SUPPLEMENTARY MATERIAL

Chemical composition and larvicidal activity of *Zanthoxylum armatum* against diamondback moth, *Plutella xylostella*

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Abstract

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) is the most serious pest of cruciferous crops grown in the world causing economic yield loss. Several synthetic insecticides have been used against *P. xylostella* but satisfactory control was not achieved due to development of resistance to insecticides. Therefore present study was carried out to screen different fractions of *Zanthoxylum armatum* for their insecticidal activities against second instar larvae of *P. xylostella*. Results indicate, all the fractions showed activity to *P. xylostella*. However, *n*-Hexane fraction of *Z. armatum* showed maximum larvicidal activity with minimum LC₅₀ value of 2988.6 ppm followed by ethanol (LC₅₀ = 12779.7 ppm) and methanol fraction (LC₅₀ = 12908.8 ppm) whereas chloroform fraction was least toxic (LC₅₀ = 16750.6 ppm). The GC-MS analysis of *n*-hexane fraction of leaf extract showed maximum larvicidal activity, which may be due to two major compounds *i.e.* 2-undecanone (19.75%) and 2-tridecanone (11.76%).

Keywords: *Zanthoxylum armatum*; fractions; insecticidal activity; *Plutella xylostella*

Experimental

Plant material

The leaves of *Z. armatum* were collected from Palampur, Himachal Pradesh, India in June 2011. The plant material was authenticated and a voucher specimen was deposited in the herbarium (voucher no. PLP 16528) of CSIR-Institute of Himalayan Bioresource Technology, Palampur, India.

Preparation of plant extracts

Air dried powder of leaves of *Z. armatum* (1 kg) was extracted with 80% aqueous MeOH (4L x 3) in a percolator at room temperature. The combined percolations were dried under reduced pressure to get crude extract (ZALMW, 348 g). The extract was suspended in water and subsequently fractionated with solvents of different polarity *n*-hexane, chloroform, ethyl acetate and *n*-butanol to get corresponding extracts ZALH (5.3 g), ZALC (18.7 g), ZALE (9.5 g) and ZALB (98.6 g), respectively.

Analysis of volatile compounds of *n*-hexane fraction

Analysis of the samples was performed on GC-MS from Shimadzu (QP 2010) series Gas Chromatogram-Mass Spectrometer (Tokyo, Japan), AOC-20i auto-sampler coupled, and a DB-5MS capillary column, (30 m x 0.25 mm i.d., 0.25 μ m). The initial temperature of column was 70 °C held for 4 min. and was programmed to 230 °C at 4°C/min., then held for 15 min. at 230 °C; the sample injection volume was 2 μ l in GC grade dichloromethane. Helium was used as carrier gas at a flow rate of 1.1 ml min⁻¹ on split mode (1:50). Relative percentages were calculated from the FID from the automated integrator. Kovats indices (KI) of the compounds relative to a mixture of *n*-alkanes (C8-C23) were calculated. Identification of compounds was first attempted using the mass spectral libraries Wiley 7 and NIST 02 (Mc Lafferty, 1989; Stein, 1990). Validation of the identification was then conducted by matching the KI of the compounds reported on a column having an equivalent binding phase.

Test insect

P. xylostella used for the experimental study was collected from infested cabbage field and reared under laboratory conditions at 25 $\pm$ 2°C, 60 $\pm$ 5% RH and photoperiod of 16:8 (L: D) for more than 30 generations. Second instar larvae of *P. xylostella* were used for the experiments.

Residual toxicity of different fractions of *Z. armatum* against *P. xylostella*

Residual toxicity of different fractions of *Z. armatum* against *P. xylostella* was tested following leaf dip bioassay (Park et al. 2002) against second instar larvae of *P. xylostella*. Briefly, 800 mg (0.8 g) of the test samples were dissolved in 2 ml of acetone-Triton (9:1) and then diluted in 40 ml distilled water containing 0.05% Triton®-X 100 LR spreader (SD Fine Chemicals Limited, www.sdfine.com) and ultra-sonicated for complete dissolution. Six concentrations of test solutions (6000-20000 ppm) were prepared from stock solutions from the solution of higher concentration for dose response bioassay. The prepared concentrations were poured in glass petri dishes. Fresh mustard leaf discs (6 cm diameter) were cut from cabbage leaves grown under field conditions without any insecticide spray. Leaf discs were dipped in test solutions for 10 seconds and then allowed to air dry at room temperature. For control, leaf disks were dipped in distilled water containing 0.05 percent Tritone. Treated leaf discs were placed individually into plastic Petri dishes with moistened filter paper to prevent desiccation. Ten larvae starved for 4 h were transferred onto the treated leaf discs in Petri dish (6 cm diameter) and then sealed with para film and kept in the laboratory conditions at 25 $\pm$ 2 °C temperature, 60 $\pm$ 5 percent relative humidity and a photoperiod of 16:8 (L: D) for observations. Three replicates of 10 larvae per concentration were maintained. The observation on mortality was recorded after 48 h of treatment.

Data analysis

The experiment was repeated for three times. The mean data from all bioassays are corrected for control mortality using Abbott formula (Abbott 1925) and analyzed using SPSS 7.5 for determining LC₅₀ values by log-probit regression.

References

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Table S1. Chemical composition of *n*-hexane fraction of *Zanthoxylum armatum* leaves.

Sl. No.	Compound	% age	KI	KI (lit.)	Identification mode
1	Sabinene	1.42	982	977	KI, MS
2	β -Myrcene	0.67	993	997	KI, MS
3	<i>n</i> -Decane	0.30	1003	1000	KI, MS
4	β -Thujene	6.77	1041	1038	KI, MS
5	<i>E</i> -Sabinene hydrate	1.79	1080	1057	KI, MS
6	Undecane	0.21	1103	1100	KI, MS
7	<i>E</i> -Sabinene hydrate acetate	2.97	1225	1226	KI, MS
8	Linalyl acetate	0.39	1258	1239	KI, MS
9	2-Undecanone	19.75	1301	1277	KI, MS
10	<i>trans</i> -Caryophyllene	9.88	1438	1429	KI, MS
11	α -Humulene	1.01	1474	1468	KI, MS
12	Germacrene D	3.44	1498	1474	KI, MS
13	2-Tridecanone	11.76	1504	1476	KI, MS
14	Caryophyllene oxide	1.58	1606	1589	KI, MS
15	Neophytadiene	6.89	1839	1806	KI, MS
16	Phytol	3.10	1862	1981	KI, MS
17	Methyl palmitate	0.62	1923	1927	KI, MS
18	Palmitic acid	5.16	1975	1962	KI, MS
19	Methyl (7 <i>E</i> ,10 <i>E</i>)-octadecadienoate	1.12	2072	-	MS
20	Methyl linolenate	1.46	2078	2050	KI, MS
21	Linoleic acid	2.64	2128	2079	KI, MS
22	Linolenic acid	7.55	2132	-	MS
	Total	90.48			

Table S2. LC₅₀ values for different fractions of *Zanthoxylum armatum* against *Plutella xylostella* (48 hrs after treatment)

Fractions	LC ₅₀ (ppm)	95% Confidence limits (ppm)		Chi-square	Slope	P value
		Lower	Upper			
Hexane	2988.6	222.8	4995.8	0.478	2.210	0.97
Ethyl acetate	12779.7	11312.0	14859.5	1.558	3.746	0.81
Methanol	14462.1	12908.8	16873.8	3.070	4.265	0.54
Chloroform	16750.6	14605.8	20956.5	2.067	3.928	0.72

Figure S1. Efficacy of different fractions of *Zanthoxylum armatum* against *Plutella xylostella*