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Insecticidal and Larvicidal Activities of the Acetone Extract, Fractions, and Volatile Compounds of *Dennettia tripetala* (G. Baker) Seeds, and Anticholinesterase Activity of the Identified Compounds

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ABSTRACT

The plant, *Dennettia tripetala* (G. Baker), is renowned for its insecticidal potential. This study aimed to isolate insecticidal and larvicidal compounds from its seeds and to explore their possible mechanism of action. Bioassay-guided fractionation of the acetone extract, tested against adult *Rhyzopertha dominica* (F.), *Sitophilus oryzae* (L.), and 3rd instar larvae of *Aedes aegypti* (L.), yielded 1-nitro-2-phenylethane (NPE), nerolidol, and linalool. Gas Chromatography–Mass Spectrometry revealed the components of the most active fraction and a selected active subfraction, while Column Chromatography and High-Performance Liquid Chromatography were employed for isolation. Electron Ionisation-Mass Spectrometry, Nuclear Magnetic Resonance, and Fourier Transform-Infrared Spectroscopy confirmed all structures. NPE was the predominant compound (83.8%) in the hexane fraction and showed strong activity against *R. dominica* (LD_{50}: 44.93 ± 0.00 µg/cm²) and *S. oryzae* (68.24 ± 0.87 µg/cm²); however, it was inactive against *A. aegypti* larvae at 200 ppm. Nerolidol showed no activity, but a synergistic larvicidal effect emerged in the second hexane subfraction. Linalool, being highly volatile, was not further assayed. None of the compounds inhibited acetylcholinesterase at 0.5 mM, suggesting that NPE acts via a non-cholinergic mechanism. These findings establish NPE as the major insecticidal principle of *D. tripetala* seeds and reveal novel synergistic interactions among its constituents. The results underscore the capacity of this plant's volatile components in developing eco-friendly insecticides, offering prospects for safer pest management strategies.

Keywords: *Dennettia tripetala*, Essential oils, 1-Nitro-2-phenylethane, Nerolidol, Insecticide, Acetylcholinesterase.

Introduction

The global burden of insect pests, from stored-product beetles causing severe grain losses to mosquito vectors transmitting diseases such as dengue and malaria, underscores the urgent need for effective control strategies.[1,2] Heavy reliance on synthetic insecticides has led to well-documented problems, including environmental persistence, human health risks, and widespread insect resistance.[3] These challenges have intensified the search for eco-friendly alternatives, particularly plant-derived products.[4,5] Plant secondary metabolites are promising candidates for pest management,[6] with compounds such as azadirachtin, salanin, karanjin, and pyrethrins demonstrating potent insecticidal activity. However, their practical application is often constrained by chemical instability, unsustainable sourcing, or toxicity concerns.[7,8]

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Thus, the discovery of novel plant-derived bioactive compounds with improved safety and effectiveness remains essential. The Pepperfruit tree (*Dennettia tripetala* G. Baker, Annonaceae) is a West African species valued for nutritional and medicinal purposes.[9,10] Beyond its ethnomedicinal uses, the plant is traditionally and scientifically recognised for insecticidal activity.[11,12] This property has been attributed primarily to its odoriferous principle, 1-nitro-2-phenylethane (NPE), which is abundant in the essential oils (EOs) of different plant parts,[13,14] and is also noted for its neuropharmacological properties.[13,15,16] However, studies directly linking NPE and other metabolites from *D. tripetala* seeds to insecticidal and larvicidal effects remain limited.

To our best knowledge, a bioassay-guided approach to isolate and characterise insecticidal and larvicidal compounds from *D. tripetala* seeds is documented for the first time, thereby addressing this knowledge gap. Gas chromatography–mass spectrometry (GC-MS) aided the identification of compounds in the most active fraction and a selected active sub-fraction. Compounds were isolated and structurally characterised by chromatographic techniques and spectroscopic studies, respectively. Furthermore, because the effective application of plant secondary metabolites requires an understanding of their biochemical targets and potential resistance mechanisms,[17,18] an *in vitro* acetylcholinesterase (AChE) inhibitory assay was performed to investigate possible modes of action.

Materials and Methods

Chemicals and Reagents

Acetone, Methanol, Ethylacetate, Dichloromethane (Analytical grade; BDH, England) Ethylacetate, Hexane (HPLC grade; Samchun Chemicals, Republic of Korea); Permethrin (GSK, England); Acetylcholine iodide, Electric eel acetylcholinesterase (EC 3.1.1.7), 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB), Dimethyl sulfoxide (DMSO), Sodium phosphate, Sulphuric Acid, Silica gel 60 (Sigma Aldrich, USA).

Equipment

Analytical weighing balance (OHAUS Corp, China), Freeze dryer (LGJ-10, Searchtech Instruments, China), Kitchen blender, Incubator (Fisher Scientific, USA), Temperature/humidity calculator, Glass Columns and tanks, Heating gun, UV lamp, TLC plates (Silica gel 60 F_{254}, Merck, Germany), Microplate reader (SpectraMax340, Molecular Device, USA), Rotary evaporator (Buchi R200 Switzerland), Recycling preparative HPLC (LC-908W, JAI, Japan), GC-MS (Agilent Technologies, USA), EI-MS (JEOL, JMS-600H, Japan), NMR (400/500 MHz, Bruker Avance Neo, Biospin USA).

Plant Material

Dennettia tripetala seeds were purchased from the New Benin Market (GPS coordinates: 6.3448371° N, 5.6340480° E), Benin City, in June 2018. Before purchase, a small branch bearing fruits and leaves was submitted for botanical authentication. The plant material was authenticated by Dr. H. A. Akinnibosun, a plant biologist in the Department of Plant Biology and Biotechnology, University of Benin, who issued the voucher specimen UBH–D484 for the identified species.

Insect cultures

The adult specimens of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) and *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) were obtained from a research colony in continuous culture on wheat and rice grains, respectively, at the Pakistan Agricultural Research Centre, University of Karachi, Karachi, Pakistan, with no previous pesticide exposure. The stocks were placed in 500 cm^3 glass vessels, covered with a muslin cloth, and maintained on wheat (*R. dominica*) and rice (*S. oryzae*) under controlled temperature, 27 ± 2 ^{0}C and 70 ± 5 % relative humidity, until oviposition was noticed before being removed. They were subsequently maintained under laboratory conditions for F1 progeny emergence. The F1 progenies were used for the study.[19]

The egg specimens of *Aedes aegypti* (L.) (Diptera: Culicidae) were obtained from a well-established laboratory colony at the Industrial Analytical Centre, University of Karachi, Pakistan, and reared at the same laboratory. The eggs were placed in 800 mL of water, contained in plastic bowls, covered with filter paper at a temperature of 27 ± 2 ºC and humidity of 75 ± 5 %. Egg hatching produced the first instars. The larvae were fed dried prawn powder to enhance their growth. The 3rd instars were used for the study.[20]

Isolation

The seed powder (10 kg) was macerated in absolute acetone (50 L) for 48 hours.[21] The acetone extract was successively fractionated into hexane, dichloromethane, ethylacetate, and residual aqueous fractions of 72.50, 5.30, 0.38, and 5.63 % of the crude extract, respectively.[21] The hexane fraction (50 g) was subsequently chromatographed on a Silica gel column. Gradient elution starting and ending with Hexane (Hex) (100 %) and Ethylacetate (EtAOc) (100 %) v/v, respectively, was performed. This gave rise to 8 main fractions, bulked based on their TLC similarities (UV visualization at 254 and 380 nm) of the eluents. The isolation of Compound **1** from sub-fractions **1** and **2** and Compound **2** from subfraction **3** has been described in an earlier report.[21] For compound **3** (linalool), consecutive gradient elution of fraction **3** (F-3) beginning with Hex-100 % to Dichloromethane (DCM) – 100 % v/v yielded 5 sub-fractions (SF-3A – E). SF-3C was passed through a Silica gel column with Hex-DCM (100:0 – 0:100); four main fractions (SF-3CI–IV) were obtained. SF-3CIII was purified via Normal Phase High Performance Liquid Chromatography (Hex-EtAOc (90:10) at 3 mL/min to obtain compound **3** (40 mg). Spectroscopic studies, including Electron Ionisation-Mass Spectrometry (EI-MS), Nuclear Magnetic Resonance (NMR), and Fourier Transform-Infrared Spectroscopy (FT-IR), were performed on the compounds to characterise their structures. NPE and nerolidol were utilised in bioassays; however, the highly volatile linalool could not be utilised.

Characterisation of the Hexane Fraction and Hexane Subfraction 2

Methyl derivatives of the hexane fraction and its second subfraction were subjected to GC-MS analyses using GC systems with GC 7890A coupled to a quadrupole MS-5975C Network, equipped with HP-5MS column (30 m x 0.32 mm x 0.25 µm) using helium as a carrier gas at 1.0 mL/min flow rate. The temperature was initially fixed at 50 °C, with a ramp rate of 5 °C/min and a dwell time of approximately 10 min, ultimately raising it to 300 °C at 10 °C/min. An EI system with high-energy electrons was used for spectroscopic detection. The spectra of individual compounds were juxtaposed with the National Institute for Standards and Technology (NIST) Reference Spectra Library with an accepted Match Factor of ≥ 700 and available literature. The peak area was used to estimate the relative percentage composition of detected compounds.

Bioassay-guided Insecticidal Assay

The impregnated filter paper method was employed as outlined by Remon *et al.*[22] with minor adjustments. The samples, including the extract, fractions, and sub-fractions at 20mg/mL, and compounds at 0.5, 2, 3.5, and 5 mg/mL, were prepared. Permethrin (2 mg/mL) and acetone served as the reference and negative control, respectively. Samples (1 mL) were loaded on filter papers cut to the size of petri dishes (60 mm diameter) and left for 2 hours for solvent evaporation. Ten non-sexed *R. dominica* and *S. oryzae* adults were subsequently placed in each petri dish; the treatments were in triplicate and incubated at 27 ± 2°C and 50 ± 5 % relative humidity. The treatments were observed within 24 hours, and dead insects in each petri-dish were counted. The percentage insect mortality was computed employing Abbott's formula (Equation 1):

$$\%\,Mortality = \left(1 - \frac{number\ of\ insects\ in\ treatment}{nnumber\ of\ insects\ in\ control}\right) x\ 100 \quad \text{(Equation 1)}$$

Bioassay-guided Larvicidal Assay

This assay was executed in line with the protocol described by Scalvenzi *et al.*[23], with minor adjustments. In this method, each sample (40 mg) was individually dissolved in 2 mL of ethanol to prepare the respective stock solutions. The acetone extract, hexane fraction, and hexane sub-fractions 1, 2, and 3 were tested at 200 ppm by adding 0.5 mL of the stock solutions in 49.5 mL of distilled water. Isolated compounds were prepared at 100 ppm (0.25 mL in 49.5 mL of distilled water) and 200 ppm as well. Afterward, batches of ten 3rd-instar *A. aegypti* larvae were placed in respective test solutions contained in different flasks. Permethrin (100 ppm) and ethanol served as the reference and negative control, respectively. The percentage mortality was computed employing Abbott's formula (Equation 1)

Anticholinesterase activity

The Ellman *et al.*[24] method, with slight alterations, was performed to assess the Anticholinesterase activity of the isolated compounds. Test compounds (10 µL, 0.5 mM), acetylcholinesterase (20 µL), and sodium phosphate buffer (150 µL, 100 mM, pH = 8), were mixed and maintained at 25 ^{0}C for 15 min. Adding DTNB (10 µL) and Acetylcholine iodide (10 µL), initiated the reaction. Galantamine hydrobromide served as the standard. All tests were executed in triplicate in a 96-well microplate, and substrate hydrolysis leading to 5-thio-2–nitrobenzoate anion formation was evaluated at 412 nm with a microplate spectrophotometer. Percentage inhibition was calculated with the formula (Equation 2):

$$\%\ inhibition = \left(100 - \left(\frac{Mean\ Optical\ Density\ of\ test}{Mean\ Optiak\ Density\ of\ reference}\right)\right) x\ 100$$

(Equation 2)

Statistical Analysis

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The data obtained in this study are presented as mean ± standard deviation (SD). LD_{50} values were calculated, and one-way ANOVA, followed by Tukey's post hoc test, was utilised for statistical analysis ($p < 0.05$), employing GraphPad Prism version 8.2.0. The IC_{50}, with a 95 % confidence interval, was estimated using EZ-Fit enzyme kinetics software.

Results and Discussion

The acetone extract of *D. tripetala* seeds showed significant insecticidal activity against non-sexed adults of *R. dominica* and *S. oryzae* after 24 h exposure (Table 1). Activity was concentrated in the hexane fraction, moderate in the dichloromethane fraction, while the ethyl acetate and aqueous fractions were inactive. These results confirm earlier reports of the plant's insecticidal potential as a grain protectant.[11,12,25]

Table 1: Percentage mortality of adult *R. dominica* and *Sitophilus oryzae* exposed to the acetone extract of the seeds of *D. tripetala* and its fractions at 756.67 µg/cm^2 after 24 hours.

Sample	Insect	Percentage of Mortality (Mean ± SD)
Acetone Extract	*R. dominica*	100.00 ± 0.00^a
Hexane Fraction		100.00 ± 0.00^a
DCM Fraction		53.33 ± 5.77^b
EtOAc Fraction		0.00 ± 0.00^c
RAQ Fraction		0.00 ± 0.00^c
Permethrin		100.00 ± 0.00^a
Acetone		0.00 ± 0.00^c
Acetone Extract	*S. oryzae*	100.00 ± 0.00^a
Hexane Fraction		100.00 ± 0.00^a
DCM Fraction		43.33 ± 5.77^b
EtOAc Fraction		0.00 ± 0.00^c
RAQ Fraction		0.00 ± 0.00^c
Permethrin		100.00 ± 0.00^a
Acetone		0.00 ± 0.00^c

* DCM = Dichloromethane, EtOAc = Ethylacetate, RAQ = Residual Aqueous
Results are presented as mean ± SD ($n = 3$)
^{a,b,c} Significant differences ($p < 0.05$) exist between means with distinct superscripts in the same column

GC–MS analysis of the active hexane fraction (Fig. 1, Table 2) unveiled EO constituents dominated by NPE (83.8 %), consistent with earlier studies that identified NPE as the most abundant volatile in the seeds, fruits, and leaves of *D. tripetala*.[13,14,26,27] The insecticidal activity of this fraction can therefore be ascribed largely to its high NPE content, a hypothesis confirmed through subsequent isolation and testing. Further sub-fractionation of the hexane fraction yielded four subfractions (HSF-1 to HSF-4). GC–MS analysis of HSF-2 (Fig. 2, Table 3) identified NPE (81.5 %) and (6E)-nerolidol (13.8 %) as the major components, with linalool present in trace amounts (<1%). HSF-1 and HSF-2 caused the highest mortalities in both *R. dominica* and *S. oryzae*, with mortality rates significantly higher ($p < 0.05$) than those observed for HSF-3 and HSF-4 (Table 4). These findings suggest that while NPE is the dominant insecticidal principle, minor constituents such as nerolidol and linalool may contribute synergistically to the overall activity. The potent activity of *D. tripetala* fractions is consistent with reports of EOs from various plants exhibiting insecticidal attributes and their use in stored-product protection.[28] More specifically, essential oils from *D. tripetala* fruits and seeds were previously shown to suppress *C. maculatus* and *S. oryzae* infestations,[25,29] which corroborates our results with seed extracts.

Compound 1 (Fig. 3A) was obtained as a faint yellow oily liquid. Its EI-MS spectrum exhibited a molecular ion peak at m/z 151 $[M]^+$, with a base peak at m/z 104 correlating with the loss of HNO_2.[21] As reported earlier,[21] the observed ^{1}H NMR (C_3D_60, 500 MHz), ^{13}C NMR (C_3D_60, 125 MHz), and FT-IR data confirmed the compound as NPE ($C_8H_9NO_2$, MW 151.16). Compound 2 (Fig. 3B) emerged as a pale-yellow oily liquid. The EI-MS spectrum revealed diagnostic fragment peaks at m/z 69 (100 %) and 204 ($[M - H_2O]^+$), while the molecular ion at m/z 222 $[M]^+$ was absent,[21] a feature characteristic of tertiary alcohols. Structural elucidation using ^{1}H NMR (C_3D_60, 500 MHz), ^{13}C NMR (C_3D_60, 125 MHz), and FT-IR data substantiated its identity as nerolidol (3,7,11-trimethyl-1,6,10-dodecatrien-3-ol, $C_{15}H_{26}O$, MW 222.37), as earlier documented.[21] Compound 3 (Fig. 3C) appeared as a faint yellow, highly volatile liquid. Its EI-MS spectrum displayed a molecular ion at *m/z* 154 $[M]^+$ with a base peak at *m/z* 71. The FT-IR spectrum displayed absorption bands at 3394, 2971, 2922, 2104, 1708, 1640, 1449, 1410, 1373, 1112, 996, 917, 834, 737, 689, and 549 cm^{-1}. These absorptions are characteristic of a tertiary alcohol (O–H stretch at 3394 cm^{-1}; C–O stretch at 1112 cm^{-1}), olefinic bonds (=C–H bending at 996 and 917 cm^{-1}), multiple methyl groups (1373 cm^{-1}), and methylene moieties (2922 and 1449 cm^{-1}). The ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum displayed downfield signals at δ 5.19 (dd, J = 1.2, 17.6 Hz) and δ 5.04 (dd, J = 1.2, 10.4 Hz), corresponding to two geminal olefinic protons attached to C-1 (δ 111.67). A doublet of doublets at δ 5.89 (J = 10.8, 17.2 Hz) was attributed to the olefinic proton at C-2 (δ 145.02), while a triplet of quartets at δ 5.10 (J = 1.2, 7.2 Hz) was assigned to the olefinic proton at C-6 (δ 124.29). Broad singlets at δ 1.58 and 1.26 and a broad doublet at δ 1.66 were assigned to methyl groups at C-8 (δ 17.68), C-10 (δ 27.07), and C-9 (δ 25.66), respectively. Multiplets at δ 1.54 and 2.00 were attributed to methylene protons at C-4 (δ 42.03) and C-5 (δ 22.78). The ^{13}C NMR ($CDCl_3$, 100 MHz) and HMBC spectra exhibited quaternary carbon signals at δ 73.47 and δ 131.96, corresponding to C-3 and C-7, respectively. The deshielded resonance at δ 73.47 confirmed oxygen substitution on a quaternary carbon, consistent with a tertiary alcohol functionality. Compound 3 was therefore confirmed as linalool (3,7-dimethyl-1,6-octadien-3-ol, $C_{10}H_{18}O$, MW 154.24), in agreement with already published values.[30]

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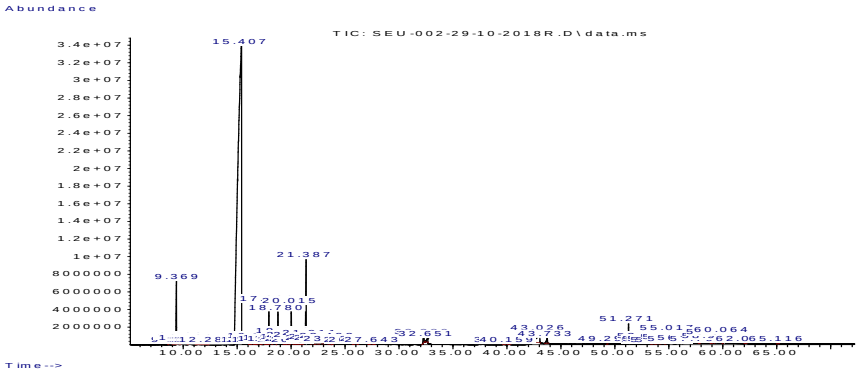

Figure 1: GC-MS spectra of the hexane fraction.

Table 2: GC-MS analysis of the hexane fraction of the acetone extract of *D. tripetala* seeds showing the 28 most occurring compounds (> 0.03 %)

S/N	Compound	Retention Index (iu)	Ret. Time (Min)	Peak area (% Composition)	Molecular Weight
1	Linalool	1082	9.3690	2.21	154
2	Phenylethyl Alcohol	1136	9.7290	0.08	122
3	Benzyl nitrile	1138	10.439	0.08	117
4	1-Nitro-2-phenylethane	1256	15.407	83.84	151
5	Copaene	1221	16.796	0.11	204
6	Caryophyllene	1494	17.939	1.26	204
7	*α*-Bergamotene	1430	18.261	0.05	204
8	Humulene	1579	18.780	0.98	204
9	*γ*-Muurolene	1435	19.299	0.04	204
10	*α*-Gurjunene	1419	19.775	0.06	204
11	1H-Benzocycloheptene, 2,4a,5,6,7,8,9,9a-octahydro-3,5,5-trimethyl-9-methylene-	1494	19.881	0.07	204
12	*α*-Farnesene	1458	20.015	0.70	204
13	(-)-*β*-Cadinene	1440	20.426	0.13	204
14	Elemol	1522	21.049	0.19	222
15	(6E)-Nerolidol	1564	21.487	3.32	222
16	Caryophyllene Oxide	1507	21.913	0.27	220
17	*β*-Guaiene	1523	22.191	0.08	204
18	Calarene epoxide	1293	22.513	0.13	220
19	*γ*-Eudesmol	1627	23.480	0.31	222
20	*α*-Eudesmol	1598	27.207	0.06	222
21	Dibutyl phthalate	2037	32.280	0.42	278
22	n-Hexadecanoic acid	1968	32.651	0.33	256
23	Cis-Vaccenic acid	2175	43.026	2.30	282
24	Octadecanoic acid	2167	43.733	0.36	284
25	Mono(2-ethylhexyl) phthalate	2162	51.271	0.52	278
26	Campesterol	2632	58.919	0.20	400
27	Stigmasterol	2739	59.309	0.30	412
28	*γ*-Sitosterol	2731	60.064	0.50	414

Table 3: GC.MS analysis of the hexane sub-fraction 2 of the acetone extract of *D. tripetala* seeds

S/N	Compound	Retention Index (iu)	Ret. Time (Min)	Peak area (% Composition)	Molecular Weight
1	Acetonyldimethylcarbinol	845	8.84	0.42	116
2	Linalool	1081	14.29	0.93	154
3	1-Nitro-2-phenylethane	1256	17.66	81.54	151
4	(6E)-Nerolidol	1564	22.33	13.83	222
5	Dibutyl phthalate	2037	38.00	3.01	278

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6	Diisocotyl phthalate	2704	45.40	0.27	390

The insecticidal activities of NPE and nerolidol against *R. dominica* and *S. oryzae* are summarised in Table 5. NPE exhibited strong toxicity toward both species, with *R. dominica* being more susceptible ($p < 0.05$), as reflected by a lower LD_{50} value (Table 6). These values compare favourably with those of permethrin and are within the range reported for other botanically derived insecticides such as azadirachtin and pyrethrins,[7] highlighting NPE as a potent natural insecticide. Characteristic symptoms of intoxication included initial hyperactivity followed by paralysis and death within hours, consistent with neurotoxic action. Given its high abundance across different plant parts and the strong bioactivity observed here, NPE can be considered the primary insecticidal principle of *D. tripetala*. Although its insecticidal potential has been rarely investigated, our findings align with an earlier report of its protective effects against *C. maculatus*.[31] Importantly, NPE's physicochemical profile supports practical application: it is a volatile yet stable oily compound that readily degrades in the environment, minimising persistence and residue accumulation, and supporting its potential for safe practical application.[32] Toxicological studies indicate moderate mammalian toxicity (LD_{50} = 470 mg/kg, i.p., in rats) but no cytotoxicity toward 3T3 fibroblasts or *Artemia salina* nauplii,[16,21] suggesting a favourable safety margin compared to many synthetic insecticides.

In contrast, nerolidol exhibited no fumigant activity against either *R. dominica* or *S. oryzae*. Data on its insecticidal properties against stored-product pests are scarce; however, it has shown activity against aphids, houseflies, and fruit flies, among others.[33-35] Nerolidol's reported

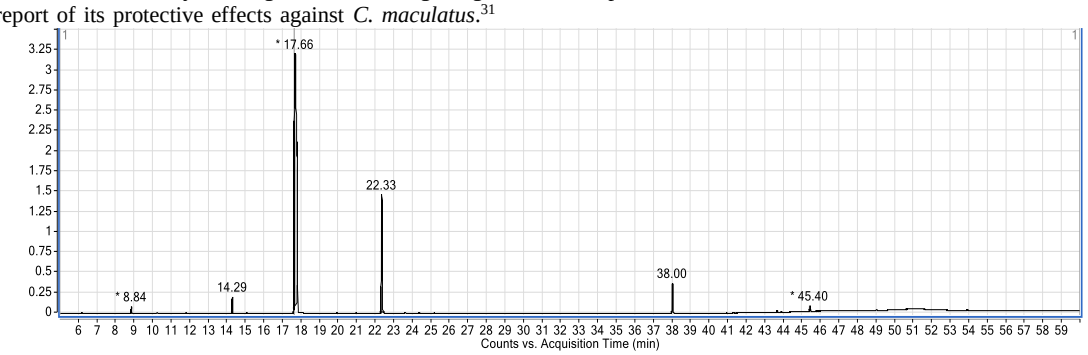

Figure 2: GC-MS spectra of the hexane sub-fraction 2

Table 4: Percentage mortality of adult *Rhyzopertha dominica* and *Sitophilus oryzae* exposed to hexane sub-fractions 1 – 4 (756.67 µg/cm^2) of the seeds of *D. tripetala* after 24 hours

Sample	Insect	Percentage of Mortality (Mean ± SD)
HSF-1	*R. dominica*	100.00 ± 0.00^a
HSF-2		100.00 ± 0.00^a
HSF-3		70.00 ± 5.77^b
HSF-4		20.00 ± 5.77^c
Permethrin		100.00 ± 0.00^a
Acetone		0.00 ± 0.00^d
HSF-1	*S. oryzae*	100.00 ± 0.00^a
HSF-2		100.00 ± 0.00^a
HSF-3		63.33 ± 6.67^b
HSF-4		13.33 ± 3.33^c
Permethrin		100.00 ± 0.00^a
Acetone		0.00 ± 0.00^d

* HSF = Hexane Sub-Fraction
Values are presented as mean ± SD (n = 3)
^{a,b,c,d} Significant differences ($p < 0.05$) exist between means with distinct superscripts in the same column

Table 5: Insecticidal activity of 1-nitro-2-phenylethane and nerolidol on *R. dominica* and *S. oryzae* after 24 hours.

Compound	Insect	Surface Density (µg/cm^2)	Percentage of Mortality (Mean ± SD)
1-nitro-2-phenylethane	*R. dominica*	18.92	0.00 ± 0.00^a
		75.67	100.00 ± 0.00^b
		132.42	100.00 ± 0.00^b
		189.17	100.00 ± 0.00^b
Nerolidol		18.92	0.00 ± 0.00^a
		75.67	0.00 ± 0.00^a
		132.42	0.00 ± 0.00^a
		189.17	0.00 ± 0.00^a
Permethrin		18.92	100.00 ± 0.00^b

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Acetone		0.00	0.00 ± 0.00[a]
1-nitro-2-phenylethane	*S. oryzae*	18.92	0.00 ± 0.00[a]
		75.67	43.33 ± 5.77[c]
		132.42	100.00 ± 0.00[b]
		189.17	100.00 ± 0.00[b]
Nerolidol		18.92	0.00 ± 0.00[a]
		75.67	0.00 ± 0.00[a]
		132.42	0.00 ± 0.00[a]
		189.17	0.00 ± 0.00[a]
Permethrin		18.92	100.00 ± 0.00[b]
Acetone		0.00	0.00 ± 0.00[a]

Values are expressed as mean ± SD (n = 3)
[a,b,c] Significant differences ($p < 0.05$) exist between means with distinct superscripts in the same column

Table 6: LD_{50} and observed insecticidal effect of 1-nitro-2-phenylethane on *R. dominica* and *S. oryzae*

Insect	LD_{50} ± SD (µg/cm^2)	Observed Insecticidal Effect
R. dominica	44.93 ± 0.00[a]	Hyperactivity with subsequent paralysis and death within an hour
S. oryzae	68.24 ± 0.87[b]	Hyperactivity with subsequent paralysis and death within an hour.
Acetone	0.00 ± 0.00[c]	

Values are presented as mean ± SD (n = 3)
[a,b] Significant differences ($p < 0.05$) exist between means with distinct superscripts in the same column

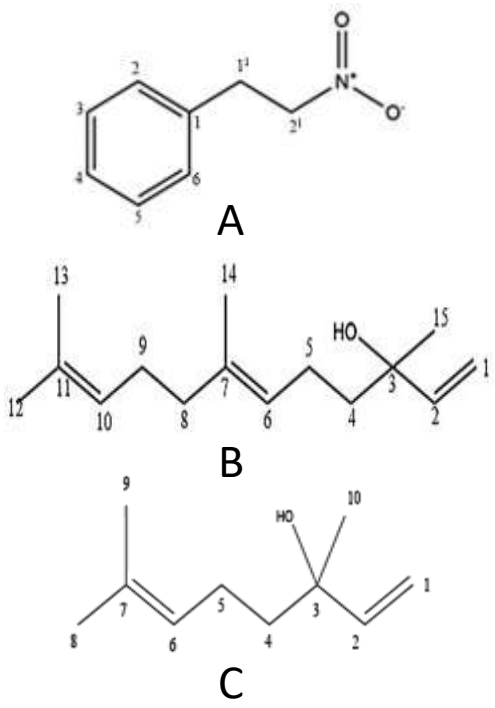

Figure 3: Chemical Structures of **A.** Compound 1: 1-Nitro-2-phenylethane[21]
B. Compound 2: Nerolidol[21] **C.** Compound 3: Linalool

Table 7: Percentage mortality of *A. aegypti* larvae exposed to the acetone extract, hexane fraction, and hexane sub-fractions 1, 2, and 3 of the seeds of *D. tripetala* at 200 ppm after 24 hours.

Sample	Percentage of Mortality (Mean ± SD)
Acetone Extract	100.00 ± 0.00[a]
Hexane Fraction	100.00 ± 0.00[a]
HSF-1	100.00 ± 0.00[a]
HSF-2	100.00 ± 0.00[a]
HSF-3	100.00 ± 0.00[a]
Permethrin	100.00 ± 0.00[a]

contact and stomach toxicity may arise from its ability to disrupt insect cuticle lipids and interfere with internal physiological processes upon ingestion. However, the observed lack of fumigant activity indicates that its efficacy is highly species-specific and exposure-route dependent. Consequently, nerolidol alone appears unsuitable as a grain protectant but could serve as a valuable synergist in formulations, enhancing the potency of more active compounds. Linalool, although not assayed in this study due to volatility, has been widely reported as insecticidal and fumigant-active against stored-product pests, mosquitoes, and fruit flies.[36-39]

The larvicidal activity of the *D. tripetala* acetone extract, hexane fraction, and its sub-fractions (HSF-1, HSF-2, HSF-3) was evaluated against third-instar larvae of *Aedes aegypti* at 200 ppm (Table 7). After 24 hours, all tested samples, along with the permethrin standard, induced 100 % mortality, confirming the potent larvicidal potential of the seeds. This finding aligns with a previous report on the fruit's hexane extract and supports the broad insecticidal value of this plant species.[40] A critical finding emerged from the bioassay of the purified compounds. Contrary to expectations, neither the major insecticidal compound NPE nor nerolidol exhibited any larvicidal activity against *A. aegypti* when tested individually at 200 ppm (Table 8). This result is particularly notable for nerolidol, which has been documented to be larvicidal on some mosquito species.[41-42] The larvicidal activity of linalool, which was precluded from testing due to its high volatility, has been established in the literature.[43-44]

Table 8: Larvicidal activity of NPE and Nerolidol on *A. aegypti* larvae after 24 hours

Compound	Concentration (ppm)	Percentage of Mortality (Mean ± SD)
1-nitro-2-phenylethane	100	0.00 ± 0.00[a]
	200	0.00 ± 0.00[a]
Nerolidol	100	0.00 ± 0.00[a]
	200	0.00 ± 0.00[a]
Permethrin	100	100.00 ± 0.00[b]
Control	0	0.00 ± 0.00[a]

Values are presented as mean ± SD (n = 3)
[a,b] Significant differences ($p < 0.05$) exist between means with distinct superscripts in the same column

The collective data reveal a clear case of synergistic interaction. The highly active hexane fraction and its sub-fractions are predominantly composed of NPE and nerolidol—compounds that are inactive alone. This apparent contradiction strongly indicates that the observed larvicidal activity is due to the concerted action of multiple constituents within the crude mixture, rather than to a single constituent. The potent activity of HSF-2, which is primarily a mixture of inactive NPE and nerolidol, serves as definitive proof of this synergy. This discovery has significant implications. It suggests that the full insecticidal potential of *D. tripetala* is encoded in its complex phytochemical profile rather than in a single molecule.

The synergistic efficacy of essential oils (EOs) is well-established, often resulting from interactions among their components that simultaneously affect multiple molecular targets, thereby enhancing the overall insecticidal and larvicidal activity.[18,45,46] In the case of *D. tripetala*, the significant contribution of minor components revealed by GC-MS analysis—such as α-terpineol, α-eudesmol, γ-eudesmol, caryophyllene, α-caryophyllene, caryophyllene oxide, and α-farnesene (Table 2)—is critical. These constituents may either exhibit intrinsic bioactivity or, as previously suggested for nerolidol, enhance the susceptibility of insect pests to the primary active compounds.[46] Furthermore, Scalerandi *et al.*[47] demonstrated that insects can metabolically detoxify the major compounds in an EO while remaining vulnerable to its minor constituents. This underscores the crucial role of these secondary components in the overall toxic effect and explains why whole EOs often demonstrate greater efficacy than their isolated primary compounds, reinforcing their value as multi-target botanical insecticides. [46] The rapid onset of neurotoxic symptoms—hyperactivity, paralysis, and death—following exposure to NPE strongly suggests a neurological

Table 9: Anticholinesterase activity of NPE and Nerolidol

Compound	Concentration (mM)	Percentage Inhibition	IC_{50} ± SD [µM]
1-Nitro-2-Phenylethane	0.5	11.33 ± 0.48	Inactive
Nerolidol	0.5	7.52 ± 1.21	Inactive
Galantamine hydrobromide	0.5	80.8 ± 0.57	3.5 ± 1.6

Values are presented as mean ± SD (n = 3)

mechanism of action. The nervous system or insects is a well-documented target for the bioactive compounds found in EOs, often acting as signaling molecules that structurally mimic endogenous biogenic amines.[1718,46] NPE, in particular, has been specifically linked to the neuropharmacological effects of *D. tripetala* and *Aniba canelilla* EOs.[13,15] To investigate a potential cholinergic mechanism, we evaluated the *in vitro* inhibitory activity of NPE and nerolidol against acetylcholinesterase (AChE). As presented in Table 9, neither compound showed significant inhibition at 0.5 mM. This finding concurs with the report by Oyemitan *et al.*[13] who concluded that the anti-AChE activity of *D. tripetala* EOs is not attributable to NPE alone but arises from synergistic interactions among its constituents. It is essential to note that while some EO constituents can inhibit AChE, most are weak inhibitors that require millimolar concentrations, and their effects are often rapidly reversible.[46,48,49] The neurotoxic symptoms observed here instead suggest disruption of octopaminergic or GABAergic systems, which are established EO targets.[46,48] Nerolidol, showing no significant AChE inhibition, further supports the view that sesquiterpenes are generally weak AChE inhibitors.[48] Moreover, the strong larvicidal activity of the HSF-2 fraction, despite the inactivity of its major isolates, underscores the importance of synergistic interactions.[47] Collectively, these observations indicate that the insecticidal and larvicidal attributes of *D. tripetala* are mediated not by cholinergic pathways but by non-cholinergic, multi-target mechanisms characteristic of complex botanical mixtures.

Conclusion

This study confirms the potent insecticidal and larvicidal properties of *D. tripetala* seeds, primarily attributed to their volatile oil composition. While the predominant compound, 1-nitro-2-phenylethane, demonstrated potent neurotoxic activity against stored-product pests through a non-acetylcholinesterase pathway, its lack of larvicidal effect against *A. aegypti* strongly indicates synergistic action with other oil constituents. Therefore, prospects for commercialisation are critically dependent on elucidating these synergistic mechanisms, developing stable controlled-release formulations, and conducting comprehensive toxicological evaluations.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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