.80 bB	9.00 ± 1.00	65.80 ± 3.69	73.42 ± 2.87 cB
200	27.33 ± 1.53	9.00 ± 1.00	66.87 ± 5.44	66.87 ± 5.44 aA	2.67 ± 0.58	90.24 ± 2.13	92.42 ± 1.65 aA
500	26.67 ± 2.52	9.00 ± 0.00	66.04 ± 3.23	68.05 ± 3.08 aA	4.67 ± 0.58	82.52 ± 0.95	86.42 ± 0.74 bA
1000	25.67 ± 2.08	8.33 ± 0.58	67.51 ± 0.73	69.43 ± 0.69 aA	4.33 ± 1.15	83.21 ± 3.86	86.96 ± 3.00 bA
Water control	21±1.00	22.33±1.53	–6.28±2.43		27.00 ± 1.00	–28.69 ± 5.42	

concentrations of the ethanol crude extract was 92.42%, which was significantly higher than the other four concentrations. The average correction control effects of 1000, 500, 100, and 10 μg/ml are 86.96, 86.42, 73.42 and 63.92 per cent, respectively.

Crude extract of *M. pachycarpa* with an organic solvent extraction was shown to have an insecticidal effect against the larvae of *P. rapae*. The is because the seeds of *M. pachycarpa* contain many insecticidal activity compounds, including rotenone. The contact toxic effects of methanol-, dry ethanol-, and acetone extract reference the larvae of *P. rapae* were strong in moisturizing dishes, and the average mortality rate of the larvae of *P. rapae* was more than 50 per cent.

All of the tested concentrations of the ethanol crude extracts of the seeds of *M. pachyarpa* demonstrated good control effects against the larvae of *P. rapae* in cabbage fields. The average correction control effect against the larvae of *P. rapae* in a 200 μg/ml concentration of ethanol crude extract was 66.87 per cent after 2 days of application. At this point, there was no significant difference between the mean control effects of 500 and 1000 μg/ml concentrations of the ethanol crude extract (68.05 and 69.43) respectively, although these values were significantly higher than those of 10 and 100 μg/ml concentrations of ethanol crude extract. After 7 days of application, the average correction control effect against the larvae of *P. rapae* of 200 μg/ml concentration of the ethanol crude extracts was 92.42 per cent, which was significantly higher than that of other concentrations of crude extracts. Considering the effect of cabbage caterpillar prevention and medication costs, we recommend using a 200 μg/ml concentration of the ethanol crude extract for field control of the larvae of *P. rapae*.

Previous research has indicated that the field correction control effect of 7.5 per cent rotenone oil 800 and 1000 times dilution after 7 days of spraying rotenone dilution was 83.8 and 81.2%, respectively (Yang *et al*. 2014). The field correction control effects of three concentrations (including 200, 500 and 1000 μg/ml) of the ethanol crude extract of the seeds of *M. pachyarpa* after 7 days of spraying were 92.42, 86.42, and 86.96 per cent, respectively. Therefore, the ethanol crude extract of the seeds of *M. pachyarpa* shows strong potential for the field control of cabbage caterpillars.

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