BIOEFFICACY OF LEAF EXTRACTS FROM POUZOLZIA ZEYLANICA (L.) BENN AGAINST DIAMONDBACK MOTH PLUTELLA XYLOSTELLA IN VIET NAM

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ABSTRACT

In Viet Nam, *Pouzolzia zeylanica* (L.) Benn is a native plant and has been demonstrated its applicability as a medical plant. Additionally, *Pouzolzia zeylanica* was used to control fly larvae during food processing due to insecticidal activity. We optimized the extraction of *Pouzolzia zeylanica* by ethanol at different conditions: concentration, the ratio of solid (material) - liquid (ethanol volume) (mg/ml) and the extraction time (hour). Results indicated that extraction yield was effected by all of the factors. The optimized extraction yield was 6.85% (Y) with ethanol concentration at 96 percent ethanol (Z_1), the ratio solid to liquid is 1: 25 (mg/ml) (Z_2) and extraction time is 4 days (Z_3). We tested the efficiency of leaf extracts from *Pouzolzia zeylanica* and antifeedant activity against diamondback moth *Plutella xylostella* at different leaf extract concentrations. Results indicated that 80% mortality induced by those compounds was recorded on *Plutella xylostella* second instars at 30% leaf extract concentration and had significant difference compared to the control (P=0.0000); the leaf extract affected the ratio of pupation, adult emergence and antifeedant activity of *P. xylostella* (P=0.0000). The obtained results promise a potential of using *Pouzolzia zeylanica* as biopesticide in Viet Nam.

Keywords: *Pouzolzia zeylanica*; leaf extract, optimized extraction; diamondback moth; antifeedant.

1. Introduction

Using overdose chemical pesticide affect not only non-target organism but also human health. In Viet Nam, developing biopesticide from natural plant-derived products play an important role in sustainable agriculture. Moreover, *Plutella xylostella* is one of the most devastating insect pests of brassica crops and have developed resistance to many chemical pesticides. Therefore, increasing the efficiency of IPM program, particularly using biopesticides to manage diamondback moth (DBM) is paid attention nationally and worldwide. Many different plant species have been discovered its useful compounds that can be used as biopesticides such as *Lantana camara* L. which contain phenolics, flavonoids, alkaloids, triterpens, saponin, terpenoids, etc. have the ability to kill mosquito 3rd and 4th instars (Kalita et al. 2011). Scientist have reported leaf extract from *Elsholtzia cristata* have antifeedant to *Pieris rapae* (Nguyen Ngoc Hoa et al. 2011). The extraction of *Pouzolzia zeylanica* (L). Benn has lot of activities such as exhibiting out-standing scavenging like DPPH, ABTS and hydroxyl radicals (Li P. 2011); cytotoxic activity (Paul S. 2012).

In Asia, *Pouzolzia zeylanica* (L). Benn is a perennial herbaceous plant belong to the *Urticaceae* family. It has been a Vietnamese traditional medicine plant used as remedy of a cough, urination difficulty, bacterial infection, helminthic, etc. In addition, study indicated that *Pouzolzia zeylanica* was isolated isoflavon from cloroform extraction of

Pouzolzia zeylanica L. named 5-metoxt-4'-hydroxy-2", 2"-demitylpyrano (3", 4", 7,8) isoflavon. This isoflavon can killed 50% bacterial cell *Escherichia coli* at 32µg.ml^{-1} (Saha et al. 2012). In VietNam, *Pouzolzia zeylanica* has been tested for anti some bacteria such as *Staphylococus aureus, Escherichia coli, Streptococcus haemolyticus* (unpublished data). Beside, *P. zeylanica* was also used to control fly larvae during processing "Sauce of macerated fish" food due to traditionally insecticidal activity. The aim of this study is to optimize the extraction conditions and to evaluate the larvicidal activities of *P. zeylanica* against *P. xylostella.*

2. Materials and Methods

Plant collection

Poulzolzia zeylanica (L.) Benn was collected at Binh Duong Province. Leaves were collected, washed and then dried under sun condition for a week. Dried leaves were stored in a dry and clean place until use.

Cold-extraction

The dried leaves were ground into powder. The plant powder was soaked in ethanol in a 250 ml becher for several days at room temperature with occasional shaking. The extract was then filtered through filter papers. The filtrate obtained was evaporated in the vacuum until dried. It rendered a greenish color concentrate paste.

Determining percent yield of the extraction: extract paste was dried into unchanged weight (m_2) then calculating the yield as the following: (H%): H% = [(m_1 – m_2) / m_1]. 100%, where m_1: unchanged weight of leaves; m_2: unchanged weight of the extract

The two-level full-factorial design was used to evaluate the effect of the three main process parameters: the concentration of ethanol solvent (^{o}), the ratio of solid (material) to liquid (ethanol) (mg/ml) and the extraction time (day) (Table 1). The levels of each factor were chosen so as to cover a range of values of practical interest. With 3 factors and 2 levels for each factor, N = 2^3 = 8 experiments were done to get the practical yield of the extraction (Table 2).

Table 1

Factors and levels of the experimental design

Factor	Unit	Level	
		-1	+1
Ethanol concentration (Z1)	(^{o})	80	96
Solid to liquid ratio (Z2)	(mg/ml)	0.067	0.04
Extraction time (Z3)	Day	3	4

Table 2

Experimental design layout

Std order	Factor			Coded varibles							Yield
	Z_1	Z_2	Z_3	X_0	X_1	X_2	X_3	X_1X_2	X_1X_3	X_2X_3	Y (%)
1	96	0.04	4	+	+	-	+	+	+	+	6.85
2	80	0.067	4	+	-	+	+	+	-	-	5.75
3	96	0.067	4	+	+	+	+	-	+	-	6.03

Std order	Factor			Coded varibles							Yield
	Z_1	Z_2	Z_3	X_0	X_1	X_2	X_3	X_1X_2	X_1X_3	X_2X_3	Y (%)
4	80	0.04	4	+	-	-	+	-	-	+	6.58
5	96	0.04	3	+	+	-	-	+	-	-	6.30
6	80	0.067	3	+	-	+	-	+	+	+	4.66
7	96	0.067	3	+	+	+	-	-	-	+	5.75
8	80	0.04	3	+	-	-	-	-	+	-	5.21

To minimize the effect of variability in the response due to extraneous factors, the experiments were performed in a random order. The analysis of results was carried out using the statistical software Design Expert 7. After run data from Table 2 with DX7, the polynomial model was derived as:

$$y = \alpha_0 + \sum_{i=1}^{3} \alpha_i x_i + \sum_{i=1}^{3} \sum_{j=I+1}^{3} \alpha_{ij} x_i x_j + \alpha_{123} x_1 x_2 x_3 \quad (1)$$

The statistically significance of the model and coefficients were also determined in DX7.

Insect culture

Plutella xylostella were collected in Cu Chi, Ho Chi Minh City and were reared until second generations on leaves of *Brassica juncea* at room temperature (28±2˚C) with 16-8 L:D condition. Second instar larvae *P. xylostella* were used for experiments.

Larvicidal and pupicidal activity

Crude extracts were dissolved in methanol and made up at different treatment at 10% (NT10), 15% (NT15), 20% (NT20), 25% (NT25), 30% (NT30) with distilled water. 10 larvae/treatment were put together with a *B. juncea* in plastic box (12 cm x 17 cm x 10 cm) with netting on the top. The larvicidal activity of crude extracts of *P. xylostella* was assessed by spraying method. Tween 80 was used as an emulsifier at concentration of 0.02% (v/v). After 6 hours exposures, the dead larvae were counted and correted by using Abbott formula (Abbott, 1925) and the percentage mortality was recored from the average of three replicates. Pupicidal activity was calculated by counting dead pupae from the total larvae. Data were transformed into arcsin√(x) to reduce variance heterogeneity and analyzed using one-way analysis of variance (ANOVA) followed by Duncan.

Antifeedant activity

Larvae were starved 3-4h before the bioassay. Leaf disc choice method was used to study the antifeedant activity of the crude extracts. Fresh *B. juncea* leaf discs of 1 cm diameter were punched using cork borer and then were dipped in 10% (NT10), 15% (NT15), 20% (NT20), 25% (NT25), 30% (NT30) concentration of crude extracts individually. The leaf discs dipped in methanol 30% (DC30) were used as control. Larvae were starved 3-4 h prior to experiment then second instar larvae were introduced to plastic petridish (1.5 cm x 9 cm) which is with wet filter paper to avoid early drying of the leaf discs. Three replicates were maintained for each treatment with 5 larvae per replicate. The antifeedant activity was calculated using the formula of Caasi, 1983:

Antifeedant index = [(C0 - Ci)/ (C0)] x 100

Where "C0" is the weight of leaf disc consumed in the control and "Ci" is the weight of leaf discs consumed in the treatment

Statistical analysis

The antifeedant, larvicidal and pupicidal activities were subjected to analysis of variance (ANOVA) followed by Duncan, Statgraphics plus 3.0 software.

3. Results

The optimized condition for the extraction The analysis variance of the experiment with DX7 was showed in Table 3

Table 3

Value and p – value for model and coefficients in Equation 2

Coefficient	Effect	Coded value	Factual value	p-value
Model				<0.0001
a_0		5.890	-16.9749	
a_1	Ethanol concentration (C)	0.340	0.2209	<0.0001
a_2	Solid to liquid ratio (R)	0.340	10.1852	<0.0001
a_3	Concentration time (T)	0.410	5.8499	<0.0001
a_{13}	C-T	-0.200	-0.0509	<0.0001
a_{23}	R-T	0.069	-10.1852	0.0003

By removing the non-significant terms from the full polynomial model, the simplified expression formed:

$Y = -16.9749 + 0.2209Z_1 + 10.1852Z_2 + 5.8499Z_3 – 0,0509Z_1Z_3 - 10.1852Z_2Z_3$ (2)

With $R^2 = 1$; cv% = 0,06

A very good agreement was found between experimental and calculated yields with R-square = 1 and p-value of model < 0.0001. The cv of regression model was 0.06, this cv value indicated that the factual yields of the experiment did not have too much variation. The equation 2 also included the coefficients associated with three main factors. The p-value of coefficients measured the strength of the relationship between the factors and the response. All of the parameters C, R and T were positive sign indicated that they had positive effect on the extraction yield. It means that the extraction yield would increase as the parameters increased. The p-value of the significant coefficients have been smaller than 0.05. Additionally, it can be deduced from the result obtained that the interaction between C and T, R and T also influenced the yield significantly. Depending on the regression model, the optimized extraction yield in this situation were 6.85% with Concentration of ethanol is 96^o; Solid – liquid ratio is 0.04 (mg/ml); Extraction time is 4 days.

Larvicidal activity

The leaf extract of *Poulzolzia zeylanica* produced high mortality to the 2nd instar larvae *Plutella xylostella*. After 12h treatment, the leaf extract at 25% concentration show significant different to other treatments ($p<0.05$ was taken to be significant) and caused almost 90% mortality in the exposed larvae after 48h (Table 4). Pupicidal activity of 21% in ethanol leaf extract was recorded in 30% concentration. Adult activity was increased corresponding with the increased concentration of leaf extract (Table 5).

Table 4

Larvicidal activity of different solvent leaf extracts against 2nd instar larvae of *Plutella xylostella* at different time interval

Leaf extract concentration	% Mortality			
	6h	12h	24h	48h
NT10	17.7±15.3a	26.5±0.0c	28.6±2.0c	65±22.9a
NT15	17.7±15.3a	30.8±7.3bc	43.0±3.3ab	65±22.9a
NT20	8.9±15.3a	35.0±7.3bc	56.9±5.3ab	80±17.3a
NT25	8.9±15.3a	42.7±13.9ab	80±17.3a	90±0.0a
NT30	8.9±15.3a	50.8±0.0a	80±17.3a	90±0.0a

Data were transformed into arcsin√(x) and were analyzed using one-way analysis of variance (ANOVA) followed by Duncan. Different letters indicate statistically significant differences among groups ($p<0.05$).

Table 5

Percent pupicial activities and adult activity observed after treatment of *Pouzolzia zeylanica* against *Plutella xylostella*

Treatments	Pucidal activity (%)	Adult activity (%)
DC 30	61,95±10.4a	61,95±10.5a
NT 10	37,16±6.8b	37,16±6.8b
NT 15	37,24b±3.4b	33,02±6.3b
NT 20	28,09±10.5bc	6,15±10.6c
NT 25	23,37±8.5bc	6,15±10.6c
NT 30	21,16±4.7c	0,0±0.0c

Data were transformed into arcsin√(x) and were analyzed using one-way analysis of variance (ANOVA) followed by Duncan. Different letters indicate statistically significant differences among groups ($p<0.05$).

Antifeedant activity

Our results indicated that the crude extract of *Pouzolzia zeylanica* showed very good antifeedant activity against *Plutella xylostella*. The present investigation revealed that all the extracts exihibited feeding deterrent activity and NT 30 treatment showed maximum antifeedant activities at 73% (Table 6).

Table 6

Antideedant activity of *P. zeylanica* leaf extract agaist 2nd instar *Plutella xylostella*

Treatments	Antifeedant index (%)
DC 30	0,90e
NT 10	25,52d
NT 15	42,42c
NT 20	46,45c
NT 25	54,35b
NT 30	73,05a

4. Discussion

Pouzolzia zeylanica is the toxic and feeding deterrent to *Plutella xylostella.* Many studies have been reported the deterrent activity of plant extract against *P. xylostella* such as *Azadirachta indica* A. Juss, *Acorus calamus* L. (Areceae), *Melia azedarach* L (Meliaceae), and *Acalypha fruticosa* F. (Euphorbiaceae) (Lingathurai et al. 2011). The highest exttraction yield from leaf of plant with ethanol as a solvent in this study proved that ethanol is a suitable solvent for terpenoids extraction from plant materials.

Many plants secondary metabolites are known antifeedant and toxic to insect larvae such as triterpenes, sequiterpene lactones and alkaloids (Paul and Saha 2012). Baskar et al. (2009, 2010) reported the antifeedant activity was due to the presence of alkaloids. In Viet Nam, Phylanthin, metil stearat, ß-sitosterol -3-O-ß-D-glucopyranosid, isovitexin, vitexin and quercetin were isolated from *Pouzolzia zeylanica* (Le Thanh Thuy et al. unpublish data). Sarkar et al. 2014 also suggested the pesticidal activity of *P. zeylanica* in their study. In this study, significant insecticidal and antifeedant activities against *P. xylostella* were first observed in crude extract of *Pouzolzia zeylanica* suggest a future exploitation for the isolation of active molecules and to develop a new botanical formulation for sustainable development in Viet Nam. The results described the role of biopesticide of *P. zeylanica* on insect pest, it suggests for further test and application against other lepidopteran pests■

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