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Oviposition deterrence effect of EC formulations of *Strychnos nux-vomica* L. plant extracts against *Plutella xylostella* Linn. under laboratory conditions

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Abstract
A laboratory bioassay was conducted to investigate the efficacy of *Strychnos nux-vomica* extracts on the oviposition deterrent effect on the Diamondback moth, *Plutella xylostella* fed on cauliflower (*Brassica oleracea* L. *var. botrytis*). The extracts from different plant parts *viz.*,leaves, seeds, stem, bark, root bark and fruit rind of *Strychnos nux-vomica*, extracted in different solvents *viz.*, ethanol, methanol, hexane, acetone, chloroform was evaluated for deterrence for oviposition on *P. xylostella*. The results revealed that EC formulations of solvent extracts of *S. nux-vomica* possessed oviposition deterrence effect and maximum effect of 69.81 per cent was observed in chloroform fruit rind extract 10.00 EC at 2 per cent concentration. Oviposition deterrence of different solvent extracts of *S. nux-vomica* was in the order of chloroform > hexane > acetone > ethanol > methanol and different plant parts were in the order of fruit rind > root bark > seed > leaf > stem bark.

Keywords: Cauliflower, diamond back moth, oviposition, eggs, formulation

Introduction
Cauliflower. is an important crucifer cultivated in India. Diamondback moth (DBM), *Plutella xylostella* (Linnaeus) (Plutellidae: Lepidoptera), is the major destructive pest of crucifers and causes significant economic losses up to 50 per cent with an estimate of US$ 168 million per year [1]. Sole reliance on chemical insecticides has facilitated the rapid build-up of resistance in the multi-voltine DBM, which undergoes 20 generations a year in the tropics [2]. To overcome resistance in DBM to insecticides, farmers often increase the doses of insecticides when insecticides alone account for between 30 and 50 per cent of the total cost of production. Climatic changes may lead to increase in severity of this pest in many regions of the country [3]. It is pertinent that a change in the insect pest management strategy may form a meaningful solution to avoid the ill-effects caused by the synthetic chemical insecticides. Therefore, in search of safer alternatives, attention has been focused on the use of botanical pesticides. Botanical insecticides were the earliest recorded insecticides used in agriculture. In nature, more than 1800 plant species are reported to have biopesticidal properties [4].
The strychnine tree (*Strychnos nux-vomica* L.), also known as nux-vomica, is a deciduous tree native to India and South east Asia. Nux-vomica belongs to the family Strychnacaceae (Loganiaceae), commonly known as poison nut, snake wood, quaker buttons, semen strychnos. *S. nux-vomica* species is a medium sized, deciduous tree, with fairly straight and cylindrical bole and dark-grey or yellowish-grey bark with minute tubercles. Flowering occurs from March to May and fruits mature up to December. Spherical fruits of the nux-vomica are large and hard-rinded. Berries contain 3 to 8 round, flattened, greyish seeds. Nux-vomica seeds contain a mixture of 13 alkaloids [5]. but the main alkaloids are strychnine and brucine [6]. Seeds of *S. nux-vomica* contain 0.4 and 0.6% strychnine and brucine, respectively. Content of strychnine and brucine may vary in different plant parts (Plate 1). Keeping above in the mind and to exploit the potential of *S. nux-vomica* as a biopesticide, oviposition deterrent effect of EC formulation of *S. nux-vomica* extracts against *Plutella xylostella* Linn. was evaluated under *in-vitro* conditions.

Plate 1: Description of *Strychnos nux-vomica*

2.Materials and Methods

2.1.Collection and processing of plant samples: Different plant parts *viz.*,fresh leaves, seeds, stem bark, root bark and fruit rind of *S. nux-vomica*, each weighing almost 3 kg were collected from the trees found in drought prone area of Krishnagiri district of Tamil Nadu. The plant samples were air dried in the Entomology laboratory, TNAU, Coimbatore up to two weeks and ground into uniform powder and packed in 3 kg plastic containers separately (Plate 2).

Plate 2: Different plant parts of *S. nux-vomica* used for the assay

2.2. Extraction and formulation

Dry powders of plant samples are extracted with organic solvents such as ethanol, methanol, acetone, hexane and chloroform using soxhelt apparatus. The extracts (miscella) were collected in 50 ml screw capped vials and excess solvents were evaporated in hot water bath (65 ºC) and concentrated miscella were stored at 4ºC for further usage. For formulation purpose, One gram of miscella was transferred to 500 ml beaker. Suggested EC formulation solvent, cyclohexanone was added drop by drop to the crude extract using micropipette, until the miscella was completely soluble in the solvent. Finally emulsifiers tween 20 and triton X added to the solution. Then this mixture was kept nearly one hour in the mechanical shaker to get mix the solution to get the EC formulation. Then mixture was subjected to emulsion stability test by following [7]. Finally, this formulated miscella were utilized for oviposition deterency bioassay.

2.4. Mass culturing of DBM

The test insects required for the bioassays were obtained from the stock culture maintained on mustard and cauliflower at Insectary, Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore by following the standard method given by [8]. Cabbage seedlings or mustard seedlings are used for the mass multiplication of *P. xylostella*. Mustard *Brassica juncea* (Linnaeus) Czern seedlings are grown in small ice cream cups with vermiculite or in wooden trays containing a equal mix of red soil, sand and farm yard manure. The seeds dribbled by either way are allowed to germinate and proper moisture to ensure maximum germination of the seedlings is provided. Five days after germination the seedlings are placed in an insect rearing cage 3 x 2.5 x 2.5 meters all sides of the' cage are covered by nylon mesh but the top is covered by acrylic sheet. Two hundred *P. xylostella* pupae are kept in small petri plate and placed inside the cage. The emerging adults are fed on 30% honey and water soaked swabs. The adults are allowed to oviposit on the leaves of mustard for 24 hours. After this time the seedlings are removed and placed in trays in a cage. *P. xylostella* larvae upon hatching feed on mustard seedlings and the seedlings are completely eaten away by the larvae. The larvae from these seedlings are shifted to another set of fresh seedlings by chopping the remnents of the old seedlings and placing them on fresh seedlings. The 'pupae are formed in about 15 days time, they are collected and placed in an oviposition cage and the production procedure continues. The pupal stock in the oviposition cages is renewed once in 5-6 days, so that continuous supply of eggs or larvae could be maintained. The mustard seedlings or trays could be raised depending upon the need.

2.5. Oviposition deterency bioassay: Eighteen days old cauliflower seedlings were transplanted in the plastic pots. Pot cultures were maintained up to 45 days and then seedlings were transferred to the experimental cages. Plants were sprayed with *S. nux-vomica* extract EC formulations at different concentration *viz.*, 0.5, 1.0, 1.5 and 2.0% and kept inside the oviposition cages. Treatment with water alone served as negative control and solvents act as a positive control. Newly emerged adults were allowed to mate in oviposition cages. After 48 h of exposure adults were collected with the help of test tubes and 10 pairs of adults were transferred to each oviposition deterency bioassay cages. Observations were taken every 24 h with the help of hand lens upto 5 days and the number of eggs laid in each treatment was pooled to calculate the oviposition deterency index. There are six treatments each treatment was replicated four times.

Treatments details: EC formulations of solvent extracts of *S. nux-vomica* used in the bioassay were as follows:

S. No	Treatments	Dosage (%)
1	Ethanol extracts of *S. nux-vomica* leaf 22.2 EC	0.5
2	Ethanol extracts of *S. nux-vomica* leaf 22.2 EC	1
3	Ethanol extracts of *S. nux-vomica* leaf 22.2 EC	1.5
4	Ethanol extracts of *S. nux-vomica* leaf 22.2 EC	2
5	Ethanol alone (positive control)	-
6	Water alone (negative control)	-

The oviposition deterrence study was conducted for all other *S. nux-vomica* solvent extract EC formulations *viz*., methanol, acetone, hexane and chloroform following the same methodology. Oviposition deterrence index was calculated as follows [9],

$$\text{Oviposition deterrency index (\%)} = \frac{B - A}{B + A} \times 100$$

Where,
B - Number of eggs laid on the treated leaf surface
A - Number of eggs laid on the untreated leaf surface

2.6.Statistical Analysis: The laboratory experiment was conducted in completely randomized design. The raw data were subjected to square root transformation and the data on percentage were transformed into arc sine values before statistical analysis. The mean values were separated using LSD through ANOVA.

3.Results and Discussion

The oviposition deterrence effect of EC formulations of different solvent extracts of *S. nux-vomica* against *P. xylostella* under laboratory condition revealed that the extracts of *S. nux-vomica* hindered oviposition ability of *P. xylostella*. The oviposition deterency effect of all the solvent extracts of *S. nux-vomica* are given below.

Oviposition deterency effect of ethanol extracts: Oviposition deterency effect of all the ethanol extracts of *S. nux-vomica* are furnished in Table 1.

Table 1: Oviposition deterrence of EC formulations of *S. nux-vomica* ethanol extracts against *P. xylostella*

Plant materials / Concentration	Leaf (22.2 EC)	Root bark (12.55 EC)	Stem bark (12.55 EC)	Seed (12.55 EC)	Fruit rind (10.00 EC)
0.5%	11.93(20.20)^{d}	15.55(35.26)^{d}	05.37(13.39)^{d}	15.38(23.09)^{d}	16.98(24.33)^{d}
1%	19.37(26.06)^{c}	22.63(23.18)^{c}	11.34(19.67)^{c}	22.63(28.23)^{c}	24.00(29.33)^{c}
1.5%	33.33(35.26)^{b}	36.07(28.40)^{b}	22.73(28.47)^{b}	36.35(37.07)^{b}	37.17(37.56)^{b}
2%	49.45(44.68)^{a}	51.27(36.91)^{a}	31.76(34.30)^{a}	51.97(46.13)^{a}	52.96(46.12)^{a}
Solvent	01.67(7.42)^{e}	01.87(08.12)^{e}	01.65(7.38)^{e}	01.78(7.66)^{e}	1.98(08.08)^{e}
Water	-	-	-	-	-
SE(d)	00.51	00.52	00.53	04.21	6.6820
CD (0.05%)	01.07*	01.10*	01.11*	08.86*	14.03*

Observations are mean of four replicates.
Figures in the parenthesis are arc sine transformed values.
In the column, means followed by common letters are not significantly different by LSD(P=0.05).

The results of experiments revealed the maximum oviposition deterency in ethanol fruit rind extract 10.00 EC at 2 per cent concentration recording 52.96 per cent followed by seed extract 12.55 EC, root bark extract 12.55 EC, leaf extract 22.22 EC and stem bark extract 12.55 EC recording 51.97, 51.27, 49.45 and 31.76 per cent oviposition deterency respectively. This is in line with the findings of [10], who reported that ethanol extracts of *Peganum harmala*L. exhibiting 0.93 per cent oviposition deterency index against *P. xylostella* at 40 mg/ml, [11] investigated oviposition deterency of *Murraya koenigii* L. and *A. indica* A.jus. against pulse beetle, *Callasobruchus maculatus* F. and reported that mixtures of *M. koenigii* L. and *A. indica* A. Juss. showed 100 per cent oviposition deterency followed by *M.koenigii* L., *A. indica* A.Juss. showed 58.92, 50.93 per cent oviposition deterency respectively.

Oviposition deterency effect of methanol extract: Oviposition deterrency effects of all the methanol extracts of *S. nux-vomica* are presented in Table 2.

Table 2: Ovipositiondeterrence of EC formulations of *S. nux-vomica* methanol extracts against *P. xylostella*

Plant materials /Concentration	Leaf (16.66 EC)	Root bark (12.55 EC)	Stem bark (14.25 EC)	Seed (11.11 EC)	Fruit rind (14.25 EC)
0.5%	12.50(19.25)^{d}	15.32(20.70)^{d}	10.77(19.15)^{d}	13.76(21.77)^{d}	15.66(23.31)^{d}
1%	20.00(27.12)^{c}	23.08(28.71)^{c}	17.65(24.84)^{c}	21.57(27.67)^{c}	23.08(28.71)^{c}
1.5%	28.57(31.27)^{b}	31.51(32.31)^{b}	25.00(30.00)^{b}	30.07(33.25)^{b}	33.33(35.26)^{b}
2%	39.34(38.12)^{a}	41.70(38.64)^{a}	36.36(37.08)^{a}	40.38(39.45)^{a}	46.94(43.24)^{a}
Solvent	01.24(06.21)^{e}	01.44(06.93)^{e}	00.74(04.93)^{e}	01.35(06.67)^{e}	1.45(06.91)^{e}
Water	-	--	-	-	-
SE(d)	00.37	00.45	00.50	00.46	00.31
CD (0.05%)	00.78*	00.95*	01.05*	00.98*	00.65*

Observations are mean of four replicates.
Figures in the parenthesis are arc sine transformed values.
In the column, means followed by common letters are not significantly different by LSD(P=0.05).

Among the methanol extracts the maximum oviposition deterency of 46.94 per cent was observed in fruit rind extract 14.25 EC at 2 per cent, followed by root bark extract 12.55 EC, seed extract 11.11 EC, leaf extract 16.66 EC, and stem bark 14.25 EC extract and their oviposition deterency values were 41.70, 40.38, 39.34 and 36.36 per cent respectively. This is in line with the finding of [12] who studied the oviposition deterency effect of aqueous and methanol extracts of *A. calamus*L. against fruitfly, *B. cucurbitae* and reported that both aqueous and methanol extracts possessed oviposition deterency. Methanol extract possessed comparatively higher oviposition deterency than aqueous extract.

Oviposition deterency effect of acetone extract : Oviposition deterrence effect of all the acetone extracts of *S. nux-vomica* are furnished in Table 3.

Table 3: Ovipositiondeterrence of EC formulations of *S. nux-vomica* acetone extracts against *P. xylostella*

Plant materials / Concentration	Leaf (20.00 EC)	Root bark (11.11 EC)	Stem bark (09.00 EC)	Seed (14.28 EC)	Fruit rind (12.50 EC)
0.5%	11.67(19.97)^{d}	16.26(23.78)^{d}	11.74(20.03)^{d}	15.10(22.86)^{d}	17.27(24.55)^{d}
1%	19.11(25.92)^{c}	24.35(29.56)^{c}	18.49(25.46)^{c}	22.61(28.39)^{c}	25.87(30.57)^{c}
1.5%	33.33(35.26)^{b}	40.20(39.34)^{b}	31.93(34.40)^{b}	37.56(37.79)^{b}	41.13(39.89)^{b}
2%	43.32(41.16)^{a}	48.96(44.40)^{a}	44.04(41.57)^{a}	46.11(42.77)^{a}	53.77(47.16)^{a}
Solvent	02.00(08.13)^{e}	02.33(08.72)^{e}	01.97(07.92)^{e}	02.10(08.33)^{e}	02.40(08.91)^{e}
Water	--	--	-	-	-
SE(d)	00.48	00.44	00.49	00.45	00.44
CD (0.05%)	01.01*	00.93*	01.03*	00.95*	00.93*

Observations are mean of four replicates.
Values in the parenthesis are arc sine transformed values.
In the column, means followed by common letters are notsignificantly different by LSD(P=0.05);

The results of the experiment showed the highest oviposition deterency in acetone fruit rind extract 12.50 EC at 2 per cent concentration recording 53.77 per cent followed by root bark extract 11.11 EC, seed extract 14.28 EC, leaf extract 20.00 EC, and stem bark 09.00 EC extract recording 48.96, 46.11, 43.32 and 44.04 per cent respectively, which is in accordance with the findings of [13] reporting the efficacy of acetone extracts of *Peganum harmala*L. recording 71.88 per cent oviposition deterency against the peach fruitfly, *B. zonata* L.

Oviposition deterrence effect of hexane extract: Oviposition deterrence effect of all the acetone extracts of *S. nux-vomica* are furnished in Table 4.

Table 4: Oviposition deterrence of EC formulations of *S. nux-vomica* hexane extracts against *P. xylostella*

Plant materials/ Concentration	Leaf (16.66 EC)	Root bark (11.11 EC)	Stem bark (12.55 EC)	Seed (14.25 EC)	Fruit rind (12.50 EC)
0.5%	18.44(25.43)^{d}	21.34(27.51)^{d}	16.50(23.96)^{d}	19.31(26.06)^{d}	23.64(29.09)^{d}
1%	30.16(33.31)^{c}	35.12(36.34)^{c}	29.03(32.60)^{c}	31.26(33.99)^{c}	37.10(37.52)^{c}
1.5%	44.19(41.66)^{b}	47.63(43.64)^{b}	39.53(38.95)^{b}	45.15(42.21)^{b}	49.78(44.87)^{b}
2%	57.13(49.10)^{a}	63.12(52.60)^{a}	49.07(44.46)^{a}	60.97(51.33)^{a}	65.05(53.76)^{a}
Solvent	02.45(09.00)^{e}	02.98(09.94)^{e}	02.33(08.78)^{e}	02.56(09.20)^{e}	03.00(09.97)^{e}
Water	-	-	-	-	-
SE(d)	00.18	00.17	00.41	00.39	00.38
CD (0.05%)	00.37*	00.37*	00.86*	00.83*	00.81*

Observations are mean of four replicates.
Figures in the parenthesis are arc sine transformed values.
In the column, means followed by common letters are not significantly different by LSD(P=0.05).

Among the hexane extracts of *S. nux-vomica* L. the maximum oviposition deterency of 65.05 per cent was observed in fruit rind 12.50 EC at 2 per cent, followed by root bark extract 11.11 EC, seed extract 14.25 EC, leaf extract 16.66 EC and stem bark extract 12.55 EC and their oviposition deterency values were 63.12, 60.97, 57.13 and 49.07 per cent respectively. The findings were in line with [14] who screened twenty five plants for their oviposition activity against *S. litura*Fab, and reported *Aegle marmelos* ethyl acetate, *Cinnamum zeylanicum* hexane and *Ocimum americanum* ethyl acetate extracts recording strong oviposition deterency activity and their oviposition index were 32.67, 50.81 and 34.64 per cent respectively.

Oviposition deterrence effect ofchloroform extract : Oviposition deterency effect of all the acetone extracts of *S. nux-vomica* are furnished in Table 5.

Table 5: Ovipositiondeterrence of EC formulations of*S. nux-vomica* chloroform extracts against *P. xylostella*

Plant materials/ Concentration	Leaf (20.00 EC)	Root bark (12.55 EC)	Stem bark (12.55 EC)	Seed (11.11 EC)	Fruit rind (10.00 EC)
0.5%	15.81 (23.43)^{d}	21.17 (27.39)^{d}	10.12 (18.54)^{d}	17.21 (24.51)^{d}	24.14 (29.42)^{d}
1%	27.43 (31.58)^{c}	36.13 (36.94)^{c}	27.12 (31.38)^{c}	30.37 (33.44)^{c}	37.40 (37.70)^{c}
1.5%	41.28 (39.97)^{b}	49.58 (44.76)^{b}	34.67 (36.07)^{b}	43.99 (41.54)^{b}	52.54 (46.45)^{b}

2%	58.17 (49.70)[a]	66.34 (54.53)[a]	43.91 (41.50)[a]	61.17 (51.45)[a]	69.81 (56.67)[a]
Solvent	03.24 (10.37)[e]	03.45 (10.70)[e]	03.12 (10.17)[e]	03.33 (10.51)[e]	03.62 (10.96)[e]
Water	-	-	-	-	-
SE(d)	00.18	00.21	00.20	00.23	00.39
CD (0.05%)	00.39*	00.45*	00.42*	00.51*	00.82*

Observations are mean of four replicates. Figures in the parenthesis are arc sine transformed values. In the column, means followed by common letters are not significantly different by LSD(P=0.05).

The results of experiments showed the highest oviposition deterency in chloroform fruit rind extract 10.00 EC at 2 per cent concentration recording 69.81 per cent followed by root bark extract 12.55 EC, seed extract 11.11 EC, leaf extract 20.00 EC, and stem bark extract 12.55 EC recording 66.34, 61.17, 58.17 and 43.91 per cent respectively. The findings were in line with the records of [15] who tested oil extracts of citrus peels of five *citrus sp* namely *Citrus tangina*, *Citrus limonium*, *Citrus paradise*, *Citrus aurantifolia* and *Citrus sinensis* for their effects on oviposition and adult emergence of cowpea weevil, *C. maculates* and revealed that oviposition deterency had maximum in *C. chinensis* (72-79%) and lowest in *C. tangina (*62-68%).

Conclusion

The results of the present study proved that EC formulations of solvent extracts of *S. nux-vomica*L. possessed oviposition deterent effect against *P. xylostella* L. Oviposition deterency effect was recorded maximum in EC formulations of chloroform fruit rind extracts of *S. nux-vomica* L. at 2 per cent concentration. The ovipostion deterency activity also varies according to different solvents and plant parts utilized for experimentation. But all the solvent extracts showed oviposition deterency against *P. xylostellaL*. From this we can conclude that *S. nux-vomica* L. posseses ovipostion deterency effect against *P. xylostella* L. So it can be utilized as a part of pest management program in cruciferous ecosystem to reduce DBM infestation.

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Author Contributions

1. C. Selvaraj, M.Sc. (Ag), Department of Agricultural Entomology, TNAU, Coimbatore-3. He actively conducted the research experiments.
2. Dr. J.S. Kennedy, Professor, Department of Agricultural Entomology, TNAU, Coimbatore-3. He frequently gave the instruction to me in the successful conduct of research experiments.
3. Dr. M. Suganthy, Assistant Professor, Department of Agricultural Entomology, TNAU, Coimbatore-3. She helped me in the successful conduct of research experiment and timely issuance of chemicals needed for the experiments.

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