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Ovicidal Activity of Crude Extracts of Indigenous Plant Species Against *Plutella xylostella* (L.) (Lepidoptera: Plutellidae)

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Abstract Studies on ovicidal effects of methanol ethyl acetate and aqueous extracts of four different plants namely, *Acorus calamus, Vitex negundo, Adhatoda vasica* and *Dioscorea deltoide* were carried out under laboratory conditions against the diamondback moth, *Plutella xylostella* (L.) which revealed that extracts of two plants had significant effect on the mortality of eggs. However, the methanol extracts were found to be better than the other extracts. Methanol and hexane extracts of *A. calamus* (rhizome) resulted in 100% egg hatching inhibition at 7.5% concentration in comparison to aqueous extract where 38.89% egg hatch was observed. This was followed by *V. negundo* leaf extract where the same concentration resulted in 19.19% and 38.35% egg hatch in methanol and ethyl acetate extracts respectively. *A. vasica* (leaf) and *D. deltoidea* (tuber) extracts were followed by these with 61.74% eggs hatch in case of ethyl acetate extract and 68.80% egg hatch in case of methanol extract. There was constant increase in the per cent kill of egg masses with the increase in the extract concentration.

Keywords *Acorus calamus, Adhatoda vasica,* Ovicidal activity, *Plutella xylostella, Vitex negundo.*

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Introduction

The diamondback moth, *Plutella xylostella* (L.) is one of the most destructive lepidopteran pest of cruciferous plants throughout the world. It occurs wherever crucifer crops are grown and is believed to be most universally distributed of all Lepidoptera [1]. Its control is very difficult as it is an internal feeder. The first instar larvae mines into leaf and the subsequent instars feed on the leaf and skeltonize it, ultimately affecting the plant growth and rendering it unfit for further use. The damage is more seious to cabbage where the larvae penetrate inside the head and destroy it completely. The pest has been reported to cause an average of 525 loss to marketable yield of cabbage in India and every year farmer spends US$ 1 billion to control this pest worldwide [2]. A number of insecticides have been recommended in the last decade to manage this pest but due injudicious and excess use of these synthetic insecticides resulted in the development of resistant strains of this insect, which ultimately resulted in cross-resistance to many

insecticides as a consequence residue problem on the crop [3]. Under such conditions, the uses of botanical insecticides (BI) are the only substitute, which not only are cost effective but also cause adverse effects on the development and reproduction of target insect. The present study has therefore been undertaken to study the ovicidal effect of plant extracts against the diamondback moth, *P. xylostella* under laboratory conditions so that information thus gathered may be utilized for the management of this pest under field conditions.

Materials and Methods

Preparation of stock solution

Four plant species namely *Acorus calamus* (rhizome), *Vitex negundo* (leaf), *Adhatoda vasica* (leaf) and *Dioscorea deltoidea* (tuber) were selected for testing ovicidal action against the diamondback moth. All the test plant materials except *D. deltoidea* were collected from locally available plants while the tubers of *D. deltoidea* were procured from the local market of Palampur. The collected material was shade dried for 6-7 days then different plant parts were grounded in an electric grinder to make powder. For each sample, 500 g of powder was taken in a conical flask (2000 ml) and 1500 ml of desired solvent (hexane, ethyl acetate and methanol) was added to it. These flasks were kept at room temperature for 48 h and were shaken occasionally in between. These extracts were filtered through Whatman No. 1 filter paper to remove insoluble debris and concentrated in vacuo (Low pressure) and reduced temperature (38-40 ^{0}C) in a rotary evaporator [4]. Finally, the crude extract was obtained. The crude extracts were kept under low temperature in the refrigerator until use. The crude extracts of different plant parts obtained above were further diluted with respective solvents to make the desire concentrations and emulsifier (Triton X-100 and Tween 80) was added to it. Water was used to have the desired concentration. For aqueous extraction 500 g of the powdered material was soaked in 2000 ml of distilled water. It was kept in a beaker for 48 h stirred occasionally in between. The solution was passed through muslin cloth and then filtered through Whatman filter paper No. 1 and the final volume was made 1000 ml which gave stock solution of 100%. Further dilutions were made from this stock solution with distilled water by single dilution method.

Insect culture

Stock culture of *P. xylostella* was maintained under laboratory conditions throughout the year. For this purpose, larvae and pupae of the *P. xylostella* were collected from the cauliflower and cabbage fields from the vegetable farm of the University. These were brought to insectary for mass rearing. *P. xylostella* was maintained on mustard seedlings and cabbage leaves as per the method elaborated earlier [5] with modifications and reared in insectary at 25 ± 2 ^{0}C, 70 ± 5% relative humidity (RH) and 16:8 (Light: Dark) photoperiod during larval to adult stage. The adults were held in oviposition cages (27 × 21 × 21 cm) and provided with 10% sugar solution as food in cotton swabs. The five day old mustard seedlings in pots (10 × 10 cm) were exposed overnight to adults for oviposition and allowed to hatch. Three day old larvae were transferred to cabbage leaves kept in plastic jars (25 × 20 cm). The larvae were regularly provided with fresh leaves without removing the infested one so as to enable them to shift to fresh leaves on their own, to improve their survival rate and reduce the handling time considerably. The seedlings were exposed daily and larvae were maintained separately for each exposure date to get specific stage of larvae regularly throughout the study period.

Ovicidal activity

To study the ovicidal activity of different plant extracts the leaf portion with egg masses of the diamondback moth was dipped instantly for 30 seconds into the solution of desired plant extracts, air dried and then placed into petri dish (9 cm diameter) having moist filter paper at the bottom. The egg masses in control were dipped in distilled water. The observations on egg hatchability were recorded up to 7 days and per cent egg hatching was calculated [6].

$$\text{Hatchability (\%)} = \frac{\text{Total number of eggs hatched}}{\text{Eggs kept for hatching}} \times 100$$

Table 1. Effect of *Acorus calamus* extracts on egg hatchability of *Plutella xylostella.* Figures in parentheses are arc sine transformed values. CD (p = 0.05), Extract = 1.56, Concentration = 1.91, Extract × Concentration = 3.83.

Solvent extract	Mean per cent egg hatch at indicated concentration (%) 7.5	5.0	2.5	1.25	0.625	0	Mean
Methanol	0.00	0.00	17.01	35.46	50.59	91.7	32.46
	(0.00)	(0.00)	(24.23)	(36.53)	(45.32)	(73.28)	(29.89)
Hexane	0.000	13.97	36.92	42.81	61.92	94.12	41.62
	(0.00)	(21.93)	(37.39)	(40.84)	(51.91)	(76.28)	(38.05)
Ethyl acetate	23.38	26.08	44.65	62.67	82.48	97.06	56.06
	(28.86)	(30.70)	(41.91)	(52.32)	(65.24)	(82.07)	(50.19)
Aqueous	38.89	49.38	59.61	81.78	89.90	92.36	68.65
	(38.56)	(44.63)	(50.52)	(64.71)	(71.53)	(74.00)	(57.33)
Mean	15.57	22.35	39.55	55.68	71.22	93.81	
	(16.86)	(24.31)	(38.51)	(48.60)	(58.50)	(76.41)	

Statistical analysis

The ovicidal activity was analyzed using two way anova.

Results and Discussion

The studies reveal that the methanol extract of *A. calamus* rhizome at 7.5% concentration gave cent per cent egg hatching inhibition whereas at 0.625% concentrations, the egg hatch was maximum (59.59%) and was significantly different with control. However the aqueous extract of this plant resulted in 38.89% egg hatch at 7.5% concentration whereas it was 89.90% at 0.625% and 92.36% in the untreated control (Table 1). The ethyl acetate extract of *V. negundo* leaf resulted in 38.35 and 79.53% egg hatch, respectively at 7.5 and 0.625% concentrations in comparison to 90.26% egg hatch in control, whereas the methanol extract of this plant gave 19.19 and 72.08% egg hatch at the respective concentrations in comparison to 92.70% in control (Table 2). The comparison of ethyl acetate and aqueous extract of *A. vasica* leaf revealed that at 7.5% concentration, the egg hatch was 61.74 and 82.50%, respectively however, at 0.625% the egg hatch was 89.12 and 89.68%, in comparison to 77.47 and 87.80% in the untreated control for the respective solvents (Table 3). The methanol and aqueous extracts of *D. deltoidea* rhizome resulted in egg hatch of 68.80 and 80.45% at 7.5% concentration whereas in the respective solvents at 0.625%, the egg hatch was 86.73 and 88.61% in comparison to 90.73 and 92.65% egg hatch in control (Table 4). On comparing different plant ex-

Table 2. Effect of *Vitex negundo* extracts on egg hatchability of *Plutella xylostella.* Figures in parentheses are arc sine transformed values. CD (p = 0.05), Extract = 2.10, Concentration = 2.58, Extract × Concentration = 5.17.

Solvent extract	Mean per cent egg hatch at indicated concentration (%) 7.5	5.0	2.5	1.25	0.625	0	Mean
Methanol	19.19	28.96	44.00	61.34	72.08	92.70	53.11
	(25.96)	(32.53)	(41.76)	(51.54)	(58.08)	(74.62)	(47.41)
Hexane	48.80	60.38	73.54	75.46	82.61	96.37	72.86
	(44.29)	(50.98)	(59.07)	(60.36)	(65.37)	(80.99)	(60.18)
Ethyl acetate	38.35	41.98	50.08	59.38	79.53	90.26	59.93
	(38.25)	(40.35)	(45.03)	(50.39)	(63.12)	(71.82)	(51.50)
Aqueous	56.42	62.07	64.70	77.63	81.69	96.25	73.12
	(48.68)	(51.98)	(53.54)	(61.77)	(64.73)	(80.97)	(60.28)
Mean	40.69	48.35	58.18	68.45	78.98	93.89	
	(39.30)	(43.96)	(49.85)	(56.01)	(62.82)	(77.10)	

Table 3. Effect of *Adhatoda vasica* extracts on egg hatchability of *Plutella xylostella*. Figures in parentheses are arc sine transformed values. CD (p = 0.05). Extract = 2.19, Concentration = 2.68, Extract × Concentration = 5.35.

Solvent extract	Mean per cent egg hatch at indicated concentration (%)						
	7.5	5.0	2.5	1.25	0.625	0	Mean
Methanol	70.75 (57.24)	76.03 (60.70)	79.86 (63.36)	84.90 (67.11)	88.24 (69.94)	90.82 (72.34)	81.76 (65.11)
Hexane	74.59 (59.72)	77.45 (61.64)	85.81 (67.86)	88.06 (69.93)	89.14 (70.74)	96.48 (81.35)	85.26 (68.54)
Ethyl acetate	61.74 (51.77)	65.27 (53.88)	72.52 (58.39)	81.37 (64.56)	89.12 (70.90)	94.78 (79.26)	77.47 (63.13)
Aqueous	82.05 (64.93)	86.07 (68.27)	86.22 (68.19)	88.92 (70.55)	89.68 (71.25)	93.86 (75.64)	87.80 (69.80)
Mean	72.28 (58.42)	76.20 (61.12)	81.10 (64.45)	85.81 (68.04)	89.04 (70.71)	93.98 (77.15)	

tracts, the extract of *A.calamus* (rhizome) was found to be more effective in causing mean 32.46% egg hatch and was statistically differ with hexane extract of *A. calamus* giving 41.62% egg hatch. This was followed by mean per cent egg hatch in leaf methanol extract of *V. negundo* (53.11%) and ethyl acetate extract (59.93%) and these were significantly different from each other. When the comparison of *A. vasica* extracts was made, the ethyl acetate extract was found to be effective in causing 77.47% egg hatch and was significantly different with the methanol extract giving 81.76% egg hatch. However, in case of *D. deltoidea* methanol found to be effective in causing 81.59% egg hatch and was significantly different with the aqueos extract giving 86.16% egg hatch (Tables 1, 2, 3 and 4). The studies thus indicated the superiority of *A. calamus* rhizome extract to all other plants extracts tested in the present studies. Aqueous extract of *M. azedarach* leaf caused 48.0% egg mortality (infertility) at 5% concentration [7]. The less efficiency of the aqueous extracts in the present study might to be due to poor extraction of active ingredients in water. Extract from plant material has the ability to penetrate the chorion of egg thus causing the death of developing embryo [8]. This fact can also be well supported by the findings of previous study [9] which reported the post ovipositional effects of kernel extract of *M. azedarach* with ethanol where less number of eggs of *P. xylostella* were hatched and subsequently those hatched resulted in mortality in larval and pupal stages and malformed adults.The results on the effectiveness of *V. negundo* leaves draw considerable supports from the findings of researcher who reported 17.0% egg hatch in *P. xylostella* when cabbage leaves were treated with volatile oils of *V. negundo* [10]. When eggs were treated with 0.06 and 0.08% methanol extracts of *A. calamus* caused 90.0 and 96.6% inhibition in egg hatching in *Bactrocera cucurbitae* (Coquillett), respectively [11].The results also corroborate the previous studies of which also reported the 47.91% inhibition of egg hatching in methanol extract of *A. indica* [12]. Similarly, aqueous extract of *A. calamus* and *V. negundo* also caused 46.40 and 39.85% egg mortality of *P. xylostella* [13].

Table 4. Effect of *Dioscorea deltoidea* extracts on egg hatchability of *Plutella xylostella*. Figures in parentheses are arc sine transformed values. CD (p = 0.05), extract = 2.76, Concentration = 4.78, Extract × Concentration = NS.

Solvent extract	Mean per cent egg hatch at indicated concentration (%)						
	7.5	5.0	2.5	1.25	0.625	0	Mean
Methanol	68.80 (56.33)	77.00 (61.33)	80.21 (63.62)	86.07 (68.17)	86.73 (68.69)	90.733 (72.36)	81.59 (65.08)
Aqueous	80.45 (63.75)	81.64 (64.91)	85.68 (67.75)	87.90 (70.01)	88.61 (70.41)	92.65 (74.68)	86.16 (68.59)
Mean	74.64 (60.04)	79.32 (63.12)	82.95 (65.68)	86.98 (69.09)	87.67 (69.55)	91.69 (73.52)	

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