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Larvicidal action of Nux-vomica (*Strychnus nux-vomica* L.) against Diamond back moth (*Plutella xylostella* L.)

C. Selvaraj*, J. S. Kennedy and M. Suganthy

Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore-641003 (Tamil Nadu), INDIA

*Corresponding author. E-mail: selvaarjun665@gmail.com

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Abstract: The present investigation reports on larvicidal efficacy of plant extracts of Nux-vomica, *Strychnus nux-vomica* against Diamond back moth (DBM). In this investigation shade dried and powdered nux-vomica plant samples (leaves, root bark, stem bark, seed and fruit rind) were extracted with organic solvents ethanol, methanol, acetone, hexane and chloroform and also formulated as Emulsifiable Concentrates (EC) using surfactant and solvents. This formulated plant extracts were tested against third instar larvae of DBM for larvicidal efficacy using leaf disc bioassay method under laboratory condition. Among the five solvent extracts tested, hexane extracts of root bark 11.11 EC @ 2 % showed highest larval mortality of 76.66 % followed by seed 14.25 EC, leaf 16.66 EC, stem bark 12.50 EC and fruit rind 12.50 EC extracts exhibited maximum mortality @ 2 % concentration recording 66.66, 63.33, 56.66 and 40.00 per cent mortality respectively. Positive and negative control such as respective solvent and water showed 10.00 and 3.33 % larval mortality respectively. The results of these experiment clearly indicate that nux-vomica plant possess promising larvicidal action against diamond back moth.

Keywords: Alkaloids, larvicidal action, Nux-vomica, Solvent extracts, *Plutella xylostella*

INTRODUCTION

Cauliflowerris important cruciferous vegetable grown in India with an area of 4,33,870 hectares (Annonymous, 2016). Diamond back moth (DBM) is notorious pest of cauliflower (NIPHM, 2014).An estimate of the total cost associated with damage and management of DBM world wide was 4-5 billion USD per annum (Zalucki *et al*., 2012).In India Krishnamoorthy (2004) reported 52% yieldloss in crucifers due to Diamond Back Moth. Management of this pest has become a remarkable task and farmers apply chemical pesticides once in a week for the effective management of this pest. However indiscriminate use of chemicals has resulted in problems like resurgence, resistance, replacement, impact on non-target organisms, including humans, environmental pollution. Sole reliance on insecticides has facilitated rapid build-up of resistance in the multivoltine DBM, which undergoes 20 generations a year in the tropics (Talekar and Shelton, 1993). 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 and health problems with farmers were also common in states where these crops are grown (Weinberger and Srinivasan, 2009).

Increasing awareness about the deleterious effects of insecticides. Now farmers and researchers are switching over to botanical pesticides, which overcome many problems associated with chemical insecticides especially in the vegetables. In nature more than 1800 plant species are reported to have biopesticidal properties (Grainge *et al.*, 1984). Our present studies in Nux-vomica (*Strychnus nux-vomica)* belongs to the family Loganiaceae commonly known as poison nut, snake wood, strychnine tree, quaker buttons andyetti. It has alkaloids such as strychnine, brucine, vomicine etc. The alkaloids content also varies according to plant parts. Seeds of nux-vomica contain 0.4 and 0.6 % strychnine and brucine, respectively. Other parts of the tree have varying percentage of these two alkaloids *viz.,* 1.7 and 2.8 % in root bark, 0.3 and 0.4 % in root-wood, 0.9 and 2.1 % in stem bark, 0.5 and 0.01 % in stem wood and 0.2 and 0.5 % in leaves, respectively (Bisset *et al*. 1976) By keeping above aspects in mind, experiments were conducted to test the larvicidal efficacy of organic solvent extracts of different plant samples of nux-vomica against Diamond back moth *Plutella xylostella* L..

MATERIALS AND METHODS

Collection and processing of plant samples: Details on availability of *Strychnus nux-vomica* L. were collected from Botanical Survey of India (BSI), Coimbatore in Tamil Nadu. Different plant parts *viz.,* leaves, seeds, stem bark, root bark and fruit rind of *S. nux-vomica* were collected from drought prone area of Krishnagiri district of Tamil Nadu. Fresh leaves, stem bark, root bark, fruits

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and seeds each weighing almost 3 kg were collected from the trees and brought to the laboratory (Plate 1). The plant samples were shade dried in the Entomology laboratory, TNAU, Coimbatore up to two weeks and grind into fine powder and packed in 3 kg plastic containers separately for further usage.

Extraction of active principles from plant samples: Dry powders of plant samples were packed in filter paper made 20 cm × 4.5 cm size cylindrical thimbles. The samples filled thimbles were kept in the cylindrical sample holder present in the soxhelt apparatus and filled with organic solvents such as ethanol, methanol, acetone, hexane and chloroform individually. Plant samples were extracted with organic solvents. When organic solvents mixed with plant samples it produced coloured solution, extraction was done upto this coloured solution became transparent. During extraction process temperature maintenance was an essential task. Over temperature leads to explosion. Temperature ranges vary according to the solvents used for extraction process, temperature maintained during extraction process were *viz.,* ethanol (79^{o}C), methanol (65^{o}C), acetone (56^{o}C), hexane (69^{o}C), chloroform (61^{o}C). This extraction process has taken approximately 12-18 h for each samples. The extracts were collected in 50 ml screw capped vials and excess solvents evaporated in hot water bath (65^{o}C) and concentrated extracts stored at 4^{o}C for further usage by following Yadav *et al.* (2014) with slight modification.

Preparation of EC formulation: Two fifty milliliter capacity beaker was kept in electronic balance, tarred the weight of plastic container and 1 g of crude extract was transferred to plastic containers from screw cap vials. Suggested EC formulation solvent cyclohexanone added drop by drop to the crude extract using micropipette, till crude extract completely soluble in the solvent. Then 1ml of surfactant such as tween 20 or triton X added to the solution. EC formulation must be stable upto 15 min and give milky appearance when dissolved in water. This test was carried out by transferring 1 ml of EC solution to 50ml test tube filled with water. Observation was done after 15 min for whether particles float or settle downside rather it completely miscible. If sometimes particles does not miscible, instead floated on surfaces, in this cases experiment was carried out again. Finally EC formulations were brought to insectary, dissolved to needed concentration with water and progressed the bioassay.

Mass culturing of diamond back moth in laboratory: The culture was started from field collected larvae and maintained on cauliflower leaves, in the laboratory at 27 ± 2^{0}C. The plastic cups were kept inversely, on those cups fresh cauliflower leaves were placed. Entire larval period, larvae were fed with enough amount of cauliflower leaves by frequently changing the leaves day by day. When the larvae pupate in the leaf, the pupae was collected using brush in a petriplate and placed in moth emergence cage, into which the cabbage leaves or mustard seedlings grown in disposable cups and 10 per cent honey solution was provided. The emerging adults were started to laying eggs in large numbers in the mustard seedlings or cabbage leaves. Fresh mustard seedlings/leaves were provided for egg laying. The neonate larvae was transferred into the cabbage leaves by gentle tapping and also by placing the cabbage leaves nearer to the mustard seedlings (with touching) upon which the eggs were laid. The larvae were provided with fresh leaves daily and pupae were collected and placed in moth emergence cage to continue the cycle.

Treatments details: EC formulations of extracts of *S. nux-vomica* used in larvicidal bioassay were following below:

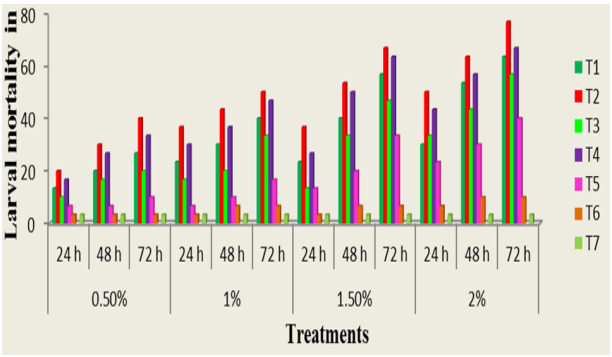
Name of the extracts	EC formulation
Ethanol extracts of nux-vomica leaf	22.22 EC
Ethanol extracts of nux-vomica root bark	12.50 EC
Ethanol extracts of nux-vomicastem bark	12.50 EC
Ethanol extracts of nux-vomica seed	12.50 EC
Ethanol extracts of nux-vomica fruit rind	10.00 EC
Solvent ethanol alone (positive control)	-
Water alone (Negative control)	-

Larvicidal bioassay: Leaf-dip bioassay as per Tabashnik *et al*. (1991) was employed for larvicidal bioassay. Cauliflower leaves were first washed with distilled water and air-dried. Leaf disc of 9 cm diameter were cut and dipped in different concentrations *viz*., 0.5, 1, 1.5 and 2 % of extracts of *S. nux-vomica* EC formulations. Each disc was dipped for 10-20 seconds and allowed to air-dry for a period of one hour. After complete evaporation, the leaves were transferred to clean bioassay containers over a moistened filter paper. The leaf discs were placed slantingly to rest on side of the container so that larvae can move on either side. Ten 3rd instar larvae (~0.8mg) of diamond back moth (*P. xylostella*) were released in each disc and three replicates were maintained per treatment. A treatment water alone served on to negative control and pure solvents at different concentration act as a positive control. Larval mortality was recorded every 24 h, consecutively for 3 days. All the experiments were carried out in a room temperature with a photoperiod of 12:12 (L:D) and experiments with control mortality more than 20% were discarded and repeated.

In the same way larvicidal bioassay were conducted in addition to ethanol, solvents extracts of methanol, acetone, hexane and chloroform also conducted. Per cent larval mortality was calculated by following (Arivoli and Tennyson, 2013).

$$\text{Per cent larval mortality} = \frac{\text{Number of dead larvae}}{\text{Total number of treated larvae}} \times 100$$

In larvicidal bioassay experiments few larvae were died naturally. This natural mortality were corrected using standard formula given by (Abbots, 1925).



T_1- Hexane extracts of *S. nux-vomica* leaf, T_2- Hexane extracts of *S. nux-vomica* root bark, T_3- Hexane extracts of *S. nux-vomica* stem bark, T_4- Hexane extracts of *S. nux-vomica* seed , T_5- Hexane extracts of *S. nux-vomica* fruit rind, T_6- Hexane alone, T_7- Water alone

Fig. 1. *Larvicidal effect of hexane extracts of Strychnus nux-vomica Linn.*

$$\text{Corrected mortality} = \frac{(P - P0)}{(100 - P0)} \times 100$$

Where,

P_0 – Larvae mortality in control

P – Larvae mortality in treatment

Statistical analysis: The data obtained from laboratory experiments were analyzed in completely randomized design (CRD) (Gomez and Gomez, 1984). The mean values were separated using Least square Difference (LSD). The median lethal dose (LD_{50}) and Median lethal concentration (LC_{50}) of insecticides used were determined by Finney's probit analysis (Finney, 1971). The corrected per cent mortality was worked out using the formula given by Abbots, 1925.

Corrected per cent mortality= P_t-P_c / 100-P_c ×100

Where,

P_t - Observed mortality in treatment

P_c - Observed mortality in untreated check

RESULTS AND DISCUSSION

The data summarized on table 1,3,5,7 and 9 represents the larval mortality of DBM varied from plant samples to plant samples and solvents to solvents. Maximum larval mortality was recorded in hexane extracts of nux -vomica root bark 11.11 EC @ 2 % concentration which is 76.66 % followed by seed 14.25 EC, leaf 16.66 EC, stem bark 12.50 EC and fruit rind 12.50 EC extracts recorded maximum mortality @ 2 % concentration which is 66.66, 63.33, 56.66 and 40.00 per cent respectively. In all plant samples order of efficacy was more or less same (Tables 1,3,5,7 and 9) (fig. 1).

Among five plant samples tested for bioefficacy study, hexane extracts of nux-vomica root bark samples was found to be more effective with LC_{50} value of 0.85 % which is followed by seed (1.07 %), leaf (1.34 %), stem (1.75 %)and fruit rind (2.78 %) @ 2 % concentration of formulations (Tables 2,4,6,8 and 10).This larval

Table 1. Larvicidal effect of EC formulation of *S. nux-vomica* ethanol extracts against *Plutellaxylostella* Linn. in laboratory.

Treatments	Larval mortality at (%)											
	0.5 %			1 %			1.5 %			2 %		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
Leaf (22.22 EC)	06.66 (14.95)^{b}	13.20 (21.30)^{b}	20.00 (26.56)^{b}	10.00 (18.43)^{c}	20.00 (26.56)^{c}	26.66 (31.04)^{c}	16.66 (24.09)^{c}	30.00 (33.21)^{c}	43.33 (41.15)^{b}	26.66 (31.08)^{b}	40.00 (39.23)^{c}	50.00 (45.00)^{c}
Root bark (12.55 EC)	10.00 (18.43)^{a}	20.00 (26.56)^{a}	26.60 (31.04)^{a}	23.30 (28.86)^{a}	33.33 (35.24)^{a}	43.33 (41.15)^{a}	23.33 (28.88)^{a}	40.00 (39.23)^{a}	56.66 (48.79)^{a}	33.33 (35.26)^{a}	53.33 (46.89)^{a}	60.00 (50.76)^{a}
Stem bark (12.55 EC)	03.33 (10.51)^{e}	10.00 (18.43)^{c}	16.60 (24.04)^{c}	06.66 (14.95)^{d}	16.66 (24.04)^{d}	20.00 (26.56)^{d}	13.22 (21.30)^{d}	23.33 (28.88)^{d}	33.33 (35.24)^{c}	20.00 (26.56)^{c}	36.66 (37.22)^{d}	43.33 (41.16)^{d}
Seed (12.55 EC)	06.66 (14.95)^{b}	13.20 (21.30)^{b}	20.00 (26.56)^{b}	13.20 (21.30)^{b}	23.33 (28.86)^{b}	30.00 (33.21)^{b}	20.00 (26.56)^{b}	33.33 (35.24)^{b}	43.33 (41.15)^{b}	26.66 (31.08)^{b}	46.66 (43.05)^{b}	53.33 (46.91)^{b}
Fruit rind (10.00 EC)	03.33 (10.51)^{e}	06.66 (14.95)^{d}	10.00 (18.43)^{d}	03.33 (10.51)^{e}	10.00 (18.43)^{e}	13.20 (21.30)^{e}	06.66 (14.95)^{e}	10.00 (18.43)^{e}	16.66 (24.04)^{d}	10.00 (18.43)^{d}	13.20 (21.30)^{e}	23.33 (28.88)^{e}
Solvent	03.33 (10.51)^{e}	03.33 (10.51)^{e}	03.33 (14.95)^{e}	03.33 (10.51)^{e}	06.66 (14.95)^{f}	10.00 (18.43)^{f}	03.33 (10.51)^{f}	06.66 (14.95)^{f}	10.00 (18.43)^{e}	06.66 (14.95)^{e}	10.00 (18.43)^{f}	10.00 (18.43)^{f}
Water	03.33 (10.51)^{e}	03.33 (10.51)^{e}	03.33 (10.51)^{e}	03.33 (10.51)^{e}	03.33 (10.51)^{g}	03.33 (10.51)^{g}	03.33 (10.51)^{f}	03.33 (10.51)^{g}	03.33 (10.51)^{f}	03.33 (10.51)^{f}	03.33 (10.51)^{g}	03.33 (10.51)^{g}
SE (d)	00.71	00.46	00.48	00.64	00.34	00.43	00.82	00.68	00.50	00.69	00.64	00.62
CD (0.05)	01.49*	00.96*	01.01*	01.33*	0.71*	00.89*	01.70*	01.42*	01.04*	01.45*	01.34*	01.30*

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

Table 2. Probit regression analysis of mortality data of *P. xylostella*treated with EC formulations of *S. nux-vomica* ethanol extracts.

Treatments	No. of larvae used	LC_{50} (%)	Fiducial limits (%)		LC_{95} (%)	Fiducial limits (%)		X^2 (cal)	Regression equation	Order of efficacy
			lower	Upper		lower	upper			
Leaf (22.22 EC)	30	2.21	1.27	3.84	23.82	2.86	198.01	00.51	Y=4.45+1.55X	3
Root bark (12.55 EC)	30	1.36	0.95	1.94	14.99	2.61	86.12	00.12	Y=4.78+1.58X	1
Stem bark (12.55 EC)	30	3.15	1.36	7.27	38.82	2.53	594.77	00.81	Y=4.24+1.44X	4
Seed (12.55 EC)	30	2.00	1.24	3.21	19.15	2.94	123.69	00.24	Y=4.49+1.63X	2
Fruit rind (10.00 EC)	30	12.74	0.64	251.45	402.90	0.28	570186.83	0.182	Y=3.79+1.07X	5
Water	30	***	***	***	***	***	***	***	***	***

Table 3. Larvicidaleffect of EC formulations of *S. nux-vomica* methanol extracts against *P. xylostella*in laboratory.

Treatments	Larval mortality at (%)											
	0.5 %			1 %			1.5 %			2 %		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
Leaf (16.66 EC)	06.66 (14.95)^{b}	10.00 (18.43)^{b}	16.66 (24.04)^{c}	06.66 (14.95)^{c}	16.66 (24.04)^{c}	26.66 (31.08)^{c}	16.66 (24.04)^{c}	26.66 (31.04)^{c}	36.66 (37.22)^{c}	23.33 (28.88)^{c}	33.33 (35.24)^{c}	46.66 (43.08)^{c}
Root bark (12.55 EC)	13.20 (21.30)^{a}	16.66 (24.04)^{a}	23.33 (28.86)^{a}	16.66 (24.04)^{a}	26.66 (31.04)^{a}	36.66 (37.26)^{a}	26.66 (31.08)^{a}	40.00 (39.23)^{a}	53.33 (46.89)^{a}	36.66 (37.26)^{a}	50.00 (45.00)^{a}	56.66 (48.82)^{a}
Stem bark (14.25 EC)	03.33 (10.51)^{c}	06.66 (14.95)^{c}	10.00 (18.43)^{d}	06.66 (14.95)^{c}	13.33 (21.38)^{d}	21.66 (27.73)^{d}	13.33 (21.38)^{d}	20.00 (26.56)^{d}	30.00 (33.21)^{d}	20.00 (26.56)^{d}	26.66 (31.04)^{d}	36.66 (37.26)^{d}
Seed (11.11 EC)	6.66 (14.95)^{b}	10.00 (18.43)^{b}	20.00 (26.56)^{b}	10.00 (18.43)^{b}	23.33 (28.86)^{b}	30 (33.21)^{b}	23.33 (28.86)^{b}	30.00 (33.21)^{b}	43.33 (41.15)^{b}	30.00 (33.21)^{b}	40.00 (39.23)^{b}	50.00 (45.00)^{b}
Fruit rind (14.25 EC)	03.33 (10.51)^{c}	03.33 (10.51)^{d}	06.66 (14.95)^{e}	03.33 (10.51)^{d}	03.33 (10.51)^{e}	13.33 (21.41)^{e}	03.33 (10.51)^{e}	10.00 (18.43)^{e}	16.66 (24.04)^{e}	06.66 (14.95)^{e}	13.33 (21.38)^{e}	16.66 (24.09)^{e}
Solvent	03.33 (10.51)^{c}	03.33 (10.51)^{d}	03.33 (10.51)$_{f}$	03.33 (10.51)^{d}	03.33 (10.51)^{e}	6.66 (14.95)^{f}	03.33 (10.51)^{e}	06.66 (14.95)^{f}	06.66 (14.95)^{f}	03.33 (10.51)^{f}	06.66 (14.95)^{f}	06.66 (14.95)^{f}
Water	03.33 (10.51)^{c}	03.33 (10.51)^{d}	03.33 (10.51)^{f}	03.33 (10.51)^{d}	03.33 (10.51)^{e}	03.33 (10.51)^{g}	03.33 (10.51)	03.33 (10.51)^{g}	03.33 (10.51)^{g}	03.33 (10.51)^{f}	03.33 (10.51)^{g}	03.33 (10.51)^{g}
SE (d)	00.88	00.96	00.68	00.94	01.01	00.63	01.02	0.8329	00.55	0.9284	00.56	00.78
CD (0.05)	01.84*	01.99*	01.42*	01.95*	02.11*	01.31*	02.12*	1.732*	01.15*	01.93*	01.17*	01.64*

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

Table 4. Probit regression analysis of mortality data of *P. xylostella*treated with EC formulations of *S. nux-vomica* methanol extracts.

Treatments	No. of larvae used	LC_{50} (%)	Fiducial limits (%)		LC_{95} (%)	Fiduciallimits(%)		X^2 (cal)	Regression equation	Order of efficacy
			lower	upper		lower	upper			
Leaf (16.66 EC)	30	2.58	1.34	4.96	28.30	2.82	283.96	0.103	Y=4.35+1.56X	3
Root bark (12.55 EC)	30	1.58	1.08	2.30	15.34	2.84	82.71	0.251	Y=4.66+1.65X	1
Stem bark (14.25 EC)	30	3.25	1.46	7.24	31.57	2.72	368.34	0.607	Y=4.12+1.76X	4
Seed (11.11 EC)	30	2.17	1.24	3.79	24.98	2.74	227.23	0.136	Y=4.48+1.52X	2
Fruit rind (14.25 EC)	30	9.08	0.98	83.70	138.31	0.74	255575.18	0.098	Y=3.65+1.44X	5
Water	30	***	***	***	***	***	***	***	***	

Table 5. Larvicidaleffect of EC formulations of *S. nux-vomica* acetone extracts against *P. xylostella*in laboratory.

Treatments	Larval mortality at											
	0.5 %			1 %			1.5 %			2 %		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
Leaf (20.00)	10.00 (18.43)^{b}	20.00 (26.56)^{b}	26.66 (31.04)^{b}	16.66 (24.04)^{c}	23.33 (28.86)^{c}	40.00 (39.23)^{b}	20.00 (26.56)^{c}	40.00 (39.23)^{c}	50.00 (45.00)^{c}	30.00 (33.21)^{c}	46.66 (43.05)^{c}	56.66 (48.82)^{c}
Root bark (11.11 EC)	20.00 (26.56)^{a}	26.66 (31.04)^{a}	33.33 (35.24)^{a}	26.66 (31.04)^{a}	33.33 (35.24)^{a}	43.33 (41.15)^{a}	26.66 (31.08)^{a}	46.66 (43.08)^{a}	60.00 (50.76)^{a}	46.66 (43.08)^{a}	56.66 (48.79)^{a}	66.66 (54.73)^{a}
Stem bark (9.00 EC)	06.66 (14.95)^{c}	10.00 (18.43)^{d}	16.66 (24.04)^{d}	13.33 (21.38)^{d}	20.00 (26.56)^{d}	26.66 (31.04)^{c}	16.66 (24.09)^{d}	26.66 (31.08)^{d}	40.00 (39.23)^{d}	26.66 (31.08)^{d}	36.66 (37.22)^{d}	46.66 (43.08)^{d}
Seed (14.28 EC)	10.00 (18.43)^{b}	16.66 (24.04)^{c}	23.33 (28.86)^{c}	20.00 (26.56)^{b}	26.66 (31.04)^{b}	26.66 (31.04)^{b}	23.33 (28.88)^{b}	43.33 (41.16)^{b}	53.33 (46.89)^{b}	43.33 (41.16)^{b}	50.00 (45.00)^{b}	60.00 (50.76)^{b}
Fruit rind (12.50 EC)	03.33 (10.51)^{d}	06.66 (14.95)^{e}	10.00 (18.43)^{e}	06.66 (14.95)^{e}	10.00 (18.43)^{e}	13.33 (21.38)^{d}	10.00 (18.43)^{e}	13.33 (21.41)^{e}	20.00 (26.56)^{e}	13.33 (21.41)^{e}	16.66 (24.04)^{e}	26.66 (31.08)^{e}
Solvent	03.33 (10.51)^{d}	03.33 (10.51)^{f}	03.33 (10.51)^{f}	03.33 (10.51)^{f}	03.33 (10.51)^{f}	03.33 (10.51)^{e}	03.33 (10.51)^{f}	06.66 (14.95)^{f}	06.66 (14.95)^{f}	03.33 (10.51)^{f}	06.66 (14.95)^{f}	06.66 (14.95)^{f}
Water	03.33 (10.51)^{d}	03.33 (10.51)^{f}	03.33 (10.51)^{f}	03.33 (10.51)^{f}	03.33 (10.51)^{f}	03.33 (10.51)^{e}	03.33 (10.51)^{f}	03.33 (10.51)^{g}	03.33 (10.51)^{g}	03.33 (10.51)^{f}	03.33 (10.51)^{g}	03.33 (10.51)^{g}
SE (d)	00.74	00.85	00.64	00.79	00.90	00.61	00.64	00.53	00.78	00.35	00.31	00.31
CD (0.05)	01.53*	01.76*	01.33*	01.65*	01.89*	01.27*	01.34*	01.11*	01.63*	00.73*	00.65*	00.64*

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

Table 6. Probit regression analysis of mortality data of *P. xylostella*treated with EC formulations of *S. nux-vomica* acetone extracts.

Treatments	No. of larvae used	LC_{50}	Fiducial limits (%)		LC_{95}	Fiducial limits (%)		X^2 (Cal)	Regression equation	Order of efficacy
			L.L	U.L		L.L	U.L			
Leaf (20.00)	30	1.61	1.01	2.57	24.95	2.35	264.31	0.0076	Y=4.70+1.38X	2
Root bark (11.11 EC)	30	1.14	0.81	1.61	13.39	2.46	72.83	0.404	Y=4.90+1.52X	1
Stem bark (9.00 EC)	30	2.41	1.33	4.35	24.25	2.94	199.64	0.112	Y=4.37+1.62X	4
Seed (14.28 EC)	30	1.62	1.15	2.28	12.11	3.05	48.05	2.07	Y=4.60+1.83X	3
Fruit rind (12.50 EC)	30	7.18	1.16	44.25	118.34	1.20	11577.28	2.08	Y=3.84+1.28X	5
Water	30	***	***	***	***	***	***	***	***	***

Table 7. Larvicidal effect of EC formulations of *S. nux-vomica* hexane extracts against *P. xylostella.*

Treatments	Larval mortality at											
	0.5 %			1 %			1.5 %			2 %		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
Leaf (16.66 EC)	13.33 (21.38)^{c}	20.00 (26.56)^{c}	26.66 (31.04)^{c}	23.33 (28.86)^{c}	30.00 (33.21)^{c}	40.00 (39.23)^{c}	23.33 (28.86)^{c}	40.00 (39.23)^{c}	56.66 (48.79)^{c}	30.00 (33.21)^{c}	53.33 (47.37)^{c}	63.33 (52.71)^{c}
Root bark (11.11 EC)	20.00 (26.56)^{a}	30.00 (33.21)^{a}	40.00 (39.23)^{a}	36.66 (37.22)^{a}	43.33 (41.15)^{a}	50.00 (45.00)^{a}	36.66 (37.22)^{a}	53.33 (47.37)^{a}	66.66 (54.69)^{a}	50.00 (45.00)^{a}	63.33 (52.71)^{a}	76.66 (61.11)^{a}
Stem bark (12.50 EC)	10.00 (18.43)^{d}	16.66 (24.04)^{d}	20.00 (26.56)^{d}	16.66 (24.04)^{d}	20.00 (26.56)^{d}	33.33 (35.24)^{d}	13.33 (21.38)^{d}	33.33 (35.24)^{d}	46.66 (43.05)^{d}	33.33 (35.24)^{d}	43.33 (41.16)^{d}	56.66 (48.79)^{d}
Seed (14.25 EC)	16.66 (24.04)^{b}	26.66 (31.04)^{b}	33.33 (35.24)^{b}	30.00 (33.21)^{b}	36.66 (37.22)^{b}	46.66 (43.05)^{b}	26.66 (31.04)^{b}	50.00 (45.00)^{b}	63.33 (52.71)^{b}	43.33 (41.16)^{b}	56.66 (48.79)^{b}	66.66 (54.69)^{b}
Fruit rind (12.50 EC)	06.66 (14.95)^{e}	06.66 (14.95)^{e}	10.00 (18.43)^{e}	06.66 (14.95)^{e}	10.00 (18.43)^{e}	16.66 (24.04)^{e}	13.33 (21.38)^{d}	20.00 (26.56)^{e}	33.33 (35.24)^{f}	23.33 (28.86)^{c}	30.00 (33.21)^{e}	40.00 (39.23)^{e}
Solvent	03.33 (10.51)^{f}	03.33 (10.51)^{f}	03.33 (10.51)^{f}	03.33 (10.51)^{f}	06.66 (14.95)^{f}	06.66 (14.95)^{f}	03.33 (10.51)^{e}	06.66 (14.95)^{f}	06.66 (14.95)^{e}	06.66 (14.95)^{f}	10.00 (18.43)^{f}	10.00 (18.43)^{f}
Water	03.33 (10.51)^{f}	03.33 (10.51)^{f}	03.33 (10.51)^{f}	03.33 (10.51)^{f}	03.33 (10.51)^{g}	03.33 (10.51)^{g}	03.33 (10.51)^{e}	03.33 (10.51)^{g}	03.33 (10.51)^{g}	03.33 (10.51)^{g}	03.33 (10.51)^{g}	03.33 (10.51)^{g}
SE (d)	00.80	00.42	00.69	00.60	00.53	00.61	00.43	00.35	00.50	00.50	01.33	00.57
CD (0.05)	01.67*	00.87*	01.44*	01.25*	01.10*	01.28*	0.9008*	00.74*	01.04*	01.05*	02.78*	01.19*

Observations are mean of three replicates; In column, means followed by common letters are not significantly different by LSD(P=0.05); Values in the parenthesis are arc sine transformed values; Total number of larvae per replication =10.

Table 8. Probit regression analysis of mortality data of *P. xylostella*treated with EC formulations of *S. nux-vomica* hexane extracts.

Treatments	No. of larvae used	LC_{50}	Fiducial limits (%)		LC_{95}	Fiducial limits (%)		X^2 (cal)	Regression equation	Order of efficacy
			L.L	U.L		L.L	U.L			
Leaf (16.66 EC)	30	1.34	0.97	1.85	12.08	2.77	52.67	0.21	Y=4.78+1.72X	3
Root bark (11.11 EC)	30	0.85	0.60	1.22	8.43	2.26	31.33	0.69	Y=5.11+1.66X	1
Stem bark (12.50 EC)	30	1.75	1.19	2.58	14.33	3.014	68.19	0.087	Y=4.56+1.77X	4
Seed (14.25 EC)	30	1.07	0.76	1.49	11.83	2.44	57.18	0.25	Y=4.95+1.56X	2
Fruit rind (12.50 EC)	30	2.78	1.59	4.85	16.76	3.35	83.86	0.412	Y=4.06+2.03X	5
Water	30	***	***		***	***	***	***	***	***

Table 9. Larvicidal effect of EC formulations of *S. nux-vomica* chloroform extracts against *P. xylostella*in laboratory.

Treatments	Larval mortality at											
	0.5 %			1 %			1.5 %			2 %		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
Leaf (20.00 EC)	10.00 (18.43)^{c}	20.00 (26.56)^{b}	23.33 (28.86)^{b}	20.00 (26.56)^{b}	26.66 (31.04)^{b}	36.66 (37.22)^{b}	20.00 (26.56)^{b}	40.00 (39.23)^{b}	56.66 (48.79)^{b}	36.66 (37.22)^{b}	53.33 (46.89)^{b}	60.00 (50.76)^{c}
Root bark (12.55 EC)	16.66 (24.04)^{a}	26.66 (31.04)^{a}	30.00 (33.21)^{a}	26.66 (31.04)^{a}	33.33 (35.24)^{a}	43.33 (41.16)^{a}	23.33 (28.86)^{b}	46.66 (43.05)^{a}	63.33 (52.71)^{a}	40.00 (39.23)^{a}	60.00 (50.76)^{a}	70.00 (56.79)^{a}
Stem bark (12.55 EC)	06.66 (14.95)^{d}	13.33 (21.38)^{d}	20.00 (26.56)^{c}	10.00 (18.43)^{d}	20.00 (26.56)^{d}	26.66 (31.04)^{d}	16.66 (24.04)^{c}	30.00 (33.21)^{d}	43.33 (41.16)^{d}	30.00 (33.21)^{d}	40.00 (39.23)^{d}	50.00 (45.00)^{d}
Seed (11.11 EC)	13.33 (21.38)^{b}	16.66 (24.04)^{c}	20.00 (26.56)^{c}	13.33 (21.38)^{c}	23.33 (28.86)^{c}	33.33 (35.24)^{c}	20.00 (26.56)^{e}	36.66 (37.22)^{c}	53.33 (46.89)^{c}	33.33 (35.24)^{c}	50.00 (45.00)^{c}	63.33 (52.71)^{b}
Fruit rind (10.00 EC)	03.33 (10.51)^{d}	06.66 (14.95)^{e}	10.00 (18.43)^{d}	06.66 (14.95)^{e}	13.33 (21.38)^{c}	16.66 (24.04)^{e}	10.00 (18.43)^{d}	16.66 (24.04)^{e}	26.66 (31.04)^{e}	16.66 (24.04)^{e}	23.33 (28.86)^{e}	33.33 (35.24)^{e}
Solvent	03.33 (10.51)^{d}	03.33 (10.51)^{f}	03.33 (10.51)^{e}	03.33 (10.51)^{f}	06.66 (14.95)^{f}	06.66 (14.95)^{f}	03.33 (10.51)^{e}	06.66 (14.95)^{f}	10.00 (18.43)^{f}	06.66 (14.95)^{f}	06.66 (14.95)^{f}	10.00 (18.43)^{f}
Water	03.33 (10.51)^{d}	03.33 (10.51)^{f}	03.33 (10.51)^{e}	03.33 (10.51)^{f}	03.33 (10.51)^{g}	03.33 (10.51)^{g}	03.33 (10.51)^{e}	03.33 (10.51)^{g}	03.33 (10.51)^{g}	03.33 (10.51)^{g}	03.33 (10.51)^{g}	03.33 (10.51)^{g}
SE (d)	00.45	00.66	00.29	00.96	00.58	00.56	00.66	00.65	00.61	00.65	00.35	00.34
CD (0.05)	00.87*	01.37*	00.60*	02.01*	01.20*	01.18*	01.37*	01.35*	01.28*	01.35*	00.74*	00.71*

Observations are mean of three replicates; In column, means followed by a common letter(s) are not significantly different by LSD(P=0.05); Values in the parenthesis are arc sine transformed values.

Table 10. Probit regression analysis of mortality data of *P. xylostella*treated with EC formulations of *S. nux-vomica*chloroform extracts.

Treatments	No. of larvae used	LC_{50}	Fiducial limits (%)		LC_{95}	Fiducial limits (%)		X^2 (Cal)	Regression equation	Order of efficacy
			L.L	U.L		L.L	U.L			
Leaf (20.00 EC)	30	1.45	1.05	2.00	11.45	2.91	45.08	0.41	Y=4.70+1.82X	2
Root bark (12.55 EC)	30	1.11	0.83	1.47	8.30	2.63	26.22	0.46	Y=4.91+1.87X	1
Stem bark (12.55 EC)	30	2.21	1.27	3.84	23.82	2.86	198.01	0.51	Y=4.45+1.55X	4
Seed (11.11 EC)	30	1.48	1.12	1.96	08.59	2.99	24.67	0.39	Y=4.63+2.11X	3
Fruit rind (10.00 EC)	30	3.97	1.53	10.26	36.60	2.67	500.47	0.09	Y=3.98+1.66X	5
Water	30	***	***	***	***	***	***	***	***	***

mortality variations are due alkaloids content variation from plant samples to plant samples and types of solvent used for extraction purposes. Literature on bio efficacy of nux-vomica against DBM is scanty or nil. So I am comparing my studies with available literature on botanicals against DBM. Morallo-Rejesus (1982) reported that ethanol extracts of *Piper nigrum* L. Against *P. Xylostella* Linn. revealed a LC_{50} value 1.819 per cent. Sood and Sharma (2010) found that ethanol extracts of *Adiantumcapillus*-Veneris against *P. xylostella* L. recorded maximum mortality 75 per cent at 20,000 ppm in 72 HAT and LC_{50}value of 6777.66 ppm.

The extracts of *Vitexnegundo, Clerodendrum inerme, Lantana camera,* and *Eupatorium odoratum* caused highest mortality against *A. janata, P. xylostella* and *S. litura* larvae in the laboratory assay (Kulkarni, 2002; Yankanchi, 2003). Methanol extracts of *Adiantumcapillus*-Veneris showed 70 per cent mortality at 20,000ppm in 72 HAT and LC_{50} value was 7,683.89 ppm (Sood and Sharma, 2010). Methanol extracts of *S. nux-vomica* showed 86.00 per cent mortality at 1,000 ppm concentration against Teak defoliator, *Hybleapurea* C. (Senthil kumar *et al*., 2012). According to Senthil kumar *et al*. (2012) acetone extracts of *S. nux-vomica* L. showed 86.67 per cent larval mortality to teak defoliator, *H. purea*C.at 1000 ppm concentration.

Hexane extracts of *S. nux-vomica* L. exhibited 100 per cent larval mortality against *C. quinquefasciatus* Say. 48 HAT at 1000 ppm concentration and recording LC_{50} Value 261.91 ppm (Arivoli and Tennyson, 2012). Chloroform extracts of *S. nux-vomica* exhibited 100 per cent larval mortality against *C. quinquefaciatus* Say. at 1000 ppm in 48 HAT treatments and the LC_{50} values was 1291.2 (Arivoli and Tennyson,2012). Obviously literatures of Arivoli and Tennyson. (2012) on larvicidal efficacy of *S. nux-vomica* against *C. quinquefaciatus* Say and Senthilkumar*et al*. (2012) on insecticidal properties of *S. nux-vomica* against Teak defoliator, *H. purea*supports to my research work.

Conclusion

Totally five plant samples of nux-vomica with five different solvents combination were tested. Among them nux-vomica root samples extracted with hexane showed highest mortality of 76.66 % against third instar larvae of Diamond back moth at root bark 11.11 EC formulation@ 2 % concentration. This result indicates the larvicidal action of nux-vomica against diamond back moth. Though root samples had maximum mortality, collecting root samples is tedious process. Instead of that we can utilize seeds for industrial purposes which also showed 66.66 % mortality against third instar larvae of the moth.

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