

Efficacy of three newly developed botanical insecticides based on pongam oil against *Plutella xylostella* L. larvae

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ABSTRACT

Efficacy of three botanical insecticides based on pongam oil (from *Pongamia pinnata*) and neem oil (from *Azadirachta indica*) against *Plutella xylostella* larvae was studied and the results are reported in this paper. All the tested botanical insecticides showed good efficacy against *P. xylostella* larvae. No significant effect on oviposition was found for any of the products. Application of the botanical insecticides caused high larval mortality and significantly lower damage feeding to the plants (ranging between 0 % – 50 %, depending on concentration and the insecticide used) compared to the control (90 % of the plants were damaged by feeding). The differences between individual botanical insecticide formulations were determined using estimated lethal concentrations. LC₅₀ of the insecticide based on *P. pinnata* oil alone were found to be significantly higher compared to LC₅₀ of insecticides designed as combinations of pongam oil and *Thymus vulgaris* or *Foeniculum vulgare* essential oil, where LD₅₀ was estimated as 0.58 % and 0.73 %, respectively. The efficacy of both botanical insecticides based on pongam oil and essential oil combinations was comparable to NeemAzal T/S (1% azadirachtin). Based on the results presented in this paper, product formulation based on the combination of *Pongamia pinnata* and *Thymus vulgaris* or *Foeniculum vulgare* essential oils can be recommended for protection of cabbage crops against *Plutella xylostella* larvae.

Key words: Botanical insecticides, *Plutella xylostella*, Pongam oil, Pongamia, mortality, *Thymus vulgaris*, *Foeniculum vulgare*, essential oils

INTRODUCTION

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is the most serious insect pest of cruciferous crops throughout the world (Sarfraz *et al.*, 2006). Chemical control of *P. xylostella* has become less effective because of the quick development of resistance to almost all groups of insecticides, including carbamates, organophosphates, organochlorines, pyrethroids, insect growth regulators, pyrazoles, abamectins, oxadiazines, neonicotinoids, and *Bacillus thuringiensis* Berliner, and also to newer active ingredients, including spinosad and indoxacarb (Shelton *et al.*, 1993; Moham and Gujar, 2003; Abdel-Razek *et al.*, 2006; Charleston *et al.*, 2005; Qian *et al.*, 2008; Zhao *et al.*, 2006; Shelton *et al.*, 2008). Intensive use of synthetic insecticides to control insect pests has led to many problems such as pest resistance and resurgence, negative effects on non-target organisms including humans, and

negative environmental impacts (Perry *et al.*, 1998). These effects have triggered the development of alternatives, including botanical insecticides (Prakash *et al.*, 2008; Binu Kumar, 2010; Oyedokun *et al.*, 2011; Sreelatha *et al.*, 2011).

Pongamia is a genus having one species only, i.e. *Pongamia pinnata* L. (= *P. glabra* Vent. *Derris indica* Lamk.), which belongs to the Leguminosae family (Krishnamurthi, 1969). Historically, *Pongamia* has been used as folk medicinal plant, particularly in Ayurveda and Siddha systems of Indian medicine (Meera *et al.*, 2003). All parts of the plant have been used as a crude drug for the treatment of tumours, piles, skin diseases, itches, abscess, painful rheumatic joints wounds, ulcers, diarrhea etc (Shoba and Thomas, 2001). Besides, it is well known for its application as animal fodder,

green manure, timber and fish poison. The seeds are reported to contain on an average about 28–34% oil with high percentage of polyunsaturated fatty acids (Sarma *et al.*, 2005). Pongam oil contains 5–6% flavonoids (Bringi, 1987), the main constituents being karanjin, a furano-flavonoid and pongamol, a diketone.

Many biological activities of *P. pinnata* seed extracts can be attributed to karanjin, the major flavonoid of the seed oil. Pongam oil is shown to possess insecticidal, repellent and antioviposition properties (Parmar and Gulati, 1969; Pavela and Herda 2007a, b). These pesticide effects and its environmental and health safety predetermine the use of pongam oil as a botanical insecticide for protection of plants. Three new formulations based on emulsified oil of *P. pinnata* fruits were developed. The first formulation was based on the emulsified oil itself, obtained by pressing *Pongamia pinnata* seeds. Essential oils obtained from *Thymus vulgaris* L. and *Foeniculum vulgare* Mill showed very good insecticidal efficacy in previous tests (Pavela, 2006; 2007; 2008b; 2009; Pavela *et al.*, 2009a); therefore, the formulations of the remaining two products were prepared with addition of these essential oils. Objective of our study was to determine of efficacy of three newly developed botanical insecticides and of a standard product based on azadirachtin against *Plutella xylostella* larvae.

MATERIALS AND METHODS

Plants

Cabbage, *Brassica oleracea* convar. *capitata* L. var Pandion was planted in plastic pots (10 cm diameter) in a mixture of 70% peat substrate and 20% perlite, pH 5,8 (Agro CS a.s., Česká Skalice, Czech Republic). Plants were watered and fertilized with water-soluble nutrients as required. Six week-old plants were used in the experiments.

Insect

Plutella xylostella larvae used in this study were obtained from a laboratory colony reared on cabbage plants (*Brassica oleracea capitata* L.) and maintained at room temperature (20 to 23°C) and a 16 h L : 8 h D light : dark regime. The colony, founded by larvae and pupae collected at cabbage

on the Crop Research Institute campus, had been maintained continuously for 3 years (>20 generations).

Chemicals

Pongamia pinnata fruit oil was purchased from the manufacturer (PARKER India Group Company, India). The oil was emulsified using TWEEN 85 (product formulation P1). Other product formulation variants were prepared by adding to pongam oil commercially available essential oils (Sigma-Aldrich, Czech Republic) of *Thymus vulgaris* (product formulation P2) or *Foeniculum vulgare* (product formulation P3) dosed 50 ml per 950 ml of pongam oil. The content of effective substances was estimated based on standard analyses of the raw materials by their respective manufacturers.

P1 - seed oil from *Pongamia pinnata* L. emulsified (Tween 85), which corresponds to the content of a.i. 18.5 g /l of karanjin; P2 - seed oil from *Pongamia pinnata* L. and essential oil from *Thymus vulgaris* (content of essential oil 50 g/l) emulsified (Tween 85), which corresponds to the content of a.i. 16.6 g /l of karanjin and 31.1 g/l of thymol; P3 - seed oil from *Pongamia pinnata* L. and essential oil from *Foeniculum vulgare* (content of content of essential oil 50 g/l) emulsified (Tween 85), which corresponds to the content of a.i. 16.6 g /l of karanjin and 24.6 g/l of *trans*-anethole and NA - preparation NeemAzal T/S[®] i (producer: Trifolio-M GmbH, Germany) emulsion of *Azadirachta indica* Juss. extract in plant oil, which corresponds to the content of a.i. 10.0 g/l of Azadirachtin A were maintained as treatments.

Bioassay

An aqueous emulsion of botanical insecticides was tested at concentrations of 0.0 (control with Tween 85), 0.5, 0.75 and 1.5% w/v. Treatments were laid out in a randomized block design, with 4 blocks and 10 plants/treatment/block. Plants were arranged in 2 benches where each bench (1.2 m wide and 12.0 m long) consisted of 2 blocks. Plants were spaced 0.25 m within rows and 0.4 m between rows in each treatment. Spaces between treatments and between blocks were 0.7 m and 1 m, respectively. Each plant was infested with a known number (Table1) third instar *P. xylostella*

larvae, obtained from the laboratory colony. A pre-spraying count was made one day after infestation and the plants prior to spraying. All treatments were applied at 3.4 bars (0.34 MPa) with 500 l/ha, using a SOLO 432 backpack sprayer with a solid cone swirl nozzle. Numbers of surviving larvae were recorded 6 and 12 days after treatment. Differences between numbers of larvae counted before and after treatment were assumed to result from larval mortality.

The cabbage plants were treated identically according to the methodology described in efficacy of botanical insecticides against of *P. xylostella* larvae. Always 5 plants were placed in cages (60 x 60 x 50 cm) 24 hours upon treatment. Subsequently, 60 adults, 5 – 7 days old, were released in the cages. After 2 days, the adults were removed from the cages and the numbers of eggs per plant was scored. The anti-oviposition index was calculated based on the numbers of eggs on treated and untreated plants according to the formula: AI (%) = (C-T)/(C+T)*100, where C is the number of eggs on untreated plants and T is the number of eggs on treated plants (Pavela *et al.*, 2009b). The plants were left in the cages, and the numbers of live larvae and the degree of plant damage due to their feeding activities were determined on day 16 after introduction of adults. The difference between oviposited egg numbers and the numbers of live larvae were considered as larval mortality.

The method of visual damage estimation was used in all experiments to estimate plant damage due to larval feeding, using a 0-6 scale where the percentage of damaged leaf area (DLA) was: 0, DMA < 10; 1, 10 < DMA < 25; 2, 25 < DMA < 50; 3, 50 < DMA < 75; 4, 75 < DMA < 90; and 5, 90 < DMA < 100 %. All experiments took place in an air-conditioned greenhouse at 22 ± 5 °C, humidity of 65 % - 82 %, and photoperiod of 16L:8D. Plants were watered and fertilized with water-soluble nutrients as required.

Data analysis

Mortality data, number of eggs or larvae and plant damage were subjected to a one-way ANOVA and the means were separated by applying the Tukey's HSD test (P<0.05). All the data that needed to be normalized were transformed before being analyzed. For the anti-oviposition index and larval

mortality data, percent were transformed to the arcsine square root before analysis to stabilize error variance (Gomez and Gomez, 1984). All analyzes were performed using Statistica 6.1 (StatSoft Inc. 1984-2003). Lethal concentrations (LC₅₀ and LC₉₀) including values within the 95% confidence limit (CI₉₅) were estimated using probit analysis based on mortality determined on day 12 or 16 after application in order to better compare the differences in efficacy of individual products (Finney, 1971). Before calculation, mortality was corrected according to Abbott's formula (Abbott, 1925).

RESULTS

Mortality of *P. xylostella* larvae

All botanical insecticides caused significant mortality compared to the control (p<0.05). As early as on day 6 after application, mortality for insecticides P2 and NA and for concentrations 0.75 % and 1.5 % was higher than 90 %. Mortality slightly increased in with time. On day 12 after application, the highest mortality was determined for insecticides P2, P3 and NA and for concentrations 0.75 % and 1.5 % in the range 95 % – 100 %. Product formulation with pongam oil alone (P1) showed the least efficacy where mortality of 77.5 % was determined even on day 12, also for the highest concentration (1.5 %), which is a significantly lower value (p<0.05) than upon treatment with P2 and NA in low concentration 0.5 % where mortality reached 90.4 % and 86.2 %, respectively. The different effect between insecticide formulations with pongam oil alone (P1) and other tested products (P2, P3 and NA) was manifested in the lethal dose estimation using probit analysis (Table 1). The highest LC₅₀ and LC₉₀ were estimated for P1, thus a significantly higher concentration (p<0.05) than that determined for other botanical insecticides. Lethal concentrations of botanical insecticides P2, P3 and NA showed no significant differences and ranged between LC₅₀ (0.28 % – 0.39 %) and LC₉₀ (0.51 % – 0.64%). Plant damage due to larval feeding was assessed on day 12 after application (Table 1) where the control plants showed damage higher than 90 % (4.9 points). Almost no damage and/or damage of approximately 10 % were

Table 1. The efficacy of botanical insecticides against *Plutella xylostella* larvae

Botanical insecticides	Conc (%)	N ¹ (Larvae)	Mortality ² (%)		Lethal concentration ³			Plant damage ⁴
			6 days	12 days	LC ₅₀ (CI ₉₅)	LC ₉₀ (CI ₉₅)	chi	
Pongam oil (P1)	1.5	76.2±8.7	70.4±2.3ef	75.5±2.4d	0.79	2.92	0.050	1.7±0.1d
	0.75	75.0±6.4	47.2±4.0c	51.2±1.0c	(0.63-0.95)	(2.02-3.89)		2.2±0.1 e
	0.5	77.3±7.9	38.8±5.5b	39.6±3.5b				2.8±0.1f
Pongam oil + thyme oil (P2)	1.5	75.5±6.1	99.2±0.9k	100.0±0.0g	0.31	0.51	0.002	0.0±0.0a
	0.75	73.1±13.3	95.3±1.5jk	99.6±3.5g	(0.06-0.40)	(0.42-0.58)		0.0±0.0a
	0.5	74.5±12.2	76.8±4.1fg	90.4±0.6ef				0.2±0.1b
Pongam oil + fennel oil (P3)	1.5	78.3±8.1	85.9±1.9hi	98.9±0.3g	0.39	0.64	2.684	0.0±0.0a
	0.75	82.3±18.9	83.8±1.8gh	96.8±0.8g	(0.26-0.45)	(0.58-0.76)		0.0±0.0a
	0.5	74.6±11.2	68.2±4.2d	77.0±4.0d				1.3±0.0c
NeemAzal T/S (NA)	1.5	68.5±9.3	96.9±2.8jk	99.7±0.3g	0.28	0.58	0.573	0.0±0.0a
	0.75	80.2±8.5	91.8±2.9ij	96.3±0.7fg	(0.09-0.38)	(0.48-0.70)		0.0±0.0a
	0.5	80.8±9.1	79.6±1.5gh	86.2±3.2e				0.3±0.2b
Control		73.3±16.5	5.2±1.3 a	9.5±0.6a				4.9±0.3g
F-value		0.37	398.24	529.15				1962.86
P-significance level		0.9637	0.001	0.001				0.001

¹ Number (mean ± S.E.) of 3rd instar larvae before application. ² Percentage (mean ± S.E.) of mortality calculated as ratio between numbers of larvae before application and the numbers of dead larvae. ³ Lethal concentration (for 50 % or 90 % mortality) in % (v/v), CI₉₅ denotes confidence intervals, compound activity is considered significantly different when the 95% CI fail to overlap. Chi-square value, significant at $p < 0.05$ level. ⁴ Plant damage (mean ± S.E.), according to the scale: 0 – is undamaged and 5 is damage > 90%. Means followed in the same column by the same letter are not significantly different ($P \leq 0.05$; Tukey's HSD test).

determined for all the tested concentrations and for the products P2, P3 and NA. The botanical insecticide P1 was the only one to show damage between 25 % - 50 %; however, even this damage level of the plants was significantly lower ($P < 0.05$) compared to the control.

Oviposition and persistence of botanical insecticides

No significant antioviposition effect ($p = 0.1857$) of the botanical insecticides was found in the undertaken non-choice tests. Antioviposition percentage was only about 20 %. However, larval mortality was significant ($P < 0.05$); it is true that the mortality was slightly lower than upon direct application of the products on the larvae (Table 1); in spite of that, it reached about 80 % for application of the insecticides P2, P3 and NA and the concentration 1.5 %. After application of the concentration 0.5 %, the mortality of only about 40 % was found (for the insecticides P2, NA) and/or 28 % (for P3). The difference in terms of

lower efficacy of the insecticide with pongam oil alone compared to other tested insecticides upon estimation of lethal concentrations was manifested in this experiment, too. The highest LC₅₀ and LC₉₀ values were estimated for P1, which is a significantly higher concentration ($p < 0.05$) than that determined for the other botanical insecticides. Lethal concentrations of botanical insecticides P2, P3 and NA showed no significant difference and ranged between LC₅₀ (0.52 % – 0.73 %) and LC₉₀ (1.37 % – 1.72 %). Overall plant damage due to larval feeding (Table 2) was minimal and ranged between 0 % to 25 % after treatment P2, P3 and NA. Significantly the lowest efficacy was determined for P1 where the plant damage was about 50 % after application of the concentration 1.5 and 0.75 %. However, even upon application of the lowest concentration of P1, significantly lower ($P < 0.05$) plant damage due to feeding was found compared to control plants where more than 90 % of the leaf area was damaged.

Table 2. Effects of the tested products on prevention of oviposition and subsequent persistency of the effect on mortality and plant damage due to feeding of *Plutella xylostella* larvae

Botanical insecticides	Conc (%)	N ¹ (Eggs)	AI ² (%)	Mortality (%) ³	Lethal concentration ⁴			Plant damage ⁵
				16 days	LC ₅₀ (CI ₉₅)	LC ₉₀ (CI ₉₅)	chi	
Pongam oil (P1)	1.5	77.5±7.7a	14.1±2.4	55.4±2.4 d	1.28	5.06	2.343	2.6±0.3 d
	0.75	75.0±2.4a	16.3±3.3	44.2±4.0 c	(0.93-1.45)	(4.28-7.26)		2.6±0.2 de
	0.5	72.5±7.7	19.7±2.8	18.7±3.5 b				3.2±0.2 e
Pongam oil + thyme oil (P2)	1.5	64.0±4.1a	28.7±0.6	81.1±2.9 e	0.58	1.72	1.225	0.0±0.0 a
	0.75	69.1±4.9a	23.2±0.1	77.1±1.9 e	(0.38-0.83)	(1.29-2.18)		0.0±0.0 a
	0.5	74.1±12.2a	18.6±7.7	39.4±4.0 c				0.3±0.2 ab
Pongam oil + fennel oil (P3)	1.5	73.3±4.1a	18.7±1.3	79.6±5.9 e	0.73	1.69	3.470	0.0±0.0 a
	0.75	78.0±8.9a	13.7±3.7	59.3±2.8 d	(0.63-0.81)	(1.41-2.26)		0.1±0.1 ab
	0.5	74.5±10.2a	17.7±5.3	28.3±3.2 b				1.2±0.4 c
NeemAzal T/S (NA)	1.5	72.0±3.2a	19.7±2.2	85.5±3.8 e	0.52	1.37	2.233	0.0±0.0 a
	0.75	76.5±4.5a	14.8±1.2	82.6±6.2 e	(0.42-0.72)	(1.21-2.18)		0.2±0.1 ab
	0.5	80.8±4.1a	10.8±1.9	42.2±2.5 c				0.6±0.3 bc
Control		90.3±6.5a		6.5±2.5 a				4.7±0.5 f
<i>F-value</i>		1.48		158.63				206.80
<i>P-signific. level</i>		0.1857		0.001				0.001

¹ Number (mean ±S.E.) of eggs oviposited on individual plants. ² Percentage (mean ± S.E.) of antioviposition indexes. ³ Percentage (mean ± S.E.) of mortality calculated as ratio between oviposited egg numbers and difference in the numbers of eggs and live larvae. ⁴ Lethal concentration (for 50 % or 90 % mortality) in %, CI₉₅ denotes confidence intervals, compound activity is considered significantly different when the 95% CI fail to overlap. Chi-square value, significant at p < 0.05 level. ⁵ Plant damage (mean ± S.E.), according to the scale: 0 – is undamaged and 5 is damage > 90%. Means followed in the same column by the same letter are not significantly different (P ≤ 0.05; Tukey's HSD test).

DISCUSSION

This paper provides the information on biological efficacy of three new formulations of botanical insecticides against larvae of an important pest of cabbage family crops, *Plutella xylostella*. All the tested botanical insecticides showed good efficacy on the mortality of *P. xylostella* larvae. Nevertheless, differences in their efficacy were found. In both formulations of insecticides, that contained essential oils, their biological efficacy increased significantly compared to the insecticide containing only the *P. pinnata* oil alone. This efficacy was clearly reflected not only in high larval mortality, but also in the overall minimal plant damage due to *P. xylostella* larvae feeding. All the 3 new botanical insecticide formulations were designed based on plant oil made of *Pongamia pinnata* seeds. Many biological activities of *P. pinnata* seed extracts can be attributed to karanjin, the major flavonoid of the seed oil. Karanjin has been shown to possess pesticidal properties (Kumar and Singh, 2002). Moreover, evidence of a significant synergistic

effect with some pyrethrins was also provided for pongam oil (Rao and Dhingra, 1997; Vastrad *et al.*, 2002) and for *Azadirach indica* seed oil (Kumar and Singh, 2002; Srinivasa *et al.*, 2003), while their mixture caused significant increase of pest mortality even upon application of sublethal doses.

Use of the synergistic effect of natural substances has also been the subject of present studies (Hummelbrunner and Isman, 2001; Pavela 2008a, 2010). Becoming familiar with and understanding this phenomenon may namely bring a practical impact not only in terms of increased efficacy of insecticides, but also particularly in terms of the potential of reducing recommended dosage or of using of these effects within the framework of antiresistance plant protection strategy, which may provide direct economic and environmental impacts. Pongam oil combinations with essential oils significantly increased biological efficacy in our tests. Upon direct contact application on *P. xylostella* larvae, we found that LC₅₀ of the oil

alone (P1) was approximately double and for LC₉₀ even more than quintuple compared to botanical insecticide formulations P2 and P3. Pongam oil combinations with *Thymus vulgaris* (P2) or *Foeniculum vulgare* (P3) essential oils showed the same efficacy as the reference product NeemAzal T/S based on azadirachtin.

Essential oils or some of their substances, respectively, may exhibit mutual synergistic effects. For example, the thyme essential oil used contained the phenol thymol as its major ingredient, which showed both good insecticidal efficacy against many pests (Isman, 2000; Pavela, 2007; 2008a, Carrubba and Catalano, 2009) and a synergic effect with other terpenes (Hummelbrunner and Isman, 2001; Al-Bayati, 2008; Pavela, 2008a and 2010). It is thus possible that thymol, in combination with other polyphenols that are included in *P. pinnata* oil (Meera *et al.*, 2003), exerts a synergic effect, thereby enhancing their insecticidal and antifeedant efficacy (Kumar and Singh, 2002; Parmar and Gulati, 1969). Nevertheless, experimental verification of this hypothesis is needed. Moreover, sublethal doses of the essential oil of *T. vulgaris* may cause significant reduction of vitality and fertility of adult pests of the subsequent generation (Pavela, 2007) and thus cause an indirect reduction of overall pest numbers in the next generation.

Repellent and antiovipositional effect of *Pongamia pinnata* oil on many pests has been known (Kumar and Singh, 2002). Although, for example, significant antiovipositional effect against *Trialeurodes vaporariorum* was found in previous papers (Pavela and Herda, 2007a, b), this phenomenon was not confirmed in the current tests against *P. xylostella* adults (Table 2). Besides others, this may have been caused also by the way of oviposition. In our tests, *Plutella xylostella* oviposited its eggs predominantly on the bases of the leaf stalks and on the stems where sticking of the products was minimal. However, *T. vaporariorum* oviposits its eggs directly onto the bottom side of the treated leaves, and thus the antiovipositional and repellent effect of secondary metabolites contained in *P. pinnata* oil may have

been manifested (Kumar and Singh, 2002; Meera *et al.*, 2003).

Persistency of the tested botanical insecticides was relatively good (Table 2). The products were applied before oviposition so that they would preserve their efficacy for a week at least, which is the approximate time duration needed for hatching of the larvae from the eggs. The products can thus be used not only for direct application onto the pests, but also as preventive spray-on treatment that reduces overall plant damage due to feeding of *P. xylostella* larvae.

Good persistency of the effects of *P. pinnata* oil was determined also for *Trialeurodes vaporariorum* where the repellence and oviposition deterrence effect in the no-choice test was higher than 50 % even before day 12 upon oil application in the concentrations of 2 to 0.5 % (Pavela and Herda, 2007 a, b). However, it should be noted that the experiments were done in a greenhouse where environmental effects are eliminated (for example, high precipitation rates, UV radiation), which may occur when the plants are grown in field conditions, and which may cause degradation of biologically active substances, thereby leading to significant reduction of efficacy of the products.

Finally, it should be emphasized that products based on extracts of the plants used herein are considered as safe both for the environment and health, and they can be recommended for use in plant protection. This claim stems from known practical use of these plants and extracts in food industry, medicine and cosmetics (Kirtikar and Basu, 1995) as well as from many experiments focused on exploring their effects on non-target organisms and the environment (Isman, 2000; Kumar and Singh, 2002; Charleston *et al.*, 2005).

Moreover, as determined by other authors, some extracts (containing similar substances to those present also in *P. pinnata* oil) may attract more natural enemies of *P. xylostella* larvae (Charleston *et al.*, 2006) and thus increase natural parasitizing levels of the larvae. Botanical insecticides based on pongam oil thus seem to possess the potential for being used together with bio-agents, for

example different *Trichogramma* species (Tabone *et al.*, 2010). Nevertheless, this possibility will be the subject of our further studies.

Based on the results presented in this paper, product formulation based on the combination of *Pongamia pinnata* and *Thymus vulgaris* or *Foeniculum vulgare* essential oils can be recommended for protection of cabbage crops against *Plutella xylostella* larvae.

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