The Original

FORMULATION AND CHARACTERIZATION OF NUTMEG ESSENTIAL OIL NANO EMULSION AND ITS BIO EFFICACY AGAINST RED FLOUR BEETLE TRIBOLIUM CASTANEUM

Anaswara ER[1,2], Dr. Jenish Paul[3*], Shabna Kanjirathingal Ibrahim[4], Jerry Jacob[5]

[1]Research Scholar, Union Christian College Aluva, Email ID: anaswaraer@uccollege.edu.in ORCID ID:0000-0002-9122-1313
[2]Quality Evaluation Laboratory, Spices Board, Ministry of Commerce and Industry, Chennai, Tamil Nadu- 601 201
[3]Associate Professor Department of Chemistry Union Christian College Aluva Email ID: jenishpaul@uccollege.edu.in
[4]Healthcare Assistant Research Scholar, Department of Chemistry, Union Christian College, Aluva. Email ID: shabnanizar11@gmail.com ORCID: 0009-0008-4334-6630
[5]Scientist Synthite Industries pvt Ltd. Email ID: jerry@synthite.com ORCID ID:0009-0000-7121-4346

Abstract
Nutmeg essential oil (*Myristica fragrans* Houtt.) possesses promising bioactive properties and has attracted considerable interest as a natural insecticidal agent. In the present study, nutmeg oil nanoemulsions were formulated using Tween 80 as a non-ionic surfactant and distilled water as the aqueous phase. Three formulations with oil-to-surfactant ratios of 1:1 (NG T80 1:1), 2:1 (NG T80 2:1), and 3:1 (NG T80 3:1) were prepared through emulsification followed by ultrasonication. The physicochemical characteristics of the emulsions were evaluated using Dynamic Light Scattering (DLS), and their insecticidal efficacy was assessed against the red flour beetle, *Tribolium castaneum*, under laboratory conditions.
DLS analysis confirmed the formation of nanosized droplets with average particle sizes of 56.80 nm, 157.8 nm, and 70.07 nm for NG T80 1:1, NG T80 2:1, and NG T80 3:1 formulations, respectively. The corresponding polydispersity index (PDI) values were 0.530, 0.391, and 0.648, indicating differences in droplet size distribution among the formulations. Contact toxicity bioassays were conducted at concentrations ranging from 1–10% (v/v), and mortality was recorded at 6,12, and 24 h after exposure. Mortality increased with both concentration and exposure period in all formulations.
Among the prepared formulations, NG T80 2:1 exhibited the highest insecticidal efficacy at 6 h, with the lowest LC_{50} value of 9.74%, followed by NG T80 3:1 with a value of 9.67%, whereas NG T80 1:1 required a higher concentration of 18.11% to achieve 50% mortality. After 12 h of exposure, all formulations were more effective, as evidenced by the reduction in LC_{50} values. The NG T80 3:1formulation showed the greatest potency, with the lowest LC_{50} 4.12%, followed by NG T80 2:1 with a value of 4.70% and NG T80 1:1 with LC 50 value of 5.40%. These findings demonstrate that prolonged exposure significantly enhanced the toxicity of the nano emulsions. At 24 h, all treated concentrations produced 100% mortality, indicating that exposure duration played a critical role in maximising insecticidal activity.
The study highlights the potential of nutmeg oil nanoemulsions as eco-friendly botanical insecticide formulations for the management of stored-product pests.

Keywords: *Myristica fragrans*; Nutmeg essential oil; Nanoemulsion; Tween 80; Dynamic Light Scattering; *Tribolium castaneum*; Contact toxicity; Insecticidal activity.

1. INTRODUCTION
Nutmeg essential oil, rich in active terpenes such as myristicin and sabinene, has demonstrated significant toxicity against various coleopteran pests, though its application remains limited by rapid volatilization and oxidation. To overcome these physiological and environmental barriers, nanoemulsion technology facilitates the encapsulation of volatile lipophilic compounds within small, stable droplets, thereby improving their water solubility and bioavailability [1]. Furthermore, the high surface-area-to-volume ratio of these nano-sized droplets facilitates superior adherence to the insect cuticle and enables a slow, persistent release of active constituents, which significantly enhances residual toxicity compared to bulk oil formulations [2]
Nanoemulsions (NE) are heterogeneous, kinetically stable colloidal systems typically consisting of oil droplets dispersed in an aqueous phase (O/W) or aqueous droplets distributed within an oil phase (W/O), with their stability maintained by surfactants. The average size of droplets ranges from 20 to 200 nm [3]. Due to their high surface area, optical transparency, and enhanced solubilization capacity for hydrophobic compounds, NE have gained recognition as potential platforms for the controlled release of bioactive agents in pharmaceutical, cosmetic, and food applications [4]. Their potential is particularly pronounced when used to encapsulate essential oils, which often exhibit potent biological activities but suffer from physicochemical limitations such as poor aqueous solubility, volatility, and chemical instability.
Among essential oils,nutmeg oil has been broadly explored for their antioxidant, antibacterial, and insecticidal properties. The primary bioactive component in nutmeg oil is notably α-pinene, β-pinene,terpinene-4-ol,myristicin,meta cymene, limonene, gamma terpinene etc.and are exhibit synergistic therapeutic potential. However, their lipophilic nature necessitates an effective delivery system to enhance their solubility, bioavailability, and stability in aqueous environments.

In this context, NE stabilized by surfactants such as Tween 80(T80) offers a viable solution. This surfactant differ in their hydrophilic-lipophilic balance (HLB) and ionic character, influencing droplet formation, interfacial tension, and long-term emulsion stability. The choice and combination of surfactants serve a pivotal function in determining the physicochemical behaviour of the NE, including droplet size distribution, zeta potential, and resistance to coalescence or Ostwald ripening. The stability of the NE also depends on the concentration of the surfactants, oil-to-water ratio, temperature, pH, agitation and proper mixing of the phases.

Concerning physicochemical properties, NE occupies an intermediate position between traditional emulsions and microemulsions. Emulsions, composed of oil, surfactant, and water, are thermodynamically unstable but kinetically stabilized systems. In these systems, surfactant molecules reduce interfacial tension and form a barrier that retards droplet coalescence. The formulation's stability is determined by the polydispersity index at 25 °C. The emulsion can be formulated using high-energy emulsification techniques, such as high-pressure and high-speed homogenization [5], ultrasonication, solvent displacement, and the phase inversion temperature method. The development of instability in NE can lead to flocculation, coalescence, separation, and sedimentation. Eliminating the charge can reduce the stability of emulsions by minimizing the forces that repel nanodroplets from one another, which arrests flocculation and coalescence [6].

Recent research has successfully synthesized and characterized nutmeg essential oil nanoemulsions, utilizing advanced techniques like dynamic light scattering and transmission electron microscopy to confirm their efficacy as novel pest control agents [7]. These formulations leverage surfactants such as Tween 80 to reduce droplet size, thereby promoting deeper penetration through the insect's tracheal system and increasing systemic insecticidal impact [8].

This research highlights the formulation and characterization of nutmeg oil NE prepared using Tween 80as emulsifying agents and evaluates the effect of different conc. of surfactants. The NEs were systematically evaluated for droplet size and their biological activity including insecticidal activity against *tribolium castaneum.* This investigation aims to elucidate the relationship between surfactant ratio against the insecticidal activity.

2. MATERIALS REQUIRED

Nutmeg essential oil (Myristica fragrans Houtt) was procured from Synthite (India). Chemicals such as Tween 80 was purchased from NICE. Double distilled water was used for all preparations.

Microorganisms: Tribolium castaneum species were obtained from Mannuthy Agricultural
University, Trichur.

2.1. METHODOLOGY

2.1.1. Preparation of Nutmeg Oil-based Emulsions using Tween 80 as surfactant (NG T80)

All emulsions were developed based on the method described in [15], with modifications. The nano emulsion was be prepared using a two-step method, spontaneous emulsification followed by ultra sonication.The formulation of essential oil emulsions (EOEs) involved varying the conc. of surfactant, a critical factor influencing NE formation, stability, and biological activity.

The influence of surfactant on NE formation was systematically evaluated to study their impact on antioxidant, cytotoxic, antibacterial and insecticidal properties.The surfactant, Tween 80 with hydrophilic-lipophilic balance (HLB) value 15.0 was used for this study. Formulations using different conc. of surfactant were prepared to investigate potential synergistic effects on emulsion formation.

2.1.2. Formulations and composition

Nutmeg based emulsions were prepared using Tween 80 surfactant and distilled water as the aqueous phase by a low-energy magnetic stirring method. Emulsion formulations were prepared at oil: surfactant ratios of 1:1, 2:1, and 3:1. Prepare the organic phase by mixing nutmeg essential oil with non-ionic surfactant Tween 80 according to predetermined rations. Magnetically stir the oil-surfactant mixture at 1000 rpm for 30 minutes at room temperature to ensure uniform dispersion and mixing. Double distilled water was added drop wise to the oil-surfactant mixture under continuous stirring for 2 hours to achieve homogenisation.The resulting pre-emulsion was subjected to ultra sonication using a Sonicator PCI TM Analytics for 30 minutes in an ice-cold water bath to prevent temperature elevation and oil degradation. Transfer the resultant emulsions into clean bottles and were stored in the low-temperature cabinet(4°C).

Table 1. Composition of Nutmeg oil emulsion

Formulation Code	Oil (%w/w)	Surfactant (%w/w)	Water (%w/w)	Oil-surfactant ratio	Color
NG T80-1:1	10	10	80	1:1	Milky White
NG T80-2:1	20	10	70	2:1	Milky White
NG T80-3:1	30	10	60	3:1	Milky White

Fig 1. Formulation before emulsion formation with oil: surfactant ratio (a)1:1 (b)2:1 (c)3:1

Fig 2. Emulsion formation with oil: surfactant ratio (a)1:1 (b)2:1 (c)3:1

2.1.3. Insect Rearing

The experiment was conducted at Union Christian college, Aluva. Mass culture of the red flour beetle, *Triblolium Castaneum*, was established in laboratory without any exposure to chemical insecticides.

The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), was reared in **wheat flour**. Adult T. castaneum was introduced into the plastic jars, filled with 200g of sterile wheat flour and 5 %(w/w) **brewer's yeast**.After 5 days of oviposition, red flour adults were transferred to fresh plastic jars. Plastic rearing jars were checked regularly to obtain adults of uniform age. The T. castaneum cultures were kept at 28 ± 2 ºC. All insects bioassays employed 2 weeks of old adults of T. castaneum and were used for the study.

Fig 3. Insect culture of *Tribolium castaneum*

2.1.4. Insecticidal Bioassay of Nutmeg Oil Nanoemulsion Against *Tribolium castaneum*

The insecticidal activity of the NG T80 emulsions were evaluated against Tribolium casteneum. All bioassays were performed under controlled conditions (26 ± 1 °C, 65 ± 5% RH).

Contact toxicity assays were conducted by diluting emulsions with water. Preliminary tests were run to determine the appropriate concentrations that can cause mortality rates ranging from 10 to 100% mortality. The insecticidal activity found at different concentrations in the range 1%,2%,4%,6%,8% and 10 % for these 3 formulations.

For testing insecticidal activity, 100 µL of each diluted emulsion was pipetted into a watch glass, swirled to evenly coat the inner surface as a thin film, and allowed to dry at room temperature before introducing the adult Tribolium castaneum insects. The control was maintained under the same conditions without emulsions. Mortality was recorded at 6, 12 and 24 hours post-exposure. Three replicates were performed for each treatment. The mortality rates were recorded after treatment. The insects were considered dead when they failed to respond to gentle probing with a fine brush. Observed mortality was corrected using Abbott's formula whenever control mortality was present:

Corrected mortality (%) = (Observed mortality − Control mortality) X 100

(100 − Control mortality)]

The corrected mortality data were transformed into probit values and plotted against the logarithm (base 10) of concentration. Probit regression analysis was performed to estimate the median lethal concentration (LC_{50}) together with the regression equation. Statistical analysis was carried out using probit analysis software, and Half-maximal lethal concentration (LC_{50}) values were calculated at different exposure periods for NG T80 1:1, NG T80 2:1 and NG T80 3:1 formulation.

3. CHARACTERISATION

3.1. Nutmeg seed oils physical characterization

At 25 °C, the specific gravity, optical rotation, and refractive index of nutmeg oil was determined. Nutmeg oil exhibited a specific gravity in the range of 0.8820-0.8970, an optical rotation of 16 to 28°, and a refractive index of 1.4795.

3.1.1. Oil profiling conditions of Nutmeg seed Oil in GC-FID

The analyses were carried out with Agilent 6890 B GC with an FID detector. Agilent nonpolar column HP1 (Methyl siloxane; Length = 60 meters, diameter = 0.25 millimeters, and film thickness = 0.25 µm) is used. The oven temperature programme is at a rate of 20 ˚C /minute, increasing from 60˚C to 260˚C. The Inlet temperature is 265˚C, Split ratio: 50:1. Temperature in the detector is 300˚C with 100Hz acquisition. The injection volume used is 0.5 µL.

Fig 4. Oil profiling of Nutmeg seed oil

Table 1. Active component of Nutmeg Seed oil by GC-FID

Peak	RT	Area %	Compound
1	2.816	4.66	Beta thujene
2	2.875	18.25	Alpha pinene
3	2.966	0.40	Camphene
4	3.092	6.40	Sabinene
5	3.137	13.25	Beta pinene
6	3.168	1.56	Beta myrcene
7	3.293	0.60	Delta-3-carene
8	3.374	2.69	Alpha terpene
9	3.392	7.35	Meta cymene
10	3.459	6.38	D-Limonene/ beta phellandrene
11	3.645	4.47	Gamma terpene
12	3.860	1.50	Iso terpinolene
13	4.202	0.17	Sabinene hydrate
14	4.455	10.99	Terpinen-4-ol
15	4.522	1.45	Alpha terpineol
16	5.116	0.16	Isobornyl acetate
17	5.163	0.15	Saffrole
18	5.297	0.15	Alpha cubebene
19	5.591	0.23	Copane
20	5.84	1.11	Methyl eugenol
21	6.621	10.33	Myristicin
22	6.771	0.63	Elemicin

3.2. Physicochemical Properties of Nanoemulsions.

Physicochemical characterization studies were carried out to characterize and control the physical stability of the formulated nanoemulsions. The pH values of the nanoemulsions were measured by immersing the pH electrode into the undiluted emulsion using a pH meter (Systronics pH Meter ,μ pH System 362). All the tests were determined at room temperature. The emulsions shown pH in the range of 3.852 to 3.964 by varing the ration from 1:1 to 3:1.

3.3. Determination of Particle Size and Polydispersity Index (PDI)of Emulsions by DLS

Nanoemulsion droplet Diameters were measured by dynamic light scattering instrument (Zetasizer Nano series, Nano ZS Model, Make Malvern). The particle size analyzer is based on Dynamic Light Scattering (DLS), which measures the changes in scattering intensity due to the Brownian motion of particles present in the suspension. DLS analysis of prepared nutmeg essential oil emulsions with varying oil ratios (NG T80 1:1, NGT80 2:1, NG T80 3:1) was carried out to study their particle size distribution. The particle size and PDI of prepared formulations were tabulated below.

Table 2. Z-average size, Polydispersity index (PDI) of Nutmeg oil emulsion

Formulation	**Average Particle Size (nm)**	**PDI**
NG T80 1:1	56.80	0.530
NG T80 2:1	157.8	0.391
NG T80 3:1	70.07	0.648

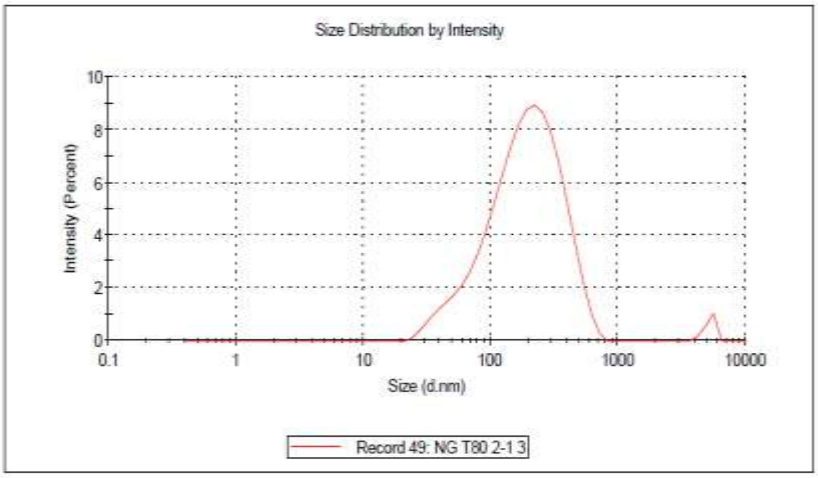

Fig 5. DLS analysis of NG T80 1:1 emulsion

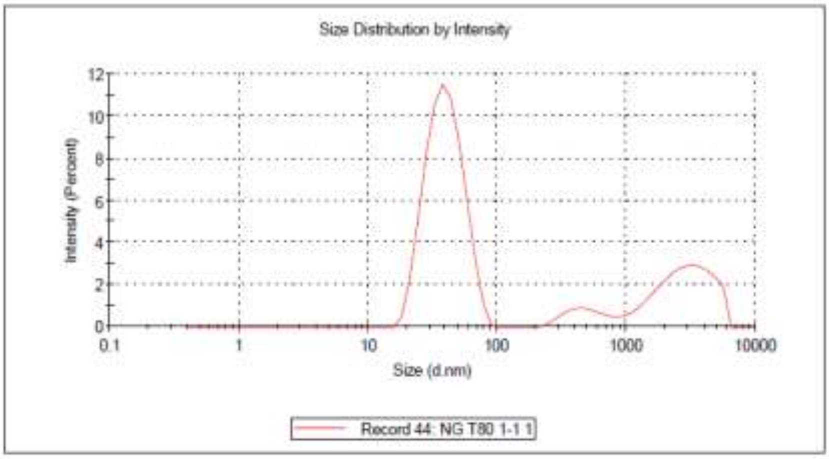

Fig 6. DLS analysis of NG T80 2:1 emulsion

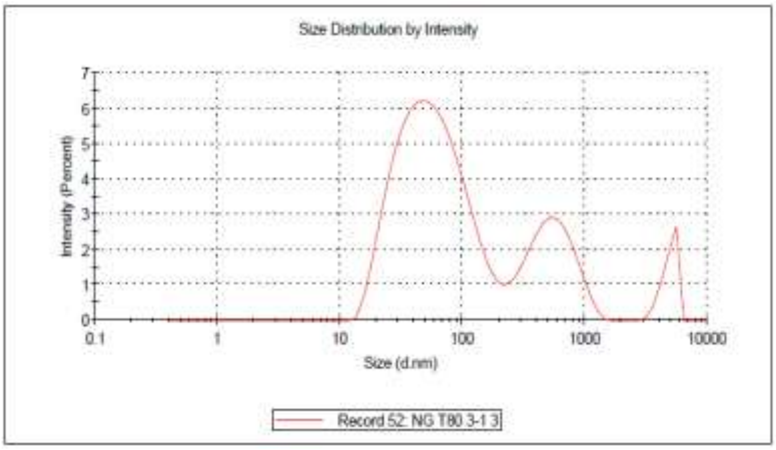

Fig 7. DLS analysis of NG T80 3:1 emulsion

3.2.1. Mortality, *Tribolium castaneum* exposed to nutmeg oil emulsion

The insecticidal activity of the NG T80 1:1was evaluated against adult Tribolium castaneum at 6h, 12h and 24 h exposure periods. Three replicate studies were carried out to calculate the LC 50 value of NG T80 emulsion against *tribolium castaneum.* Mortality increased progressively with increasing nanoemulsion concentration and exposure duration. At 24 h, all treated concentrations produced 100% mortality. Consequently, the concentration–response relationship reached a plateau, resulting in an approximately horizontal probit line (slope ≈ 0). Because all tested concentrations exceeded the median lethal concentration, LC_{50}, regression slope, 95% confidence limits, and goodness-of-fit statistics could not be estimated reliably for the 24 h exposure period.

Table 3. Replicate studies on mortality against T. Castaneum of NG T80 1:1 emulsion

Conc. (%)	Total no. of insects	No.of Dead REP1			No.of Dead REP 2			No.of Dead REP 3		
		6h	12h	24h	6h	12h	24h	6h	12h	24h
Control	10	0	0	1	0	0	1	0	0	1
1	10	0	2	10	0	1	10	0	2	10
2	10	1	3	10	1	3	10	1	4	10
4	10	1	4	10	1	3	10	1	4	10
6	10	2	5	10	2	5	10	2	5	10
8	10	2	6	10	2	6	10	2	5	10
10	10	4	7	10	4	8	10	4	6	10

The corrected mortality and standard deviation is plotted in Table 4.

Table 4. Abbott mortality against T. Castaneum of NG T80 1:1 emulsion

Conc. (%)	Mean mortality (%)		Abbott-corrected mortality (%)		Mean ± SD	Mean ± SD
	6h	12h	6h	12h	6h	12h
1	0	16.7	0.00, 0.00, 0.00	11.11, 0.00, 11.11	0.00 ± 0.00	7.41 ± 6.42
2	10	33.3	0.00, 0.00, 11.11	22.22, 22.22, 33.33	3.70 ± 6.42	25.93 ± 6.42
4	10	36.7	0.00, 0.00, 0.00	33.33, 22.22, 33.33	0.00 ± 0.00	29.63 ± 6.42
6	20	50.0	11.11, 11.11,11.11	44.44, 44.44, 44.44	11.11 ± 0.00	44.44 ± 0.00
8	20	56.7	11.11, 11.11,11.11	55.56, 55.56, 44.44	11.11 ± 0.00	51.85 ± 6.42
10	40	70.0	33.33, 33.33,33.33	66.67, 77.78, 55.56	33.33 ± 0.00	66.67 ± 11.11

At 6 h, untreated control exhibited 10% natural mortality (1 insect out of 10), which was corrected using Abbott's formula. No corrected mortality was observed at the 1% and 4% concentrations, whereas corrected mortality increased to 3.70 ± 6.42% at 2%, 11.11 ± 0.00% at both 6% and 8%, and 33.33 ± 0.00% at the highest concentration 10%. These findings indicate that the insecticidal activity of the 1:1 formulation was relatively low during the initial exposure period, with appreciable mortality occurring only at higher concentrations.

Following 12 h of exposure, mortality increased markedly across all concentrations. Abbott-corrected mortality increased from 7.41 ± 6.42% at 1% concentration to 66.67 ± 11.11% at 10%. The highest mortality was recorded at 10%, demonstrating that prolonged exposure substantially enhanced the insecticidal efficacy of the nanoemulsion.

Table 5. Replicate studies on mortality against T. Castaneum of NG T80 2:1 emulsion

Conc. (%)	Total no. of insects	No.of Dead REP1			No.of Dead REP 2			No.of Dead REP 3		
		6h	12h	24h	6h	12h	24h	6h	12h	24h
Control	10	0	0	1	0	0	1	0	0	1
1	10	1	2	10	1	2	10	1	2	10
2	10	1	3	10	2	4	10	2	3	10
4	10	2	4	10	2	4	10	2	4	10
6	10	3	5	10	3	5	10	4	5	10
8	10	4	6	10	4	6	10	4	7	10
10	10	5	7	10	5	8	10	6	7	10

Table 6. Abbott mortality against T. Castaneum of NG T80 2:1 emulsion

Conc. (%)	Mean mortality (%)		Abbott-corrected mortality (%)		Mean ± SD	
	6h	12h	6h	12h	6h	12h
1	10	20	0.00, 0.00, 0.00	11.11, 11.11, 11.11	0.00 ± 0.00	11.11 ± 0.00
2	16.7	33.3	0.00, 11.11, 11.11	22.22, 33.33,22.22	7.41 ± 6.42	25.93 ± 6.42
4	20	40	11.11, 11.11,11.11	33.33, 33.33,33.33	11.11 ± 0.00	33.33 ± 0.00
6	33.3	50	22.22, 22.22,33.33	44.44, 44.44,44.44	25.93 ± 6.42	44.44 ± 0.00
8	40	63.3	33.33, 33.33, 33.33	55.56, 55.56,66.67	33.33 ± 0.00	59.26 ± 6.42
10	63.3	73.3	44.44, 77.78, 55.56	66.67, 77.78,66.67	59.26 ± 16.98	70.37 ± 6.42

At 6 h, no corrected mortality was observed at the lowest concentration (1%). However, corrected mortality increased to 7.41 ± 6.42% at 2%, 11.11 ± 0.00% at 4%, 25.93 ± 6.42% at 6%, 33.33 ± 0.00% at 8%, and 59.26 ± 16.98% at the highest concentration (10%). The gradual increase in mortality with concentration indicates that the insecticidal activity of the nanoemulsion.

Following 12 h of exposure, insect mortality increased substantially across all concentrations. Abbott-corrected mortality ranged from 11.11 ± 0.00% at 1% concentration to 70.37 ± 6.42% at concentrations 10%. The highest corrected mortality was observed at 10%, indicating enhanced toxic action following prolonged exposure. The relatively low standard deviation values obtained for most concentrations indicate good reproducibility of the experimental results.

Table 7. Replicate studies on mortality against T. Castaneum of NG T80 3:1 emulsion

Conc. (%)	Total no. of insects	No.of Dead REP1			No.of Dead REP 2			No.of Dead REP 3		
		6h	12h	24h	6h	12h	24h	6h	12h	24h
Control	10	0	0	1	0	0	1	0	0	1
1	10	1	2	10	1	2	10	1	2	10
2	10	2	3	10	2	3	10	2	3	10
4	10	3	**5**	10	3	**4**	10	3	**5**	10
6	10	4	6	10	4	5	10	4	6	10
8	10	4	**6**	10	5	**6**	10	5	**7**	10
10	10	4	8	10	6	8	10	5	8	10

Table 8. Abbott mortality against T. Castaneum of NG T80 3:1 emulsion

Conc. (%)	Mean mortality (%)		Abbott-corrected mortality (%)		Mean ± SD	
	6h	12h	6h	12h	6h	12h
1	10	20	0.00, 0.00, 0.00	11.11, 11.11, 11.11	0.00 ± 0.00	11.11 ± 0.00
2	20	30	11.11, 11.11, 11.11	22.22, 22.22, 22.22	11.11 ± 0.00	22.22 ± 0.00
4	30	46.7	22.22, 22.22, 22.22	44.44, 33.33, 44.44	22.22 ± 0.00	40.74 ± 6.42
6	40	56.7	33.33, 33.33, 33.33	55.56, 44.44, 55.56	33.33 ± 0.00	51.85 ± 6.42
8	46.7	63.3	33.33, 44.44, 44.44	55.56, 55.56, 66.67	40.74 ± 6.42	59.26 ± 6.42
10	50	80	33.33, 55.56, 44.44	77.78, 77.78, 77.78	44.44 ± 11.11	77.78 ± 0.00

At 6 h, the untreated control showed 10% natural mortality, and all mortality values were corrected using Abbott's formula. No corrected mortality was observed at the lowest concentration (1%), whereas corrected mortality increased steadily to 11.11 ± 0.00% at 2% to 44.44 ± 11.11% at 10%.

Following 12 h of exposure, mortality increased markedly at all concentrations. Abbott-corrected mortality reached 77.78 ± 0.00% at 10% from 11.11± 0.00% at 1%. The highest corrected mortality was observed at 10%, where identical mortality values among the three replicates resulted in zero standard deviation, demonstrating excellent reproducibility. The substantial increase in mortality after prolonged exposure indicates that the toxic constituents of the nanoemulsion continued to exert insecticidal effects over time.

Compared with the 1:1 and 2:1 formulations, the 3:1 nanoemulsion produced higher mortality at intermediate concentrations, indicating enhanced insecticidal activity associated with the increased proportion of nutmeg oil in the formulation. The slightly higher standard deviation observed at 10% concentration reflects normal biological variation among the experimental replicates.

LC 50 (%) calculated by fitting a probit regression model to mortality data in which the concentration is plotted on a $\log_{10}$ scale in x-axis where as y-axis is Abbott-corrected mortality (%) calculated from the three replicates (control mortality = 10%).The Dose response curve against T.castaneum is shown below.

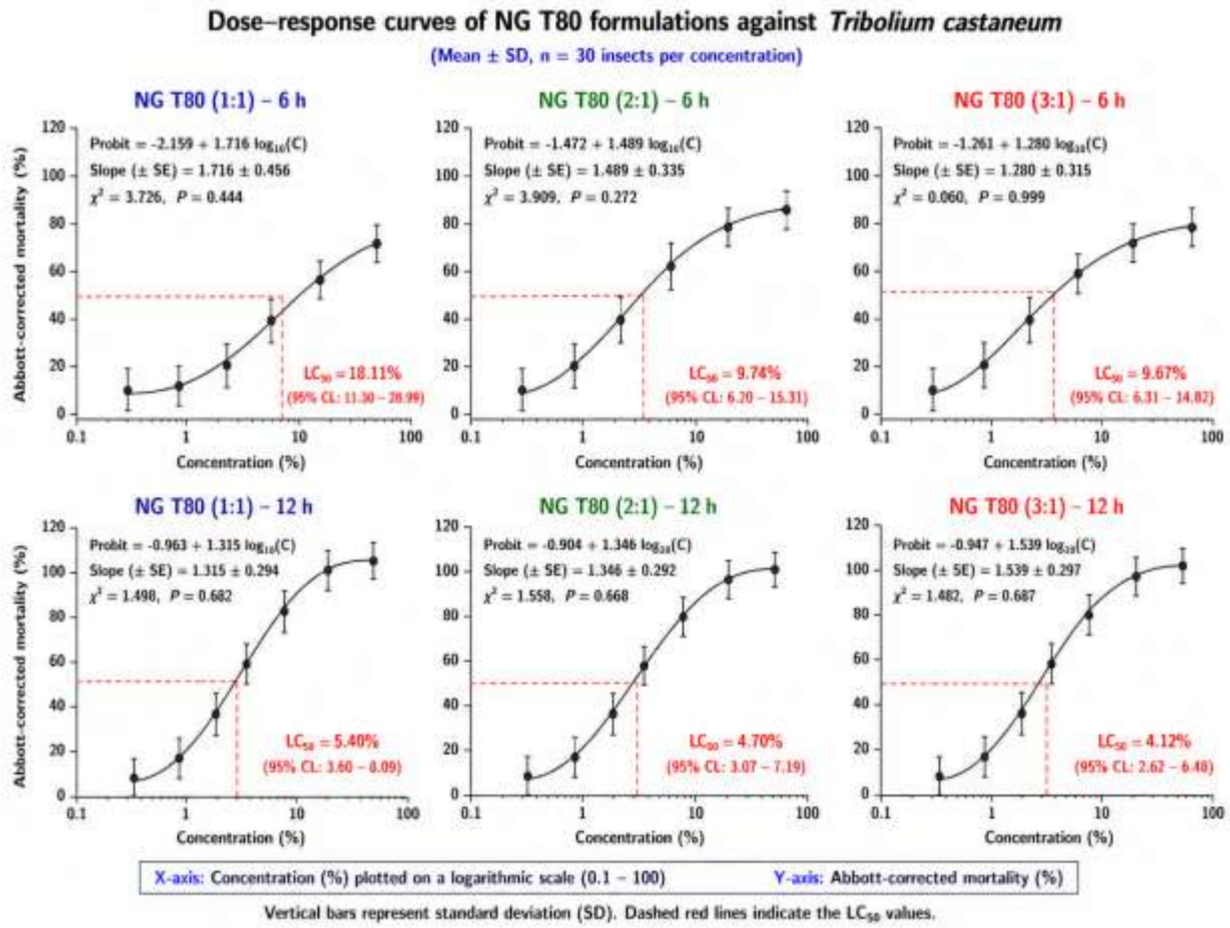

Fig 8. Dose response curve of NG T80 emulsion against T.castaneum

Table 9. Probit regression analysis of nutmeg oil nanoemulsions against *Tribolium castaneum*

Formulation	**Time**	**Regression equation***	**Slope ± SE**	**LC_{50} (%)**	**Pearson χ^2**
NG T80 (1:1)	6 h	Probit = −2.159 + 1.716 × $\log_{10}$(C)	1.716 ± 0.456	18.11	3.726
NG T80 (1:1)	12 h	Probit = −0.963 + 1.315 × $\log_{10}$(C)	1.315 ± 0.294	5.40	1.498
NG T80 (2:1)	6 h	Probit = −1.472 + 1.489 × $\log_{10}$(C)	1.489 ± 0.335	9.74	3.909
NG T80 (2:1)	12 h	Probit = −0.904 + 1.346 × $\log_{10}$(C)	1.346 ± 0.292	4.70	1.558
NG T80 (3:1)	6 h	Probit = −1.261 + 1.280 × $\log_{10}$(C)	1.280 ± 0.315	9.67	0.060

Formulation	Time	Regression equation*	Slope ± SE	LC_{50} (%)	Pearson χ^2
NG T80 (3:1)	12 h	Probit = −0.947 + 1.539 × $\log_{10}(C)$	1.539 ± 0.297	4.12	1.482

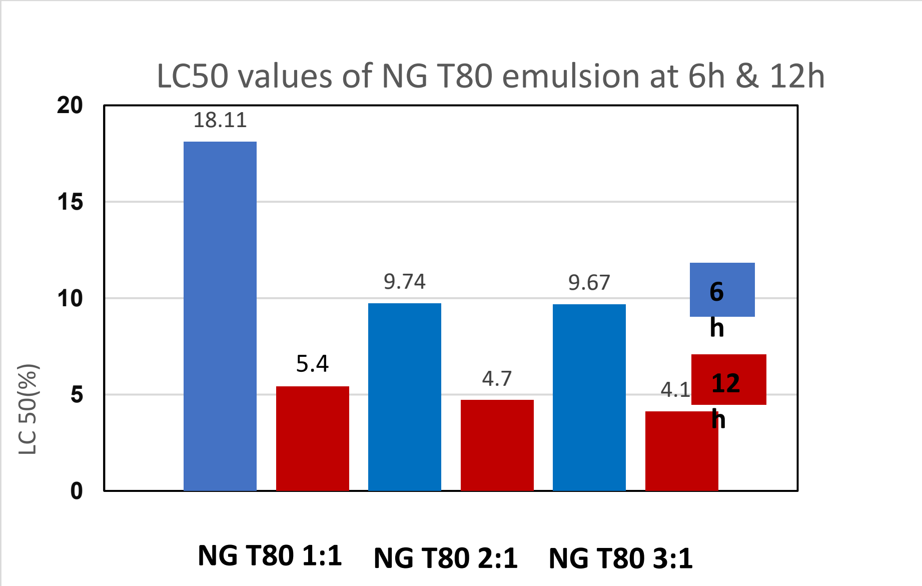

Fig 9. LC 50 value of NG T80 emulsion against T.castaneum

CONCLUSION

The oil profiling of nutmeg seed oil was performed in GC-FID. The compounds present in the nutmeg seed oil such as α-Pinene,β-Pinene,Sabinene,Myristicin,Safrole,Elemicin and Limonene contributing to the insecticidal, repellent and contact toxicity. Nutmeg seed essential based nanoemulsion enhances its insecticidal activity compared with the oil due to physicochemical changes in the formulation rather than changes in the chemical composition of the oil.

Nano emulsion with different oil to surfactant ration was prepared using Tween 80 as surfactant. NG T80 1:1 emulsion exhibit particle size of 56.80 nm, NG T80 2:1 with 157.8nm and 70.07 nm for NG T80 3:1 emulsion.

Oil-to-surfactant ration and exposure time play a vital role in the insecticidal activity of NG T80 emulsion against *Tribolium castaneum.* Probit analysis confirmed a clear dose-dependent increase in the mortality for all formulations *at* 6h and 12 hr exposure. From the linear probit regression models revealed a good agreement with the observed mortality data, which indicates the selected combination is appropriate for calculating the LC50.

Among the prepared formulations, NG T80 2:1 exhibited the highest insecticidal efficacy at 6 h, with the lowest LC_{50} value of 9.74%, followed by NG T80 3:1 with a value of 9.67%, whereas NG T80 1:1 required a higher concentration of 18.11% to achieve 50% mortality.After 12 h of exposure, all formulations were more effective, as evidenced by the reduction in LC_{50} values. The NG T80 3:1formulation showed the greatest potency, with the lowest LC_{50} 4.12%, followed by NG T80 2:1 with a value of 4.70% and NG T80 1:1 with LC 50 value of 5.40%. These findings demonstrate that prolonged exposure significantly enhanced the toxicity of the nanoemulsions. At 24 h, all treated concentrations produced 100% mortality, indicating that exposure duration played a critical role in maximising insecticidal activity. Because complete mortality occurred at every concentration after 24 h, an LC_{50} value could not be estimated for this exposure period, as the dose–response relationship no longer permitted probit analysis.

The slope values (1.280–1.716) showed a consistent increase in mortality with increasing in concentration, suggesting a uniform susceptibility of *T. castaneum* to the nanoemulsion formulations. The relatively low chi-square (χ^2) values further suggested an acceptable fit between the observed and predicted mortality responses.

Overall, the results demonstrate that NG T80 emulsions are highly effective botanical insecticides against *Tribolium castaneum*. The NG T80 3:1 formulation showed the greatest efficacy after prolonged exposure, while the NG T80 2:1 formulation produced the most rapid toxic response during the early exposure period. These findings suggest that optimizing the oil-to-surfactant ratio can improve the insecticidal performance of nutmeg oil nanoemulsions an environmentally friendly alternatives to conventional synthetic grain protectants.

REFERENCE:

1. Gharsan, M., Alghamdi, S., Alshammari, A., et al. Nanoemulsion-based delivery systems for essential oils: Preparation methods, stability, and agricultural applications. *Heliyon, 10*(3),(2024). e24789. https://doi.org/10.1016/j.heliyon.2024.e24789

2. González, M. F., et al., Nanoemulsions containing essential oils: Formulation, properties, and applications. In D. J. McClements (Ed.), *Nanoemulsions: Formulation, Applications, and Characterization* (2024). (pp. 121–140). Academic Press.

3. C.Solans, P.Izquierdo, J.Nolla, N.Azemar, M.J.Garcia Celma, Nanoemulsions, Current opinion in colloids and interfaces.10(2005)102-110, https://doi.org/10.1016/j.cocis.2005.06.004
4. Davidson, P.Food antimicrobials: Back to nature", in First International Symposium on Natural Preservatives in Food System. (2005).
5. Bi D., et al. "Unsaturated guluronate oligosaccharide used as astabilizer of oil-in-water nanoemulsions loaded with bioactive nutrients". Food Chemistry: X 16 (2022): 100469,
6. https://doi.org/10.1016%2Fj.fochx.2022.100469
7. Filippin Cossetin L., et al. "Development of nanoemulsions containing Lavandula dentata or Myristica fragrans essential oils: Influence of temperature and storage period on physical chemical
8. properties and chemical stability". Industrial Crops and Products 160 (2021):113115, http://dx.doi.org/10.1016/j.indcrop.2020.113115
9. Nasser, A., et al. (2024). Preparation and characterization of nutmeg (*Myristica fragrans*) essential oil nanoemulsion as a botanical insecticide**.** *Heliyon*, **10**, 1292272.
10. Hashem, A. S., & Ramadan, M. F. Preparation, characterization and biological applications of essential oil nanoemulsions: A review. *Journal of Applied Research on Medicinal and Aromatic Plants*, 21, (2021). 100291.