"Chemical Composition and Larvicidal Activities of *Zanthoxylum armatum* (Rutaceae) against Diamondback Moth, *Plutella xylostella* "

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Introduction

❖Diamondback moth, *Plutella xylostella* (L.) is the most serious damaging insect pest of cruciferous crops throughout the world causing economic yield loss.

- Cabbage, *Brassica oleracea* var. *capitata* L., is cultivated throughout the year in many parts of India with approx. 2.4 lakh ha planted and 52% yield loss was reported due to *P. xylostella* (Krishnamoorthy 2004).

- Nearly two decades ago, the annual cost of controlling *P. xylostella* on a worldwide basis was estimated to be US $1 billion (Talekar and Shelton 1993) but in a recent study the overall management costs were estimated at US $4 billion (Zalucki et al., 2012).

- Intensive use of chemical pesticides in its control has led to this pest developing resistance to a wide range of insecticides and caused serious damage to natural enemies (Talekar and Shelton 1993).

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Introduction (Contd.)

❖**The natural plant products can be an excellent alternative source of novel insecticides which are considered to be less toxic to non-target species and more environment friendly because of their biodegradable nature.**

❖***Zanthoxylum armatum*** **growing abundantly in the mid hills of Western Himalayas; extensively used in the Indian system of medicine (carminative, stomachic and anthelmintic). The bark is pungent and stick from the plant is used in preventing toothache. The fruits and seeds are employed as an aromatic tonic in fever, dyspepsia, and expelling roundworms.**

❖ **Essential oil of fruits showed good antibacterial, antifungal, anthelmintic and insecticidal activity against stored grain pests.**

❖**In the present study, the chemical composition and insecticidal activity of different fractions of leaf extracts of *Z. armatum* was tested against second instar larvae of *P. xylostella.***

❖**The results of the present study would be useful in promoting research aiming at the development of new agents for insect pest control based on natural products.**

Methodology

Plant material

❖The leaves of *Z. armatum* were collected from Palampur (H.P.) 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, 348g).

❖The extract was suspended in water and sequentially fractionated with *n*-hexane, chloroform, ethyl acetate and *n*-butanol to get corresponding extracts ZALH (5.3g), ZALC (18.7g), ZALE (9.5g) and ZALB (98.6g), respectively.

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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., 0.25µm).

- 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.

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Toxicity of different fractions of *Z. armatum* against 2nd instar larvae of *P. xylostella* by leaf dip bioassay (Park et al., 2002)

Dose-response bioassay

- Test samples (materials) were prepared at different concentrations either separately or by serial dilution from the solution of higher concentration.

- 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.

- From stock solutions six different concentrations (6000, 8000, 10000, 12000, 15000, 20000 ppm)

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Leaf dip method

- Three cabbage leaf disks (6 cm diameter) were cut and dipped in either individual test or control solutions for 10 seconds then allowed to air dry at room temperature.

- Ten second instar larvae of *P. xylostella* starved for 4 hours were transferred to treatment and control leaf disks kept on the moist filter paper in petri plates (9 cm diameter) then sealed with para film and kept in the laboratory at 25 ± 2°C, 60 ± 5% RH and a photoperiod of 16:8 (L:D).

- The expt. was repeated thrice with three replications/treatment.
- Observations on mortalities were recorded at 48 hr after treatment.

Data analysis

- The 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.

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Efficacy of different fractions of *Zanthoxylum armatum* against *Plutella xylostella*

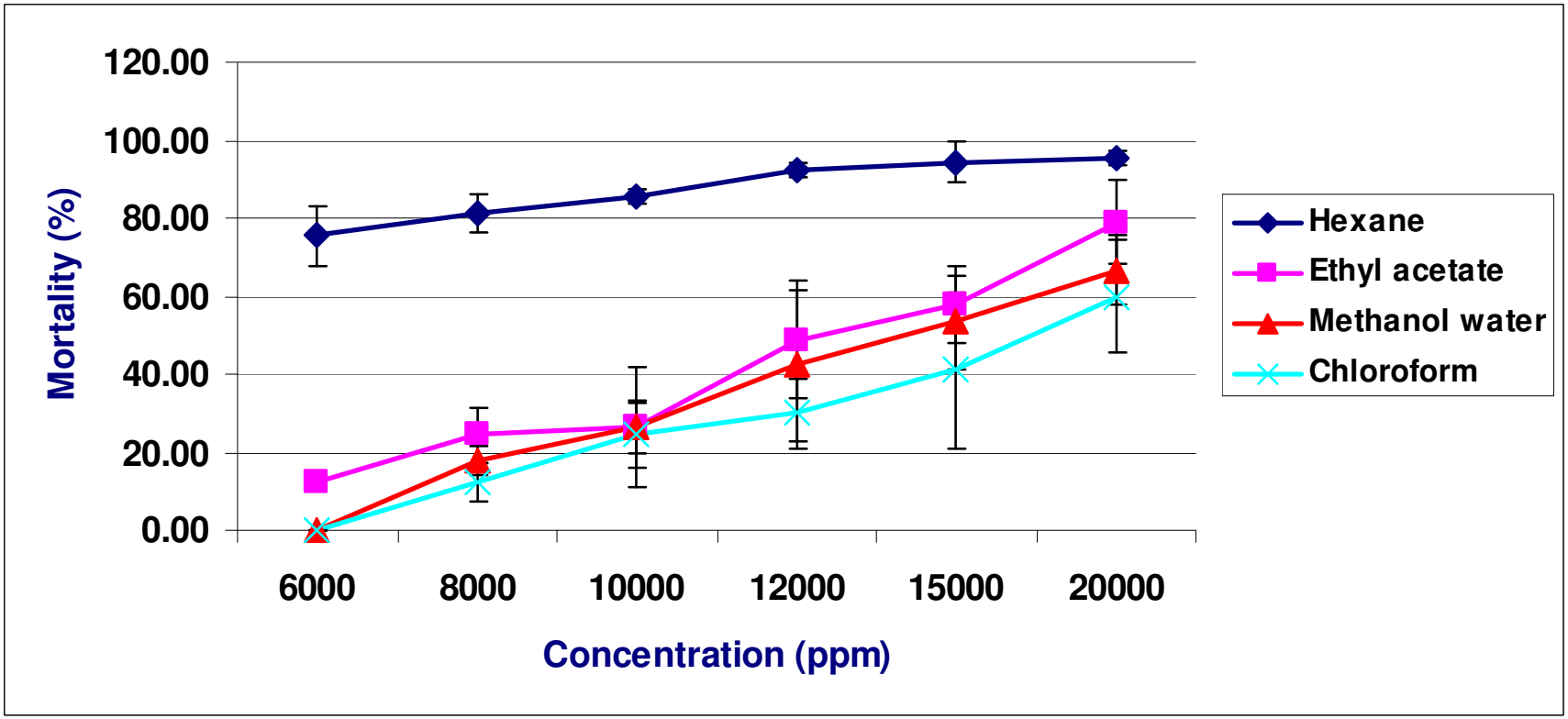

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Table 1. LC_{50} (ppm) values for different fractions of *Z. armatum* against *P. xylostella* (48 hrs after treatment)

Fractions	LC_{50} (ppm)	95% Confidential limits (ppm)		Chi-square	Intercept	Slope	P value
		Lower	Upper				
Hexane	2988.6	222.8	4995.8	0.478	7.68	2.21	0.97
Ethyl acetate	12779.7	11311.1	14859.5	1.558	15.38	3.74	0.81
Methanol water	14462.1	12908.8	16873.8	3.070	17.74	4.26	0.54
Chloroform	16750.6	14605.8	20956.5	2.067	16.59	3.92	0.72

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Table 1. Chemical composition of *n*-hexane fraction of *Zanthoxylum armatum* leaves

Sl. No.	Compound	% age
1	Sabinene	1.42
2	β-Myrcene	0.67
3	n-Decane	0.30
4	β-Thujene	6.77
5	E-Sabinene hydrate	1.79
6	Undecane	0.21
7	E-Sabinene hydrate acetate	2.97
8	Linalyl acetate	0.39
9	2-Undecanone	19.75
10	trans-Caryophyllene	9.88
11	α-Humulene	1.01
12	Germacrene D	3.44
13	2-Tridecanone	11.76
14	Caryopyllene oxide	1.58
15	Neophytadiene	6.89
16	Phytol	3.10
17	Methyl palmitate	0.62
18	Palmitic acid	5.16
19	Methyl (7E,10E)-octadecadienoate	1.12
20	Methyl linolenate	1.46
21	Linoleic acid	2.64
22	Linolenic acid	7.55
	Total	90.48

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Thank you