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Ecofriendly naturally occurring neem derivatives: As an omnipotent source of Bihar hairy caterpillar, *Spodoptera litura* Fabricius management

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Abstract

A laboratory experiment was conducted out to assess the larvicidal biopotency of neem and its derivatives against third instars larvae of Bihar hairy caterpillar, *Spodoptera litura* Fabricius. The impact neem seed kernel extracts (NSKE), Neem leaf extracts (NLE) and neem based insecticide viz azadirachtin, bioneem, econeem, neemazal, neemarin, nimbecidine, azadirone and neemgold were tested by feeding *Spodoptera litura* Fabricius of mustard at Dept. of Zoology, DBS P.G.College Kanpur. The spraying of the insecticidal preparation was done in glass petridishes (10cm diameter) by potters spray tower, using 1.0 ml. of solution per petridish. Three treatments were introduced and each treatment with three replications, along with one control (Benzene + emulsified water). Ten third instars larvae of *S. litura* were released for feeding inside each pair of petri-dishes and allowed to remain there up to 6hr, 12hr and 24hours under control conditions (25 ± 1 °C temp. $75 \pm 2\%$ relative humidity). The result on the insecticidal biopotency of different neem based insecticidal treatments showed that the treatment with Azadirachtin 68.63 per cent was found highly effective for the control of this pest followed by Bioneem which killed 67.12% against 3rd instar larvae of larvae of *S. litura*. The insecticidal biopotency of remaining neem based formulations were arranged in the following descending order on the basis of their respective relative mean mortality percentage of third instars larvae of larvae of *S. litura* i.e. Econeem (66.39%) >Neemgold (66.20%) > Neemazal (63.28%) > NSKE (60.75%) >Neemarin (56.49%) > NLE (54.87%) >Nimbecidine (50.26%) > Azadirone (44.98%) and control (12.22%), respectively. Thus the above mentioned meliaceous based insecticides was found to be the most economical insecticide against sawfly infesting mustard.

Keywords: Azadirachtin, bioneem, *Spodoptera litura*, Econeem and Neemgold

Introduction

Under the intensive cropping systems, the indiscriminate and unilateral use of synthetic chemicals was the only plant protection tool for sustaining of agricultural production potential of the high yielding varieties [1-3]. This has led to health hazards to human and animal, ecological imbalance and environmental pollution as well as destruction of bio-control agents etc [4, 5, 6, 7].

Mustard, rapeseed, cabbage, cauliflower, knol-khol, turnip, radish to name a few are some important cruciferous vegetables being raised for food. Most cruciferous vegetables are rich in vitamins A and C and minerals like as folate and vitamin K. Dark green cruciferous veggies are contain phytonutrients [8, 9].

Bihar hairy caterpillar, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae), is an important pest of mustard, which attacks all types of cruciferous plants. Larvae alone are destructive and start to feed from margin of leaves [10]. The grown up larvae make holes preferably on young leaves and skeletonise them. Sometimes they also feed on the epidermis of the tender shoots, flowers and fruits. The infected seeds are unfit for human consumption and oil extraction [11]. The population of *Spodoptera litura* in *Brassica campestris* increased with increased dose of nitrogen whereas high doses of P2 O5 and K2 O reduced incidence of the pest [12]. The insect is prevalent during December and January and May-june [13]. These pesticides are also expensive and out of reach of the poor farmers. Therefore, there is a need to develop alternative ecofriendly, biodegradable cheap and indigenous methods of pest management [14].

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Meliaceous biorational extractives and their derivatives possess a large variety of anti-insect biopotency including, repellent, insecticidal and antifeedant activity^[15, 16]. *Neem* oil, *Neem* oil extractive (NOE), *neem* leaves extracts (NLE) and *neem* seed kernel extracts (NSKE) from *Azadirachta indica* A. Juss have been used in India since ancient times for the protection of plants from insect attack. Biopesticides made from neem are biodegradable, non-toxic, eco-friendly and have no residual effect on agriculture produce^[17-19]. This concept has the bearing upon the present investigation that evaluates the bioefficacy of neem derivatives. This remarkable observation had aroused the interest of chemists and biologists all over the world during the past two decades, and as a result, more than 100 compounds like Nimbin, Azadiradione, Nimbin and Salannin have been isolated from all parts of the tree^[20-23].

Neem extracts make an ideal insect control for farmers worldwide by providing a safe, inexpensive and very effective insect control for both ends of the agricultural spectrum^[24]. Biopesticides made from neem are biodegradable, non-toxic, eco-friendly and have no residual effect on agriculture produce. This concept has the bearing upon the investigation that evaluates the bioefficacy of neem derivatives on okra fruit borer, *Helicoverpa armigera* Hubner. Some similar plants are known to contain bioactive metabolites, which show antifeedant, repellent and toxic effects on a wide range of insect pests^[25-27].

Farmers of India and abroad are facing a major problem of growing vegetables fruits and crops due to the infestation of Bihar hairy caterpillar, *Spodoptera litura* Fabricius^[28, 29]. Therefore, the search of indigenous Bio-pesticides of meliaceous botanical origin have become the focus of attention today to combat the problem of the Bihar hairy caterpillar, *Spodoptera litura* Fabricius. Problems in an eco-friendly manner^[30, 31]. The use of botanical pesticides is now emerging as one of the prime means to protect crop, vegetables and stored grains. Botanical pesticides can greatly decrease the use of conventional insecticides or can be used in rotation or in combination with other insecticides, potentially lessening the overall quantities applied and possibly mitigating or delaying the development of resistance in pest populations^[32, 33].

Material and methods

The present study was conducted in the Department of Zoology, D.B.S. College, affiliated to Chhatrapati Shahu Ji Maharaj University, Kanpur, India. The laboratory culture of Bihar hairy caterpillar, *Spodoptera litura* was initiated from the eggs collected from fields at C.S. Azad university of Agriculture and Technology, Kanpur. The insects were reared in the laboratory at $27 \pm 2^\circ \text{C}$ on a diet of mustard leaves^[34]. The collected eggs were placed in a well ventilated plastic container and mustard leaves were provided to newly hatched larvae. The laboratory reared third instar larvae of Bihar hairy caterpillar, *Spodoptera litura* Fabricius were used for the present investigation to evaluate the insecticidal efficacy of neem derivatives.

Mass culturing of Bihar hairy caterpillar, *Spodoptera litura*

The larvae of hairy caterpillar, *Spodoptera litura* were obtained from the experimental farms of CSAZad University of Agriculture and Technology, Kanpur and maintained in

the laboratory on natural diets. The collected larvae were kept for at least 5 days in the laboratory to check, whether or not, there are any other infections before using them for experiments. Bihar hairy caterpillar, *Spodoptera litura* were mass reared on mustard leaves in the laboratory. The mass culturing was initiated by confining 10-20 grubs of mustard sawfly in the plastic containers of 59 x 21 x 18 cm having green mustard leaves which were then covered with muslin cloth and secured tightly with rubber band. Mass culture of *Spodoptera litura* Fabricius larvae was done at $28 \pm 2^\circ \text{C}$ temperature in the plastic container and observed daily.

Procurement of raw plant materials: In the present investigation the neem plant materials were collected while their derivatives were obtained from local market and used for their insecticidal effectiveness against third instar larvae of *Spodoptera litura* in the laboratory trials.

Preparation of powder: Fresh collected green plant parts (leaves and seeds etc) were washed with distilled water and kept in the laboratory for 7 days for air drying followed by one day sun drying before making powder. Electric grinder was used to have coarse powder then these were passed through a 60-mesh sieve to get fine powder. Powders were kept in polythene bags at room temperature and properly sealed to prevent quality loss.

Extraction of Botanical Materials: For the extraction, Soxhlet Apparatus was used; about 20g powder of each category of powder were extracted with 300 ml of different solvents (n-hexane, acetone, methanol, petroleum ether and distilled water). Extraction of each powder was done in about 12 hrs. After Soxhlet extraction, the material was run on rotary evaporator. The extracts were concentrated on rotary evaporator by removing the excess solvent under vacuum. After evaporation of solvent with rotary evaporator the remaining extracted material was kept on water bath for removing remaining solvent from the extracts. The extracts were stored at 4°C prior to application.

Stock Solution Preparation: For stock solution, 50ml. extract in each case was taken into reagent bottles and 50ml. benzene was added in it to dissolve the constituents of the materials. The mouth of the bottles were stopped with airtight corks after which, these bottles containing the solutions were kept in refrigerator. The alcoholic extracts of *Neem* seed kernel extracts (NSKE) and *Neem* leaf extracts (NLE) and *neem* based insecticide viz azadirachtin, bioneem, econeem, neemazal, neemarin, nimbecidine, azadirone and neemgold were tested under laboratory condition against third instar starved larvae of *Spodoptera litura*, which is a noxious insect pest of cruciferous vegetables and crops. The details of which, are described as under:-

The Insecticidal Concentrations: Five concentrations of neem extract and their derivatives (0.25, 0.5, 1.0, 1.5, 2.0 percent) and were used for experiments on repellent tests in the laboratory conditions. The different concentrations of the herbal extracts were prepared from the stock solution using benzene as solvent and Triton X-100 as emulsifier. The level of solvent and emulsifier were kept constant.

Table 1: Detailed List of Neem & Neem Based Insecticides

Botanical Name	Solvent	Concentration with
<i>Neem</i> seed kernel Extracts	Alcohol	Benzene+emulsified + H ₂ O
<i>Neem</i> leaf Extracts	Alcohol	Benzene+emulsified + H ₂ O
Deoiled <i>Neem</i> Seed kernel powder suspension	Alcohol	Benzene+emulsified + H ₂ O
Neem Based Insecticides		
Azadirachtin	—	Benzene+emulsified + H ₂ O
Azadirone	—	Benzene+emulsified + H ₂ O
Bioneem	—	Benzene+emulsified + H ₂ O
Econeem	—	Benzene+emulsified + H ₂ O
Neemazal	—	Benzene+emulsified+ H ₂ O
Neemgold	—	Benzene+emulsified + H ₂ O
Neemarin	—	Benzene+emulsified + H ₂ O
Nimbecidine	—	Benzene+emulsified + H ₂ O

Table 2: Formulations of Extracts

Concentration (%)	Amount of Stock Solution (ml)	Amount of Benzene (ml)	Amount of Emulsifiable Water (ml)	Total Amount (ml)
0.25	1.25	23.75	475.00	500.00
0.50	2.50	22.50	475.00	500.00
1.00	5.00	20.00	475.00	500.00
1.50	7.50	17.50	475.00	500.00
2.00	10.00	15.00	475.00	500.00

Experimental Protocol

The alcoholic extracts of *Neem* seed kernel extracts (NSKE) and *Neem* leaf extracts (NLE) and *neem* based insecticide viz azadirachtin, bioneem, econeem, neemazal, neemarin, nimbecidine, azadirone and neemgold were tested under laboratory against third instar starved larvae of *Spodoptera litura*, which is nuisance insect pest of cruciferous vegetables and crops. For testing the insecticidal effect the mustard leaves were used as food against the third instar larvae of *Spodoptera litura* treated with different concentrations of eleven *neem* extracts and *neem* based insecticides. The treated foods were kept in jar (23cm x

10cm) on moist filter paper. Then third instar, 24 hours starved larvae of *Spodoptera litura* were released in each jar. In each set of extract and one control was introduced, where the leaf pieces were dipped in Benzene + emulsified water only. After four hours of the release of larvae the data was collected on the number of larvae reached at each treated food. Three replication of treatment were made. The mortality effect of all the extracts was judged by counting the number of larvae after 4 hours, present on the treated leaf in each treatment and the percentage of repellency were adjudged over control. All the values were calculated as per Abbott formula [35].

Table 3: Mortality % of larvae of *Spodoptera litura* different periods/concentration of neem products

Treatment (Plant Extracts)	Con. (%)	Mean mortality % after					
		6 Hrs.		12 Hrs.		24 Hrs.	
		T ₁	TBV ₁	T ₂	TBV ₂	T ₃	TBV ₃
Azadirachtin	0.5	59.01	73.1	61.22	76.8	63.44	80.0
Azadirachtin	1.0	59.01	73.1	63.44	80.0	66.15	83.7
Azadirachtin	2.0	71.56	90.0	83.84	98.8	90.00	100.0
Bioneem	0.5	54.78	66.7	56.79	70.0	59.01	73.4
Bioneem	1.0	61.22	76.8	63.44	80.0	66.15	83.4
Bioneem	2.0	75.00	93.0	77.71	95.5	90.00	100.0
Econeem	0.5	50.77	60.0	52.78	63.4	59.01	73.4
Econeem	1.0	61.22	76.8	63.93	80.7	68.85	68.8
Econeem	2.0	71.56	90.0	77.71	95.5	90.00	100.0
Neemazal	0.5	52.78	63.4	54.78	66.7	61.22	76.8
Neemazal	1.0	61.22	76.8	63.44	80.0	66.15	83.7
Neemazal	2.0	66.15	83.7	68.85	87.0	75.00	93.0
Neemarin	0.5	43.08	46.6	45.00	50.0	46.92	53.3
Neemarin	1.0	54.78	66.7	59.01	73.4	59.07	80.0
Neemarin	2.0	61.22	76.8	66.15	84.7	68.85	87.0
Nimbecidin	0.5	37.22	36.5	39.23	40.0	41.15	43.3
Nimbecidin	1.0	46.92	53.3	50.85	60.0	52.78	63.4
Nimbecidin	2.0	59.01	73.4	61.22	76.8	63.93	80.7
Azadirone	0.5	41.07	43.2	45.00	50.0	46.92	53.4
Azadirone	1.0	48.85	56.8	50.77	60.0	54.78	66.7
Azadirone	2.0	54.78	66.7	57.00	70.3	54.78	70.0
Neemgold	0.5	41.15	43.3	45.00	50.0	46.92	53.3
Neemgold	1.0	61.22	76.3	66.15	83.7	71.56	90.0
Neemgold	2.0	83.85	98.8	90.00	100.0	90.00	100.0
N.S.K.E.	0.5	41.15	43.3	45.00	50.0	48.85	56.8

N.S.K.E.	1.0	52.78	63.4	59.01	73.4	66.15	83.7
N.S.K.E.	2.0	68.85	87.0	75.00	93.0	90.00	100.0
N.L.E	0.5	37.22	36.5	43.08	46.6	46.92	53.3
N.L.E	1.0	46.92	53.3	48.85	56.8	52.78	63.4
N.L.E	2.0	68.85	87.0	71.56	90.0	77.71	95.5
Control	-	00.00	0.00	18.44	10.0	18.44	10.0

(T₁, T₂, T₃ = Treatments and T.B.V.₁, T.B.V.₂, T.B.V.₃=Transformed Back Values)

C.D. for treatment x period means=0.078

C.D. for treatment means (plant extra) = 0.037

C.D. for treatment means (control) = 0.162

Table 4: Mean Mortality % of larvae of *Spodoptera litura* different period irrespective of concentration

Treatment	6	Mean	Mortality	Percent	after		Mean	Mor-
(Plant Extracts)	6	hrs.	12	hrs.	24	Hrs.	tality	(%)
	T ₁	TBV ₁	T ₂	TBV ₂	T ₃	TBV ₃	G.T.	TBV
Azadirachtin	63.19	79.6	69.50	87.7	73.19	91.6	68.63	86.7
Bioneem	63.67	86.8	65.97	83.4	71.71	90.1	67.12	84.9
Econeem	61.18	75.3	64.80	81.9	73.19	91.6	66.39	84.0
Neemazal	60.05	75.0	62.35	78.4	67.45	85.3	63.28	79.8
Neemarin	53.03	63.8	56.71	69.9	59.73	74.6	56.49	69.5
Nimbeecidin	47.72	54.7	50.43	59.4	52.62	63.1	50.26	59.1
Azadirone	41.71	44.2	44.97	49.9	48.26	55.7	44.98	49.9
Neemgold	61.18	75.3	64.80	81.9	72.62	91.1	66.20	83.7
N.S.K.E.	54.26	65.9	59.66	74.5	68.33	86.3	60.75	76.1
N.L.E.	51.00	60.4	54.49	66.2	59.13	73.7	54.87	66.9
D.N.S.K.P.S.	56.95	70.2	59.80	74.7	65.95	83.4	60.90	76.3
Control	00.00	0.00	18.44	10.0	18.44	10.0	12.22	04.5

(T₁, T₂, T₃ = Treatments and T.B.V.₁, T.B.V.₂, T.B.V.₅=Transformed Back Values)

C.D. for treatment x period means=0.078

C.D. for treatment means (plant extra) = 0.037

C.D. for treatment means (control) = 0.162

Table 5: Mean mortality percentage of larvae of *Spodoptera litura* different concentration irrespective of treatments under In-vitro.

Concentration %	6	Mean	mortality	Percent	after		Mean	Mortality
Neem Products	6	hrs.	12	hrs.	24	Hrs.		(%)
	T ₁	TBV ₁	T ₂	TBV ₂	T ₃	TBV ₃	G.T.	TBV
0.5/0.01	46.43	52.5	49.51	57.9	52.52	63.0	49.48	57.8
1.0/0.03	55.53	68.0	58.71	68.3	60.23	70.4	59.16	73.7
2.0/0.05	68.80	86.9	72.89	91.4	79.00	96.4	73.56	92.0

(T₁, T₂, T₃ = Treatments and T.B.V.₁, T.B.V.₂, T.B.V.₅=Transformed Back Values)

C.D. for period means (extract = 0.026)

C.D. for concentration means (Control = 0.173)

Results and discussion

The data depicted in text indicated that all the neem extracts and neem based insecticides have proved to be more or less effective in controlling the larvae of *Spodoptera litura* under laboratory trials. The larvae of *Spodoptera litura* infestation was recorded only at an early stage of crop growth and it was limited for a month only. The pooled mean data presented in Table-3 and figure 1-3 reveals that biopotency of different neem based insecticidal treatments showed that the treatment with Azadirachtin 68.63 per cent was found highly effective for the control of this pest followed by Bioneem which killed 67.12% against 3rd instar larvae of larvae of *S. litura*. The insecticidal biopotency of remaining neem based formulations were arranged in the following descending order on the basis of their respective relative mean mortality percentage of third instars larvae of larvae of *S. litura* i.e. Econeem (66.39%) >Neemgold (66.20%) > Neemazal (63.28%) > NSKE (60.75%) >Neemarin (56.49%) > NLE (54.87%) >Nimbicidine (50.26%) > Azadirone (44.98%) and control (12.22%), respectively.

Many plants can protect themselves against insects by producing their own chemical defenses that are toxic or repellent [36, 37]. The consideration for the use of extracts of

plants origin is that they are easily biodegradable, effective on some pests and considered safe in pest control operations as they minimize pesticide residues, ensure safety of the consumers of the treated grains and the environment [38]. Further, the production of organic extracts of plant origin for pest control may be easier and less expensive than the synthesis of some complex chemical based pesticides. Moreover organic extract of plant origin possess many of the attributes of an ideal biological control agent, including broad host range, high virulence, host seeking capability, ease of mass production, recycling ability, non- hazardous to environment, etc. [39].

Neem based products is a natural insecticide, absolutely non-toxic, 100% biodegradable and environmentally friendly in nature [40]. If required, it can be mixed with other synthetic pesticides. Gradually, the ratio of Neem content in the mixture can be increased and synthetics reduced till a stage is reached where synthetics become redundant. Neem consists of several compounds hence development of resistance is impossible. Neem does not destroy natural enemies of pests thereby allowing these natural enemies to keep a check on the pest population [41, 42]. Neem also has a systemic action and seedlings can absorb and accumulate

the neem compounds to make the whole plant pest resistant. Neem has a broad spectrum of action active on many species of pests^[43]. Neem is harmless to non-target and beneficial organisms like pollinators, honey bees, mammals and other vertebrates.

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