Pesticide activity of unripe neem fruit milk on chosen sucking and defoliators pests

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ABSTRACT

Many phytochemicals have been used to manage the economically important insect pest. One of the most promising phytochemicals is azadirachtin, a bioactive principle of *Azadirachta indica.* This key ingredient is present in the leaves, stem, fruit, flower, and bark of the neem tree. There is numerous research work that has been done against insect pests using azadirachtin as a pesticide. So far no work has been done by using unripe neem fruit milk against insect pests. In this work, unripe neem fruit milk is used as the toxicant at different concentrations (0.1, 0.2, 0.4, and 0.8%) against *Dysdercus koenigii* fourth nymphal instars, *Spodoptera litura* third stadium larvae, *Helicoverpa armigera* third stadium larvae, *Aulacophora foveicollis* adults, *Sphenarches caffer* larvae, and *Dysdercus koenigii* eggs. From this study, the result reveals that unripe fruit of neem milk at 0.8% is highly effective against *S. litura* third-stadium larvae (100±0.00%) and *A. foveicollis* adults (100±0.00%), followed by *D. koenigii* fourth nymphal instars (91.67±8.33%); *S. caffer* larvae (68.75±11.97%) at 96 hours; and the toxicant is least effective against *H. armigera* third-stadium larvae (12.00±4.89). The ovicidal activity is effective at 0.8% against *D. koenigii* eggs at 72 and 96 hrs. The test material also caused morphological deformities, and hence we suggest utilizing unripe neem fruit milk as a pesticidal material.

Key words: Unripe neem fruit milk, toxicant, leaf dip method, Insect pests, LCs

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INTRODUCTION

Azadirachta indica, commonly known as neem, is an eminent botanical source for sustainable pest management, belonging to the Mahogany family (Meliaceae) and cultivated in tropical regions of the Indian subcontinent (Paul *et al.,* 2011; Kumar and Navaratnam, 2013). The escalating threat of insecticide resistance in agricultural pests has intensified the search for eco-friendly alternatives, establishing neem-based products as promising options for bio-intensive integrated pest management (BIPM) programs.

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants that protect against disease and contribute to plant defense mechanisms (Saxena *et al.,* 2013). The neem tree contains more than 300 phytochemicals, including triterpenoids, alkaloids, flavonoids, carotenoids, phenolic compounds, and ketones (Akila and Rani, 1999; Biswas *et al.,* 2002). Azadirachtin, a tetranortriterpenoid limonoid and the principal insecticidal constituent, is present in seeds, leaves, fruits, flowers, and bark, with highest concentrations in seed kernels (Dawkar *et al.,* 2019). Azadirachtin operates through multiple molecular targets, including juvenile hormone esterase, apolipophorin III, and fatty acid binding protein, rendering it distinctly different from conventional synthetic insecticides and dramatically reducing the likelihood of resistance

development (Dawkar *et al.,* 2019; Dhakad *et al.,* 2025).

The insecticidal activity of azadirachtin manifests through diverse mechanisms: reduction of feeding activity, suspension of molting, disruption of egg, larval, and pupal development, blocking of molting, disruption of mating, sterilization of adults, and direct poisoning of larvae and adults (Dawkar *et al.,* 2019). Metabolomic analysis demonstrates that azadirachtin exposure causes significant alterations in lipid and citrate cycle metabolism, generating phenotypes including bursting, blackening, and half-molting during larval development (Dawkar *et al.,* 2019).

While extensive research has established the efficacy of neem extracts from leaves, bark, and seeds, neem fruits remain considerably unexplored. Neem fruits are small drupes (1–2 cm) with green exteriors and white milky juice when unripe, comprising a thin epicarp, mucilaginous fleshy mesocarp, and hard endocarp containing ovoid oil seeds (Puri, 1999; Orwa *et al.,* 2009). The mucilaginous nature of unripe neem fruit suggests bioactive compounds amenable to extraction as fruit milk, yet no systematic evaluation of this fraction's pesticidal potential exists. Recent research demonstrates that plant extracts can substantially reduce pest populations through multiple mechanisms, affecting digestive physiological enzymes (amylase, protease, lipase, and invertase) and inducing morphological abnormalities, including abnormal wing formation and defective appendages (Lee *et al.,* 2015; Madasamy *et al.,* 2023). Neem-based formulations demonstrate ovicidal activity by damaging chorionic layers, sealing aeropyles, and disrupting vitellogenesis (Kala *et al.,* 2019).

This investigation targets economically significant agricultural pests: *Dysdercus koenigii* (red cotton bug, Hemiptera), *Spodoptera litura* (tobacco cutworm, Lepidoptera), *Helicoverpa armigera* (cotton bollworm, Lepidoptera) affecting more than 200 plant species, *Aulacophora foveicollis* (red pumpkin beetle, Coleoptera), and *Sphenarches caffer* (red locust tree seed-eater, Lepidoptera). The present study evaluates the impacts of unripe neem fruit milk at different concentrations (0.1, 0.2, 0.4, and 0.8%) on mortality of larval/nymphal stages and ovicidal activity against *Dysdercus koenigii* eggs using the leaf dip method, addressing a significant gap in botanical pest management literature.

MATERIALS AND METHODS

Collection and Maintenance of Insect Pests

Third instar larvae of *H. armigera,* third instar larvae of *S. litura*, and fourth instar nymphs and adults of *D. koenigii* were collected from *Abelmoschus esculentus* (okra) fields in Kuthukkal, Tirunelveli district, Tamil Nadu, India. Larvae of *Sphenarches caffer* and adults of *Aulacophora foveicollis* were collected from *Lagenaria siceraria* (bottle gourd) fields in the same region. All insects were reared in the Crop Protection Research Centre (CPRC) at 27 ± 2°C, 65 ± 5% RH, and a 12:12 hrs L:D photoperiod. *H. armigera* and *S. litura* larvae were maintained in plastic containers (15 × 10 × 5 cm) and fed fresh okra pods and castor (*Ricinus communis*) leaves, respectively. Pupae were transferred to oviposition chambers (30 × 25 × 20 cm) containing host plants dipped in 5% sucrose solution, while adults were provided a 5% sucrose-vitamin C mixture (1:10 w/v). *D. koenigii* adults and nymphs were fed water-soaked cotton seeds in plastic containers (15 × 10 × 5 cm). *S. caffer* larvae received a 5% sucrose-vitamin C mixture, and *A. foveicollis* adults were fed fresh bottle gourd leaves. F1 progeny of uniform age and physiological condition were used for all bioassays.

Collection of Unripe Neem Fruit Milk

Unripe green fruits of *Azadirachta indica* were collected from NGO-A Colony, Tirunelveli district, Tamil Nadu. Fruits were surface-cleaned with distilled water, and white milky exudate (fruit milk) was extracted by gentle squeezing into sterile 1.5 mL Eppendorf tubes. Fresh milk was prepared daily and used immediately for bioassays to prevent degradation of bioactive compounds.

The following section describes the preparation of Neem Fruit Milk Test Solutions.

Neem fruit milk solutions were prepared at 0.1%, 0.2%, 0.4%, and 0.8% (v/v) concentrations. To make a 0.1% solution, 10 µL of neem milk was mixed with 9.9 mL of DMSO and 1 µL of Teepol as an emulsifier. Higher concentrations were prepared proportionally by increasing neem milk volume while maintaining the DMSO:Teepol (99:1) ratio. The commercial neem product Vijayneem (Madras Fertilizers Ltd., Chennai) was diluted to the field-recommended 0.03% concentration in distilled water.

Preliminary Phytochemical Screening

Qualitative phytochemical screening of neem fruit milk was performed following standard protocols of Brindha *et al.* (1981) and Harborne (1984) for alkaloids, flavonoids, phenols, tannins, saponins, terpenoids, steroids, glycosides, and cardiac glycosides. Total phenols were quantified by extracting 0.5 mL neem milk with 10 mL 80% ethanol at 40°C for 4 hrs, centrifuging at 4500 rpm for 15 min, then mixing 3 mL supernatant with 0.5 mL Folin-Ciocalteu reagent (1:1 with water) and 2 mL 20% Na_2CO_3. Absorbance was measured at 650 nm against a blank and expressed as mg catechol equivalents/g sample using a standard curve (Mallick and Singh, 1980). Total tannins were determined by mixing 1 mL neem milk with 75 mL distilled water, 10 mL 35% Na_2CO_3, and 5 mL Folin-Denis reagent in a 100 mL volumetric flask, incubating for 30 min, and measuring absorbance at 700 nm. Results were expressed as mg tannic acid equivalents/g sample (Aparna, 2000).

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR analysis of neem fruit milk was conducted using a PerkinElmer Spectrum RX I spectrometer (Japan) scanning 4000-400 cm^{-1} at 4 cm^{-1} resolution to identify functional groups of bioactive compounds.

GC-MS Analysis

Active fractions and formulations were analyzed using Clarus 500 GC-MS (PerkinElmer) equipped with an Elite-5MS column (30 m × 0.25 mm × 0.25µm). Oven temperature was programmed from 110°C (2 min isothermal), 10°C/min to 200°C, then 5°C/min to 280°C (9 min isothermal). Helium was used as a carrier gas (1 mL/min), with 3 µL injection (10:1 split ratio), injector temperature 250°C, and ion source 280°C. Electron ionization was at 70 eV; the mass range was 45–450 m/z. Compounds were identified by NIST 2005 library matching based on retention time, mass spectra, and peak area percentage using Turbomass 5.2 software.

Insecticidal Bioassays

Leaf-dip bioassays were conducted following Mansour *et al.* (2010). Fresh host leaves (okra for *H. armigera,* castor for *S. litura*, and bottle gourd for *A. foveicollis* and *S. caffer* were dipped in test solutions for 5 min and air-dried for 10 min. For *D. koenigii*, cotton seeds were soaked for 10 hours. Replications comprised H. *armigera* (5 × 2 third instars) and others (4 × 4 individuals/treatment). The controls received distilled water. Mortality was recorded at 24, 48, 72, and 96 hrs. Corrected mortality was calculated using Abbott's formula (1925). LC_{30}, LC_{50}, and LC_{90} values with fiducial limits were determined by probit analysis (Finney, 1971; SPSS 20.0).

Morphogenetic Studies

Alive larvae were maintained until pupation, and emerged adults were maintained in plastic containers for further data recording. After four days, live insects were fed with the respective untreated plant parts, and morphological changes, if any, were recorded and photographed.

Ovicidal Bioassay

Freshly laid (0-day-old) *D. koenigii* eggs (n=60/treatment, 5 replicates) were placed on Whatman No. 1 filter paper discs (4.5 cm diameter) in plastic containers (5.5 × 4.3 cm). Test solutions (2 mL/10 eggs) were sprayed uniformly using a hand sprayer, ensuring complete egg coverage by rotation. The controls received distilled water. Hatching inhibition was recorded at 72 and 96 hrs, corrected by Abbott's formula (1925). Treated and control eggs were photographed using an Olympus phase contrast microscope (Japan).

Statistical Analysis

LC_{50} values were calculated by probit analysis (SPSS 16). Mortality data were subjected to one-way ANOVA followed by Tukey's HSD test (SPSS 20.0) at $p<0.05$ significance level.

RESULTS

Qualitative analysis of neem milk

Qualitative analysis of *Azadirachta indica* unripe fruit milk (AIURFM) showed the presence of triterpenoids, reducing sugars, tannins, phenolic compounds, phytosterols, cardiac glycosides, terpenoids, carbohydrates, and leucoanthocyanins.

Table 1. Qualitative phytochemical analysis of *Azadirachta indica* unripe fruit milk

Test	Observations
Alkaloids	-
Steroids	-
Cardiac Glycosides	+++
Triterpenoids	+++
Reducing sugars	+++
Tannins	+++
Phenolic compounds	+++
Xanthoprotein	-
Phytosterols	+++
Pholobatannins	-
Saponins	-
Amino acids	-
Aromatic acid	-
Chalcones	-
Anthroquinone	-
Anthocyanins	-
Quinones	-
Terpenoid	++
Flavonoid	-
Essential oils	-
Carbohydrates	+
Coumarins	-
Emodins	-
Polyphenols	-
Lecuoanthocyanins	++

Indicates absence of a compound; + shows presence of a compounds, + less intense, ++ moderately intense and +++ highly intense

The result exhibited the absence of the following compounds: Alkaloids, steroids, xanthoprotein, pholobatannins, saponins, amino acids, aromatic acid, chalcones, anthroquinone, anthocyanins, quinones, flavonoid, essential oils, coumarins, emodins, and polyphenols (Table 1).

Quantitative phytochemical analysis

The quantity of phytochemical analysis of both total phenol and tannins present in AIURFM was determined by spectrophotometric techniques and is tabulated in Figure 1. Total phenols are expressed in gallic acid equivalents of mg/mL. The total phenolic content of neem milk was 0.8422 ± 0.0036 GAE mg/mL. Total tannins are expressed in gallic acid equivalents of mg/mL. The total tannic content of neem milk was 0.639 ± 0.0016 GAE mg/mL.

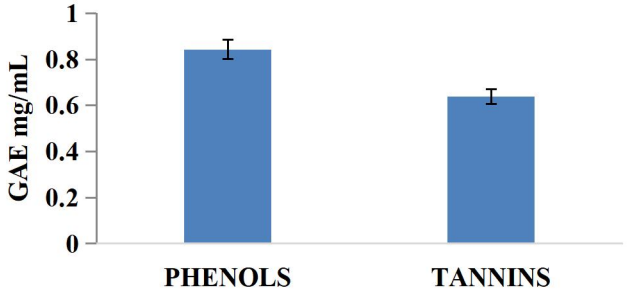

Figure 1. Quantitative analysis of total Phenols (GAE mg/mL) and Tannins (GAE mg/mL) present in *Azadirachta indica* unripe fruit milk.

FTIR analysis

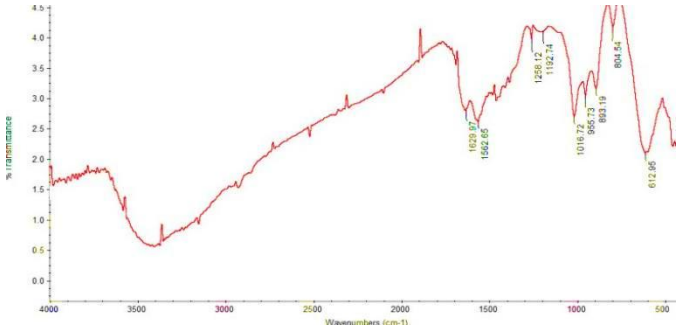

Figure 2. FTIR analysis of *Azadirachta indica* unripe fruit milk.

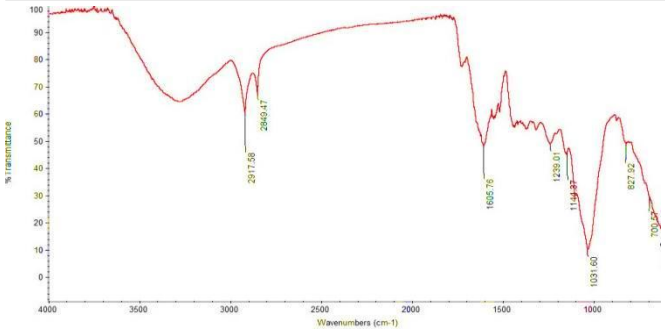

Figure 3. FTIR analysis of *Azadirachta indica* unripe fruit milk with DMSO.

The C–O stretch in the hydroxyl compound can be ascribed to the strong band that appeared at 1026 cm^{-1}. The band around 1238 cm^{-1} is due to the presence of C=O groups in aromatic esters and C-N groups in amine moieties. In the unripe extract, the strong peak appeared at 1407 cm^{-1} that could be assigned to stretching vibrations of C-H and O-H bonds. The band around 1500-1600 cm^{-1} indicates the C=C stretching vibrations of aromatic rings, which are enhanced by the presence of polar functional groups (Table 2).

Table 2. FTIR analysis of *Azadirachta indica* unripe fruit milk and *Azadirachta indica* unripe fruit milk with DMSO

***Azadirachta indica* unripe fruit milk**		***Azadirachta indica* unripe fruit milk with DMSO**	
Frequency (cm^1)	Functional group	Frequency (cm^1)	Functional group
2917.58	O-H stretching alcohol	1629.97	C=C of benzene
2849.47	O-H bond, Hydrogen phosphate group	1562.65	C=C stretching aromatic ring
1605.76	C=O stretching	1258.12	S=O sulfate
1239.01	C-O stretching	1192.74	C-O stretching ester
1144.37	C-O stretching	1016.72	Unknown
1031.60	C-O stretching ether	955.731	Unknown
827.92	Unknown	893.19	Unknown
700.57	Unknown	804.54	Unknown
-	-	612.95	Bending C-OH surface

GC-MS analysis

Major phytochemical constituents present in the *A. indica* neem milk- are silicic acid, diethyl bis(trimethylsilyl) ester (19.76), Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl- (15.31) (Table 3; Fig. 4).

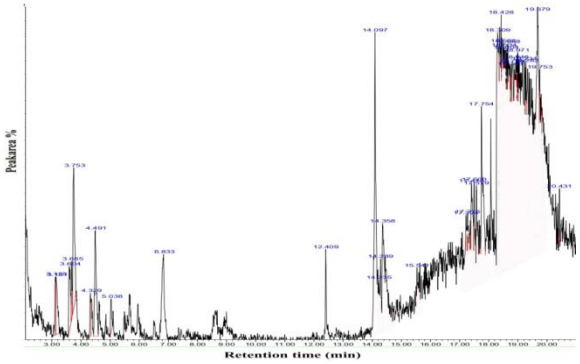

Figure 4. GC-MS analysis of *Azadirachta indica* unripe fruit milk.

Insecticidal Bioassay

Dysdercus koenigii

The significantly highest nymphal mortality was observed in 0.8% of *A. indica* unripe fruit milk (AIURFM) (df= 5,17; F= 15.194; P= 0.000) at 96 hrs when compared to the control. Similarly, at 72 hrs, same concentration significantley caused more mortality (df= 5,18; F= 8.838; P= 0.000); at 48 hrs (df= 5,18; F=9.000; P= 0.000) (Table 4). The Probit analysis confirms mortality results of 96 hrs (LC_{50} = 0.277%) followed by 72 hrs (0.457%), 48 hrs (0.602%) and 24 hrs (0.926%) (Table 5).

Spodoptera litura

Significantly highest larval mortality was observed in higher concentration of AIURFM (0.8%) (df= 5,11; F= 57.006;p= 0.00) and also vijayneem (df= 5,11; F= 57.006; P= 0.00) at 96 hrs when compared to the control (Table 4). The Probit analysis of AIURFM showed best larvicidal (LC_{50}

Table 3. Chemical composition of *Azadirachta indica* unripe fruit milk using GC-MS analysis

S.No	Name of the component	Retention time (min)	Molecular Formula	Molecular weight (g/mol)	Peak area (%)
1	Thymine	3.127	$C_5H_6N_2O_2$	126.11	0.47
2	3H-Pyrazol-3-one, 2,4-dihydro-4,4,5-trimethyl	3.155	$C_{12}H_{14}N_2O$	202.2524	1.26
3	2-Butanamine, (S)-	3.600	$C_4H_{11}N$	73.1368	1.31
4	Propane, 1-(ethenylthio)-	3.685	$C_5H_{10}S$	102.198	0.52
5	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	3.751	$C_6H_6O_4$	144.1253	2.82
6	4-Ethyl-4-methyl-1-hexene	4.328	C_9H_{18}	126.24	0.42
7	3-Hexyn-2-ol, 5-methyl-	4.488	$C_7H_{10}O$	110.15	2.28
8	Cyclohexanone, 2,3-dimethyl-	5.037	$C_8H_{14}O$	126.1962	0.41
9	Pentanoic acid, 4-methyl-	6.833	$C_6H_{12}O_2$	116.1583	2.86
10	n-Hexadecanoic acid	12.412	$C_{16}H_{32}O_2$	256.4241	0.97
11	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	14.095	$C_{21}H_{40}O_4$	356.5399	6.67
12	7-Octen-1-ol, 2,6-dimethyl-	14.237	$C_{10}H_{20}O$	156.2652	0.46
13	Oleic Acid	14.294	$C_{18}H_{34}O_2$	282.5	1.01
14	2H-Pyran-5-carboxylic acid, 6-methyl-2-oxo-, methyl ester	14.360	$C_7H_6O_4$	154.1201	4.85
15	Dodecahydropyrido[1,2-b] isoquinolin-6-one	15.542	$C_{13}H_{21}NO$	207.31	0.43
16	Plumbane, triethylmethyl-	17.225	$C_7H_{18}Pb$	309	1.44
17	Silicic acid, diethyl bis(trimethylsilyl) ester	17.272	$C_{10}H_{28}O_4Si_3$	296.58	19.76
18	3-Methoxy-2,4,5-trifluorobenzoic acid, nonadecyl ester	17.414	$C_{27}H_{43}F_3O_3$	472.6237	2.40
19	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene	17.499 18.747	$C_{17}H_{14}O_4$	282.29	1.46 2.06
20	Ethyl 4,4,6,6,8,8-hexamethyl-11-oxo-3,5,7,9,12-pentaoxa-4,6,8-trisilatetradecan-1-oate	17.575	$C_{14}H_{32}O_8Si_3$	412.65	1.90
21	1,1,1,3,5,5,5-Heptamethyltrisiloxane	17.754 18.605	$C_7H_{21}O_2Si_3$	221.50	7.7
22	tert-Butyl (5-isopropyl-2-methylphenoxy) dimethylsilane	18.312	$C_{16}H_{28}OSi$	264.48	6.26
23	3-Quinolinecarboxylic acid, 6,8-difluoro-4-hydroxy-, ethyl ester	18.426	$C_{12}H_9F_2NO_3$	253.20	8.90
24	1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester	18.501	$C_{23}H_{29}NO_5$	399.49	1.90
25	Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl-	18.974 19.683 18.539	$C_{11}H_{13}NO_3$	207.23	15.31
30	Acetamide, 2-chloro-N-((5-chloro-8-hydroxy-7-quinolinyl) methyl]-	19.749	$C_{12}H_{10}Cl_2N_2O_2$	285.1	3.83
31	1-Benzazirene-1-carboxylic acid, 2,2,5a-trimethyl-1a-[3-oxo-1-butenyl] perhydro-, methyl ester	20.430	$C_{15}H_{23}NO_3$	265.35	0.35

Table 4. Impacts of *Azadirachta indica* unripe fruit milk at different concentrations (0.1, 0.2, 0.4 and 0.8%) and Vijayneem (0.03%) against *Dysdercus koenigii* fourth nymphal instar, *Spodoptera litura, Helicoverpa armigera, Aulacophora foveicollis and Sphenarches caffer*

Hours	Concentration (%)	*Dysdercus koenigii*	*Spodoptera litura*	*Helicoverpa armigera*	*Aulacophora foveicollis*	*Sphenarches caffer*
24	0.1	6.25 ± 6.25^{d}	0.00 ± 0.00	0.00 ± 0.00^{b}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}
	0.2	12.50 ± 7.22^{cd}	8.33 ± 8.33^{c}	0.00 ± 0.00^{b}	8.33 ± 8.33^{c}	0.00 ± 0.00^{e}
	0.4	31.25 ± 6.25^{ab}	33.33 ± 8.33^{b}	0.00 ± 0.00^{b}	16.67 ± 8.33^{b}	6.25 ± 6.25^{d}
	0.8	37.50 ± 12.50^{a}	41.67 ± 8.33^{a}	4.00 ± 4.00^{a}	25.00 ± 14.43^{a}	12.50 ± 7.22^{c}
	Vijayneem	18.75 ± 11.97^{c}	41.67 ± 8.33^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{d}	18.75 ± 11.97^{a}
48	0.1	12.50 ± 7.21^{e}	16.67 ± 8.33^{e}	0.00 ± 0.00^{b}	8.33 ± 8.33^{d}	6.25 ± 6.25^{e}
	0.2	25.00 ± 10.20^{cd}	33.33 ± 8.33^{d}	0.00 ± 0.00^{b}	50.00 ± 14.43^{c}	12.50 ± 12.50^{d}
	0.4	37.50 ± 7.22^{c}	58.33 ± 8.33^{b}	0.00 ± 0.00^{b}	58.33 ± 8.33^{b}	25.00 ± 10.21^{c}
	0.8	62.50 ± 7.22^{a}	66.67 ± 8.33^{a}	4.00 ± 4.00^{a}	83.33 ± 8.33^{a}	37.50 ± 7.22^{b}
	Vijayneem	50.00 ± 10.21^{ab}	50.00 ± 14.43^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{e}	43.75 ± 11.97^{a}
72	0.1	25.00 ± 10.21^{de}	41.67 ± 8.33^{e}	0.00 ± 0.00^{d}	25.00 ± 14.43^{d}	25.00 ± 0.00^{e}
	0.2	37.50 ± 7.22^{d}	50.00 ± 0.00^{d}	0.00 ± 0.00^{d}	58.33 ± 8.33^{c}	25.00 ± 10.21^{e}
	0.4	56.25 ± 6.25^{c}	66.67 ± 8.33^{c}	4.00 ± 4.00^{b}	66.67 ± 8.33^{b}	43.75 ± 6.25^{c}
	0.8	75.00 ± 10.21^{a}	91.67 ± 8.33^{a}	4.00 ± 4.00^{b}	91.67 ± 8.33^{a}	50.00 ± 0.00^{b}
	Vijayneem	68.75 ± 11.97^{b}	75.00 ± 14.43^{b}	8.00 ± 4.89^{a}	25.00 ± 14.43^{d}	56.25 ± 11.97^{a}
96	0.1	31.25 ± 6.25^{d}	50.00 ± 0.00^{e}	0.00 ± 0.00^{e}	41.67 ± 8.33^{d}	25.00 ± 0.00^{e}
	0.2	43.75 ± 6.25^{c}	66.67 ± 8.33^{d}	4.00 ± 4.00^{c}	75.00 ± 14.43^{c}	31.25 ± 15.73^{d}
	0.4	75.00 ± 0.21^{b}	91.67 ± 8.33^{b}	4.00 ± 4.00^{c}	91.67 ± 8.33^{b}	50.00 ± 10.21^{c}
	0.8	91.67 ± 8.33^{a}	100 ± 0.00^{a}	12.00 ± 4.89^{a}	100 ± 0.00^{a}	68.75 ± 11.97^{b}
	Vijayneem	75.00 ± 10.21^{b}	100 ± 0.00^{a}	8.00 ± 4.89^{b}	41.67 ± 8.33^{d}	75.00 ± 10.21^{a}

Table 5. Impacts of *Azadirachta indica* unripe fruit milk at different concentrations (0.1, 0.2, 0.4 and 0.8%) on probit analysis data of *Dysdercus koenigii, Spodoptera litura, Helicoverpa armigera, Aulacophora foveicollis* and *Sphenarches caffer*.

Pest	Hours	LC_{30}	LC_{50}	LC_{90}	Chi-Square	df	Significance (p-value)
Dysdercus koenigii	24	0.580	0.926	1.772	8.667	3	0.013
	48	0.327	0.602	1.274	2.083	3	0.353
	72	0.174	0.457	1.150	3.047	3	0.218
	96	0.101	0.277	0.706	3.141	3	0.208
Spodoptera litura	24	0.381	0.469	0.684	2.718	3	0.099
	48	0.178	0.468	1.179	2.033	3	0.002
	72	0.320	0.197	0.756	0.083	3	0.959
	96	0.070	0.103	0.374	0.084	3	0.959
Helicoverpa armigera	24	1.669	2.079	3.080	0.004	3	0.998
	48	1.669	2.079	3.080	0.004	3	0.998
	72	1.239	1.601	2.487	5.396	3	0.670
	92	1.026	1.122	1.388	3.214	3	0.201
Aulacophora foveicollis	24	0.587	1.077	2.275	2.711	3	0.258
	48	0.118	0.349	0.911	9.047	3	0.011
	72	0.015	0.209	0.757	3.012	3	0.222
	96	0.038	0.138	0.382	6.048	3	0.049
Sphenarches caffer	24	1.058	1.310	1.927	5.150	3	0.076
	48	0.614	0.953	1.783	3.508	3	0.173
	72	0.325	1.009	2.679	1.454	3	0.483
	96	0.174	0.546	1.453	3.825	3	0.148

Table 6. Impacts of *Azadirachta indica* unripe fruit milk at different concentrations (0.1, 0.2, 0.4 and 0.8%) and Vijayneem (0.03%) against *Dysdercus koenigii* eggs.

Hours	Concentration (%)	Mortality (%)	df	F value	Significance
24	0.1	2.00 ± 1.33^{c}	5,54	1.029	0.899
	0.2	2.00 ± 1.33^{c}			0.899
	0.4	3.00 ± 1.53^{b}			0.618
	0.8	4.00 ± 1.63^{a}			0.303
	Vijayneem	3.00 ± 1.53^{b}			0.618
48	0.1	4.00 ± 1.63^{d}	5,54	2.868	0.405
	0.2	5.00 ± 1.67^{c}			0.177
	0.4	6.00 ± 1.63^{b}			0.062
	0.8	7.00 ± 1.53^{a}			0.018
	Vijayneem	6.00 ± 1.63^{b}			0.062
72	0.1	6.00 ± 1.63^{d}	5,54	8.345	0.010
	0.2	7.00 ± 1.53^{c}			0.002
	0.4	8.00 ± 1.33^{b}			0.000
	0.8	10.00 ± 0.00^{a}			0.000
	Vijayneem	8.00 ± 1.33^{b}			0.000
96	0.1	7.00 ± 1.53^{c}	5,54	27.840	0.000
	0.2	9.00 ± 1.00^{b}			0.000
	0.4	10.00 ± 0.00^{a}			0.000
	0.8	10.00 ± 0.00^{a}			0.000

Table 7. Impacts of *Azadirachta indica* unripe fruit milk at different concentrations (0.1, 0.2, 0.4 and 0.8%) on probit analysis data of *Dysdercus koenigii* eggs.

Hours	LC_{30}	LC_{50}	LC_{90}	Chi-Square	Significance
24	0.417	0.874	1.992	0.354	0.552
48	0.248	0.353	1.474	1.477	0.478
72	0.156	0.200	0.483	3.972	0.137
96	0.061	0.170	0.209	0.681	0.712

= 0.103% at 96 hrs; 0.197% at 72 hrs; 0.468% at 48 hrs; 0.469% at 24 hrs) (Table 5).

Helicoverpa armigera

As observed for the *H. armigera,* significantly highest mortality was observed in higher concentration (0.8%) of AIURFM (df= 5,24; F= 1.000; P= 0.465) at 96 hrs when compared to the control (Table 4). The LC_{50} value of 1.689% for 96 hrs followed by 1.947% at 72 hrs; 1.088% at 48 hrs and 24 hrs (Table 5).

Aulacophora foveicollis

Maximum adult mortality was observed in higher concentration of AIURFM (0.8%) (df=5,12; F= 15.229; P= 0.00) at 96 hrs of observation when compared to the control (Table 4). The Probit analysis of neem milk showed best adulticidal LC_{50} value was 0.138% at 96 hrs followed by 0.222% at 72 hrs; 0.356% at 48 hrs and 1.113% at 24 hrs (Table 5).

Sphenarches caffer

The significantly highest larval mortality was observed in vijayneem (df=5,17; F= 5.053; P= 0.008) at 96 hrs when compared to the control, followed by 0.8% (df=5,17; F= 5.053; P= 0.040) at 96 hrs. The standard vijayneem (df= 5,18; F= 5.169; P= 0.007) at 72 hrs; 0.03% (df= 5,18; F= 3.726; P= 0.032) at 48 hrs; 0.03% (df= 5,18; F= 1.600; P= 0.320) at 24 hrs showed significant mortality (Table 4). The Probit analysis of neem milk showed best adulticidal LC_{50} value of 0.558% 96 hrs followed by 0.923% at 72 hrs; 0.953% at 48 hrs; 1.310% at 24 hrs (Table 5).

Ovicidal activity

Ovicidal activity of *A. indica* neem milk at different concentrations against *D. koenigii* was also recorded. Ovicidal activity of tested concentrations showed dose dependent manner. When compared to all the concentrations, the 0.8% showed significantly higher ovicidal activity (df= 5,54; F= 27.840; P= 0.00) at 96 and 48 hrs (Table 6). The Probit analysis of neem milk showed best ovicidal LC_{50} value of 0.17% at 96 hrs followed by 0.20% at 72 hrs; 0.353% at 48 hrs; 0.874% at 24 hrs (Table 7).

Morphogensis

In *D. koenigii*, control category of both nymphs and adults are normal (a), since, morphogenesis is not taken place properly leads to abnormal animal (b) at higher concentration (0.8%), either the legs are with abnormality (c) or wings are not formed properly; as a result, it protruded outside the body (d) by the lower concentration (Figure 5). Impact of various botanicals on the abnormalities of *S. caffer* is not available in the literature. But our results reveal that application of unripe neem fruit milk caused enlargement of abdomen (b), slowly change of colour of the abdomen (b-d) and reduced body size (e) (Figure 7).

Figure 5. *Dysdercus koenigii*; a- Normal nymph, b-Normal adult, c and d-Abnormality adult caused by higher concentration (0.8%), e and f- leg abnormality caused by lower concentrations.

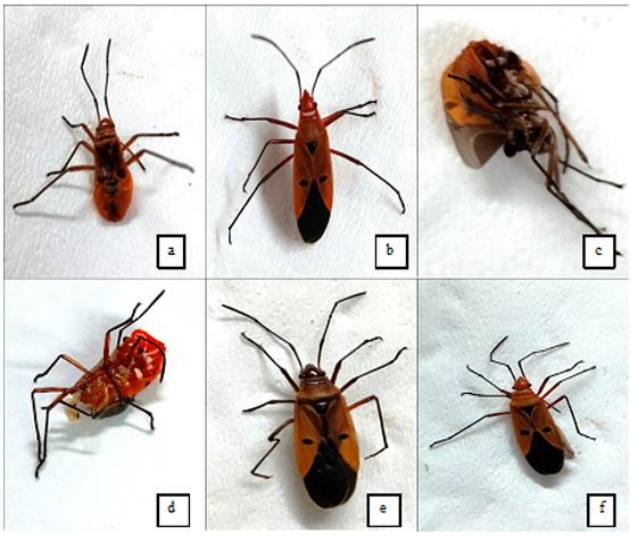

Figure 6. *Helicoverpa armigera*- a-, b-black spots all over the body, c-unripe neem milk leads to slowly blocking of spiracles, d-oozing out of heamolymph from the body and the body turns into black colour, e-higher concentrations caused larval pupal intermediate.

DISCUSSION

By identifying triterpenoids, tannins, phenolic compounds, phytosterols, cardiac glycosides, terpenoids, reducing sugars, and leucoanthocyanins, the phytochemical profiling of AIURFM established a strong bioactive basis (Biswas *et al.,* 2002; Subapriya and Nagini, 2005; Saxena *et al.,* 2013). GC-MS analysis identified 29 chemicals that contributed to the observed bioactivity, including silicic acid, diethyl bis(trimethylsilyl) ester (19.76%), 3-quinolinecarboxylic acid, 6,8-difluoro-4-hydroxy-, ethyl ester (8.90%), and indole-2-one derivatives (7.43-3.36%) (Table 3).

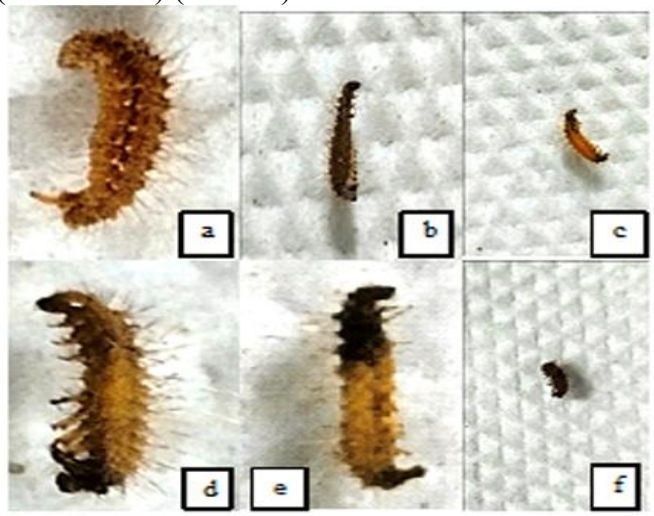

Figure 3. *Sphenarches caffer* **a-**Adult, **b-**unripe neem fruit milk caused enlargement of abdomen, **c** to **e-** indicates slowly change of colour of the abdomen and reduced body size, **f-** unripe neem fruit milk caused entire body dark.

Tetranortriterpenoids, such as azadirachtin, are strong antifeedants and insect development regulators, and they are consistent with more than 300 known neem limonoids (Siddiqui *et al.,* 2009; Dawkar *et al.,* 2019). FTIR spectra supported this profile with broad O-H/N-H bands at 3296 cm^{-1}, C-H alkyl stretches at 2929/2884 cm^{-1}, and C=O ketone/aldehyde/lactone vibrations at 1731 cm^{-1} (Prahas *et al.,* 2008), which are indicative of hydroxylated phenols and triterpenoids that have been previously reported in neem matrices (Dubey and Kashyap, 2014). AIURFM proved to be more effective against five economically significant pests. Against *Spodoptera litura*, a concentration of 0.8% produced LC_{50} = 0.103% (96 hrs, Table 5), which was higher than the literature values (0.2-0.5%) and commercial Vijayneem (0.03%). *Aulacophora foveicollis* adults performed significantly better than neem leaf extracts, which needed 1.5% for 90% mortality (Sirohi and Tandon, 2014), with LC_{50} = 0.138% (Table 5). Nymphs of *Dysdercus koenigii* had LC_{50} = 0.277% (Table 5), which was higher than the botanical ranges of 0.5-1.0 percent that have been recorded (Kayesth and Gupta, 2018). These findings imply that fatty acid esters, indole compounds, and quinoline derivatives work in concert to increase membrane disruption and neurotoxicity (*Khan et al.,* 2015; Guchhait *et al.,* 2022). In line with cytochrome P450 detoxification in late instars reported across the neem literature, *Helicoverpa armigera* showed relative resistance (LC_{50} = 1.689%, Table 5) (Jaglan *et al.,* 1997). However, azadirachtin-induced ecdysteroid/juvenile hormone pathway interference that targets juvenile hormone esterase and apolipophorin III is exactly mirrored by severe morphogenetic disruptions such as dermal melanization, spiracle occlusion, hemolymph exudation with blackening, and larval-pupal intermediates (Figure 6) (Dawkar *et al.,* 2019; Lee *et al.,* 2015; Madasamy *et al.,* 2023). Pymbagin-induced neuroendocrine-molting disruptions and *Lantana camara* morphogenetic effects are paralleled by *D. koenigii* nymphal abnormalities, including as deformed legs and externally protruding wings (Figure 5) (Gujar and Mehrotra, 1988; Kayesth and Gupta, 2018). The unreported triterpenoid effects of the new *S. caffer* abdominal hypertrophy, gradual melanization, and size suppression (Figure 7) call for species-specific toxicological research.

In comparison to neem oil formulations that seal aeropyles and disrupt vitellogenesis, AIURFM showed dose- and time-dependent ovicidal effects against *D. koenigii* eggs (LC_{50}= 0.17% at 96 hrs, Table 7), exhibiting chorionic penetration and embryogenesis suppression (Kala *et al.,* 2019). Through a variety of mechanisms, the progressive LC_{50} reduction (0.874% at 24 hrs → 0.17% at 96 hrs) validates time-cumulative toxicity. AIURFM is positioned as a sustainable IPM component by avoiding expensive seed extraction while competing with synthetic pesticides (Kumar and Navaratnam, 2013; Dhakad *et al.,* 2025). While targeted isolation of phytosterols and quinoline carboxylic acids provides new avenues for pesticide research, nanoemulsion formulations may improve photostability and field persistence. The resistance risk associated with single-mode synthetics is reduced by the multi-target profile (antifeedant, IGR, ovicidal, and morphogenetic). These results demonstrate unripe neem fruit milk as a feasible commercial biopesticide that addresses the global insecticide resistance crises and promotes agroecological sustainability by validating traditional knowledge with contemporary analytics (Puri, 1999; Orwa *et al.,* 2009).

Azadirachta indica unripe fruit milk is a powerful natural biopesticide that outperforms conventional formulations in terms of insecticidal efficacy against several economically significant pests, including total mortality in major species. Its strong pesticidal potential is confirmed by the rich phytochemical profile that includes triterpenoids, phenolics, tannins, and a variety of bioactive chemicals found by FTIR, GC-MS, and qualitative screening. Strong ovicidal effects and profound morphogenetic abnormalities across embryonic stages demonstrate multi-mechanism activity that

interferes with nutrition, growth control, and reproduction. Unripe neem fruit milk is positioned as an important part of integrated pest management programs by an understudied fruit matrix, which provides a sustainable, environmentally friendly substitute for expensive seed extraction procedures while verifying traditional knowledge with contemporary scientific validation.

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