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# Preparation, Characterization and Biological Efficacy of Eucalyptus Oil Nanoemulsion against the Stored Grain Insects

**Tahany G. M. Mohammed[1*] and M. E. H. Nasr[2]**

[1]*Central Agricultural Pesticides Laboratory, Department of Pesticide Formulations, Agriculture Research Center (ARC), 12618, Dokki, Giza, Egypt.*
[2]*Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.*

***Authors' contributions***

*This work was carried out in collaboration between both authors. Authors designed the study, wrote the protocol, wrote the first draft of the manuscript and managed literature searches. Both authors read and approved the final manuscript.*

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## ABSTRACT

Nanotechnology has enormous potential for developing alternative pest control strategies and reducing the risk of insecticide molecules. The present study aimed to develop a stable nanoemulsion (NE) of eucalyptus oil (EO) by the spontaneous emulsification method and evaluate its insecticidal and repellent effect against *Sitophilus oryzae* (L.), *Rhizopertha dominica* (F.) and *Tribolium-castaneum* (Herbst.). The prepared nanoemulsion formulation having a small particle size 8.57 nm with polydispersity index (PDI) 0.28. The study of the stability and physicochemical properties showed that the prepared formulation had good physical stability without any change in the macroscopic parameters. In addition, results showed that theinsecticidal activity of the prepared NE was higher than the original EO against the three tested insect speciesand the mortality increased with increasing concentrations and extending of exposure time. The contact toxicity of NE film revealed that, adults of R. dominica was more susceptible to all treatments followed by *S. oryzae* adults, while adults of *T. castaneum* was the least susceptible one, however, adults of *S. oryzae* was more susceptible followed by *T. castaneum* adults while, adults of

**Corresponding author: Email: tahanymohamad2@gmail.com;*

*R. dominica* was the least susceptible one according to the fumigant toxicity and repellency. Our results suggested that the prepared formulation may be used in an integrated pest managementprogramfor controlling stored grain insects.

*Keywords: Eucalyptus oil; formulation; nanoemulsion; Sitophilus oryzae; Rhizopertha dominica; Tribolium castaneum.*

## 1. INTRODUCTION

Significant storedgrain losses in developing countries during the storage period may exceed 20% and are mainly due to insect pests affecting the quantity and quality of grain [1]. *Rhizopertha dominica* (Fab.) and *Sitophilus oryza*e (L.) are universally recognized as the most devastating primary insect pests of stored cereals feed on the whole and healthy grains and cause considerable losses by reducing the quality and quantity of stored cereals [2,3]. Among the stored product beetles, *Tribolium castaneum* Herbst., is a cosmopolitan and serious pest of many stored products. Larvae and adults beetle cause both quantitative and qualitative damages to the stored products [4].

To control the stored product pests, excessive use of fumigant chemical pesticides (e.g. phosphine and methyl bromide) may increase the emergence of resistant insects, residues in agricultural products and the environment [5], enhanced pest resurgence and development of resistance, toxicological implications to human health and increased environmental pollution [6,7]. Hence, it is necessary to find more ecofriendly alternatives or viable methods to replace the use of synthetic chemicals [8]. Essential oils (EOs)are interesting alternatives to synthetic insecticides because of their limited persistence in the environment, low probability of giving resistant insect pests generation [9] and low toxicity of residues during application [8]. One of the most promising essential oils is eucalyptus oil. Eucalyptus oil has been placed under GRAS (Generally Regarded as Safe) category by Food and Drug Authority of USA and classifiedas non-toxic [10]. Eucalyptus oil (EO) is effective against storage pests e.g. *Sitophilus oryzae*, *Rhizopertha dominica* and *Tribolium castaneum* etc. [11,12]. Pesticide property of EO is due to the presence of volatile compound “eucalyptol or 1,8-cineol” [13].

Despite these promising properties, problems related to the EO high volatility, poor water solubility and aptitude for oxidation have to be resolved before they are used as an alternative pest control system [1,14]. The formulation of EOs as nanoemulsions improves both the stability and effectiveness of botanical insecticides. Indeed, nano-formulations can solve problems related to EOs volatility, poor water solubility, and the tendency to oxidize [15]. Furthermore, these formulations can release the active compounds at the site of action gradually [16] and minimize the toxic effects on non-target organisms [2].

Several methods have been detailed on preparing nanoemulsions for agriculture. Recently, one of the most popular ideas is to prepare nanoemulsions using spontaneous emulsification method because of its low-cost, simplicity of preparation and without any specialized homogenization equipment [17,18]. The study aimed to develop nanoemulsion of eucalyptus oil using the spontaneous emulsification method withthe help of biodegradable non-ionic surfactants and the physicochemical characterization of the prepared nanoemulsion wasstudied by measurements of various parameters such as stability, thermostability, droplet size, polydispersity index, pH, refractive index, surface tension, density, viscosity and evaluate its insecticidal and repellency effect against three main stored grainsinsects;*Sitophilus oryzae* (L.); *Rhizopertha dominica* (Fab.) and *Tribolium castaneum* (Herbst).

## 2. MATERIALS AND METHODS

### 2.1 Chemicals

Eucalyptus essential oil was purchased from Kato Aromatic Company for Flavors, Fragrances, Coloring Extracts, and Essential Oils, Giza, Egypt, non-ionic surfactant; Alkamuls 14/R (Castor oil (ethoxylate)$_{60}$, HLB 14.9) was kindly supplied by Rhodia-Home, Personal Care & Industrial Ingredients, Milano, Italy. Deionized water was used for preparing the nanoemulsion.

### 2.2 Insects

Laboratory strains of the rice weevil *Sitophilus oryzae* (L.), the lesser grain borer *Rhizopertha* *dominica* (Fab.) and the red flour beetle *Tribolium castaneum* (Herbst.) were used in these experiments. All species were reared for several generations in thelaboratory of Stored Grain & Product Pests Res. Dept., Plant Protection Res. Ins., Agricultural Research Center, Dokki, Egypt.

## 2.3 Nanoemulsion Preparation

Nanoemulsion formulation was prepared using eucalyptus oil, nonionic surfactant and deionized water by spontaneous emulsification method. Nanoemulsion was formulated in two steps;first, the oil phase was prepared by mixing 3% (v/v) of oil and biodegradable surfactant in the ratio of 1:3, second, the organic phase was added dropwise to water phase by stirring the systemat room temperature.

## 2.4 Characterization of Nanoemulsion

The nanoemulsion formulation was subjected to the evaluation of various parameters as per the standard procedures.

### 2.4.1 Stability and thermostability analysis

A 25 ml of each nanoemulsion was placed into a glass bottle with a screw cap and kept at room temperature (25°C) for 90 days, 0°C for 7 days and at 54°C for 14 days, as the Food and Agricultural Organization (FAO) approved standard evaluation for agrochemical products to evaluate the stability of the formulation in a tropical climate. The physical appearance of the prepared nanoemulsion formulation was visually investigated. Also, the nanoemulsion was subjected to centrifugation at 10,000 rpm for 30 min, then observed for any creaming, phase separation, or cracking [19]. The thermodynamic stability was also analyzed by freeze-thaw cycle method, in which the formulation was stressed to simulate the conditions that are encountered in warehouse storage; test tube filled with the prepared formulation and hermetically closed was vertically stored for 12 h in a freezer at -20°C, then, for 12 h at room temperature 25±2°C, nanoemulsion was exposed to such four cycles and observed for any physical changes like creaming, coalescence and phase separation. The formulation is considered "stable" if there is no substantial separation after such four cycles.

### 2.4.2 Nanoemulsion particle size distribution measurement

The average particle size of droplets and polydispersity index of the nanoemulsion was measured using Zetasizer Nano ZS® (Malvern Instruments, UK) at room temperature. The sample was diluted with milli-Q® (Millipore Corporation) deionized water before measurement to avoid multiple scattering effects.

### 2.4.3 pH measurement

The pH of a 1% solution of the prepared NE formulation was measured using a pH meter (Jenway® model pH 3510). It was recalibrated before testing using a standard buffer solution at pH 7.0 and pH 4.0; the measurements were carried out at 25°C by direct immersion of pH glass electrode into NE formulation samples (CIPAC Handbook MT 75.3) [20].

### 2.4.4 Refractive index

The Refractive index of the NE formulation was measured using a digital ABBE Refractometer, ATAGO®, Co., LTD, Japan by placing one drop of the NE formulation on the slide at 25°C [21].

### 2.4.5 Surface tension

The surface tension was measured by the Wilhelmy plate method using “Sigma 700” (Biolin Scientific). The platinum plate was burned under an alcohol flame after being washed by deionized water to remove impurities before each measurement.The instrument was recalibrated before testing and the measurements were-conducted until the surface tension values remained unchanged, indicating that equilibrium had been reached. The surface tension of the NE samples was recorded [22].

### 2.4.6 Density

The density was measured using a digital density meter model DDM 2910 with a touch screen. Rudolph Research Analytical, USA [23].

### 2.4.7 Viscosity determination

The viscosity of the prepared nanoemulsion formulation was measured without dilution using ″Brookfield DV II+ PRO″ digital Viscometer (Brookfield, USA) UL rotational adaptor. The temperature was kept at 25°C during the measurement by water bath (Model: TC-502 USA) and each reading was recorded after the equilibrium of the sample. Five replicates were conducted for the sample and the average was reported and expressed as milli pascal-second (mPas) [24].

## 2.5 Insecticidal Activity against Stored Grain Insects

### 2.5.1 Contact toxicity

The contact toxicity of the original and nanoemulsion eucalyptus oil was evaluated against the three insect species. 0.5 ml of each 10, 5, 2.5, 1.25 and 0.0 µl/ml oilconcentrations in acetone were applied on each filter paper (Whatman No. 1) then each filter paper was placed in a Petri dish (9 cm), and left for two hours to evaporate the acetone solvent. Twenty adults (a week-old) of *S. oryzae*, *R. dominica* and *T. castaneum* were introduced to each dish and covered with the lid. All replicates were kept at 25±2°C and 60 ± 5% RH. Mortalities were recorded after 24, 48 and 72 hours from treatment date.

### 2.5.2 Fumigant toxicity

The fumigant toxicity of eucalyptus oil nanoemulsion at the same previously mentioned concentrations was evaluated against the three insect species. Glass jars (approx. 250 cc) were used as a fumigation chamber; a filter paper (Whatman No.1) was treated with 0.5 ml of each concentration and placed in the bottom of the jar. Twenty adults (a week-old) of *S. oryzae*, *R. dominica* and *T. castaneum* were introduced to small nylonbags containing about 10 g of sterilized wheat kernels in case of the rice weevil and the lesser grain borer or wheat flour in the case of the red flour beetle. Bags were fixed with a rubber band and suspended over filter paper to prevent direct contact of tested insects with treated paper. Each jar sealed using a polyethylene layer. All replicates were kept at 25 ± 2°C and 60 ± 5% RH. Mortalities were recorded after 24, 48 and 72 hours from treatment. Mortalities were corrected [25].

### 2.5.3 Repellency test

Whatman filter papers (9 cm) were cut into two identical halves, the first half treated with 0.5 ml from each tested oil nanoemulsion concentrations (treatments) and the second half treated with 0.5 ml acetone solvent (control). Treated and untreated discs were left until completely dried, then attached using adhesive and placed in a Petri dish. Twenty adults (a week- old) of *S. oryzae*, *R. dominica* and *T. castaneum* were introduced separately to each dish in the central area of the disks and covered with the lid. The number of adults present on both treated and untreated discs were recorded after 72 hours from treatment. Repellency percentages were calculated as follows:

$$\text{Repellency\%} = [(NC-NT)/ (NC+NT)] \times 100 \qquad (1)$$

Where, NC = Number of adults on an untreated half.
NT= Number of adults on treated half.

## 2.6 Data Analysis

The lethal concentrations of the adults of the three stored grain insects were statistically analyzed according to Finney [26]. The obtained data were analyzed by the ANOVA test and significant means were separated by Duncan's multiple range tests using a computer program of SPSS 19.0.

# 3. RESULTS AND DISCUSSION

## 3.1 Characterization of the Prepared Nanoemulsion Formulation

### 3.1.1 Stability and thermostability analysis

Visual assessment of the prepared nanoemulsion concerning to the appearance and potential phase separation was performed.The prepared nanoemulsion had good physical stability without any change in the macroscopic parameters, phase separation, creaming, and flocculation or color change during the experimental period, when tested for stability (at 25°C for 90 days), thermostability (at 54°C for 14 days) and cold storage (at 0°C for seven days). The stabilization of the prepared nanoemulsion is due to the presence of surfactant that forms a layer at the interface of the droplet, thus, separating the oil from the aqueous phase owing to the stability of emulsion [27-29]. Also, the attractive forces between the droplets are proportional to the size of the droplets and so when the size of the droplets is in the nano range, the attractive forces between the droplets are weak, thereby, preventing particle aggregation and helps in making the nanoemulsions more stable.

### 3.1.2 Mean particle size and polydispersity index (PDI)

Based on the thermodynamic stability study, the prepared nanoemulsion exhibited a translucent and homogeneous appearance; the droplet size distribution of the prepared NE was 8.57 nm with PDI 0.28. Fig. 1. Droplet size and its distribution play an important role in the stability of the emulsions [30]. The PDI is the measure of size distribution and stability of droplet size in the emulsion, the lower polydispersity of colloidal systems signifies their higher stability attributed to the lower ripening rate [31].

#### 3.1.3 Physicochemical characterization of the prepared NE

The physical properties of nanoemulsions are very important to expand their applicability and to elucidate the interaction between nanoscale materials and insects [32]. The physicochemical parameters: pH, refractive index, surface tension, viscosity and density of eucalyptus oil nanoemulsion as shown in Table 1. The pH value of the nanoemulsion over the storage period in the range of 4.03 to 4.09. The determination of this parameter is an important test for monitoring the stability of the nanoemulsionbecause a change in pH values indicates the occurrence of chemical reactions that may compromise the quality of the final product [33]. The variation of refractive index was in the range of 1.3486 to 1.3492, it reflects the NE formulation appears nearly transparent in the visible spectrum and the formulation had a surface tension range in the range of 32.77 to 33.07 mN/m; the lower surface tension is a desirable characteristic for most agricultural sprays because it facilitates the spreading of droplets upon impact on leaves or other target surfaces, to increase the surface-active area and improve penetration and uptake of the product into the plants [34]; the density value was in the range of 1.0073 to 1.0077 g/cm$^3$ and the apparent viscosity was low, which in the range of 2.24 to 2.35 mpascal, which might be due to the low oil content.

### 3.2 The Insecticidal Activity of Nanoemulsion Formulation against the Three Tested Insect Species

#### 3.2.1 Contact toxicity

In all treatments of eucalyptus oil, the mortality percentage of all tested insects exposed to a film of oil increased with an increase in the treatment concentrations and exposure time. Also, NE formulation was more effective than EO against all insect species Table 2. Adults of *R. dominica* were more susceptible to all treatments followed by *S. oryzae* adults and *T. castaneum* was the most tolerant insect. No mortality obtained against all insect species after 24 hrs from exposing to EO at concentrations 10, 5, 2.5 and 1.25 µl/ml. While the corrected mortality percentages for *S. oryzae, R. dominica* and *T. castaneum* were 41.7, 53.3 and 20.0, 28.3, 43.3 and 16.7, 11.7, 41.7 and 6.7, 5.0, 20.0 and 3.3% after exposing to aforementioned concentrations from NE formulation, respectively. After 48 hrs and a for ementioned concentrations, mortality percentages of *S. oryzae* recorded 8.3, 6.7, 5.0 and 3.3; 51.7, 35.0, 31.7 and 25.0% of EO and NE formulation, respectively. These values were increased after 72 hrs to reach 15.0, 11.7, 5.0 and 6.7; 78.3, 63.3, 55.0 and 46.7% of EO and NE formulations, respectively.

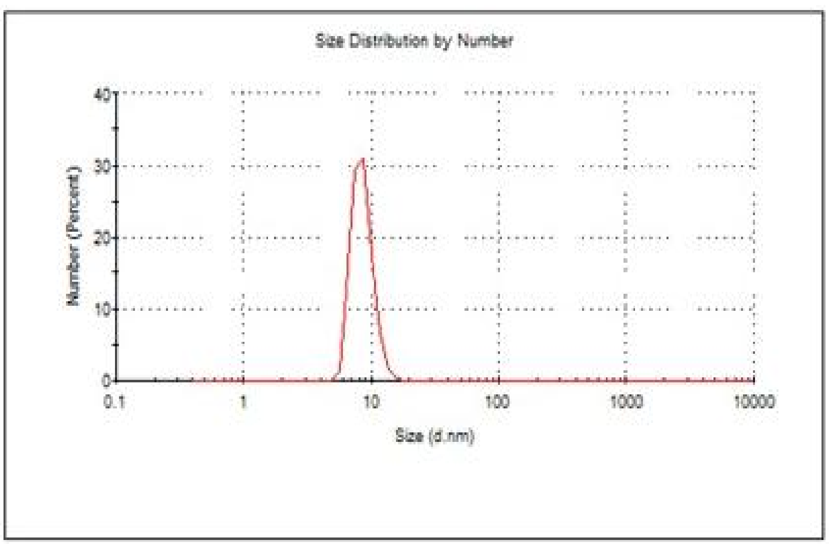


**Fig. 1. The particle size distribution of eucalyptus oil nanoemulsion**

**Table 1. Physicochemical properties of eucalyptus oil nanoemulsion**

| Parameters | Before storage | After storage | |
|---|---|---|---|
| | Fresh formulation | 7 Days | 14 Days |
| Temperature | Room temp. | 0°C | 54°C |
| pH (1%) | 4.03 | 4.09 | 4.06 |
| Refractive Index | 1.3486 | 1.3489 | 1.3492 |
| Surface tension | 32.92 | 33.07 | 32.77 |
| Density | 1.0076 | 1.0073 | 1.0077 |
| Viscosity | 2.35 | 2.27 | 2.24 |

**Table 2. Contact toxicity of eucalyptus oil formulations against three stored grain insects**

| Conc. µL/ml | Tested oils | % adult mortality ± S.E at indicated periods (hours) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 24 | | | 48 | | | 72 | | |
| | | S. o. | R. d. | T. c. | S. o. | R. d. | T. c. | S. o. | R. d. | T. c. |
| 10 | O | 00.0±0.00 | 00.0±0.00 | 00.0±0.00 | 8.3±0.33 | 11.7±0.33 | 5.00±0.58 | 15.0±0.58 | 20.0±0.58 | 8.3±0.67 |
| | NE | 41.7*±0.88 | 53.3 *±0.88 | 20.0*±0.58 | 51.7 *±0.88 | 73.3*±0.88 | 33.3 *±0.33 | 78.3 *±0.88 | 83.3*±0.88 | 46.7 *±0.33 |
| 5 | EO | 00.0 ±0.00 | 00.0 ±0.00 | 00.0 ±0.00 | 6.7±0.33 | 10.0±0.58 | 3.3±0.33 | 11.7±0.33 | 15.0 ±0.00 | 5.0±0.58 |
| | NE | 28.3*±0.88 | 43.3 *±0.67 | 16.7*±0.33 | 35.0 *±0.58 | 58.3*±0.88 | 26.7 *±0.33 | 63.3 *±0.67 | 73.3 *±0.88 | 41.7*±0.33 |
| 2.5 | EO | 00.0 ±0.00 | 00.0 ±0.00 | 00.0 ±0.00 | 5.0±0.00 | 6.7±0.33 | 1.7±0.33 | 6.7±0.33 | 8.3±0.33 | 3.3±0.33 |
| | NE | 11.7*±0.67 | 41.7 *±0.67 | 6.7 *±0.33 | 31.7**±0.33 | 51.7*±0.33 | 21.7 *±0.33 | 55.0 *±0.58 | 71.7 *±0.67 | 35.0*±0.58 |
| 1.25 | EO | 00.0 ±0.00 | 00.0 ±0.00 | 00.0 ±0.00 | 3.3±0.3 | 5.0±0.00 | 1.7±0.33 | 5.0 ±0.00 | 5.0 ±0.58 | 1.7±0.33 |
| | NE | 5.0 *±0.57 | 20.0 *±0.58 | 3.3 *±0.33 | 25.0 *±0.58 | 45.0*±0.58 | 16.7 *±0.33 | 46.7 *±0.33 | 58.3 *±0.88 | 30.0*±0.58 |
| control | | 00.0±0.00 | 00.0±0.00 | 00.0±0.00 | 00.0±0.00 | 00.0±0.00 | 00.0±0.00 | 00.0±0.00 | 00.0±0.00 | 00.0±0.00 |

*EO: Original oil, NE: nanoemulsion formulation S. o: S. oryzae R. d. : R. dominica T. c. : T. castaneum S.E. :Standard Error*
**:significant difference in the same column between NE and EO concentration at P < 0.05 level*

The effect of eucalyptus oil formulations was high on *R. dominica* adults; the mortality percentages after 48 hrs of exposure were 11.7, 10.0, 6.7 and 5.0. 73.3, 58.3, 51.7 and 45.0%of EO and NE formulation concentrations, respectively. Mean while the corresponding mortality values after 72 hrs were 20.0, 15.0, 8.3 and 5.0; 83.3, 73.3, 71, 7 and 58.3%, respectively. The lowest effect of eucalyptus oil formulation was observed against *T. castaneum* adults, mortality percentages after the longest exposure period (72 hrs) were 8.3, 5.0, 3.3 and 1.7, 46.7, 41.7, 35.0 and 30.0% for the of EO and NE formulation concentrations, respectively.

### 3.2.2 Fumigant toxicity

Data of thefumigant toxicity of eucalyptus oil NE formulation on the three insect species are presented in Table 3. The mortality percentages after 24 hrs of exposingto the highest concentration (10 µl /ml) were 95.0, 70.0 and 71.7% for *S. oryzae, R. dominica* and *T. castaneum*, respectively. The mortality values increased with prolonging exposure time to 72 hrs and recorded 98.3, 85.0 and 85.0%, respectively. While, the corresponding mortality values for the tested insect species at the lowest concentration (1.25 µl /ml) were 51.7, 45.0 and 45.0, 60.0, 56.7 and 55.0% after 24 and 72 hrs of exposure, respectively.

The lethal concentrations of eucalyptusoil NE formulation for different tested insect species are shown in Table 4. The mean values of $LC_{50}$ and $LC_{95}$ after 24 hrs of exposure were 1.2 and11.9 µl /ml for *S. oryzae*. After 48 hrs of exposure the mean values of the $LC_{50}$ and $LC_{95}$ were 1.1 and 8.6, 1.4 and 58.0 and 1.2 and 59.6 µl /ml for *S. oryzae, R. dominica* and *T. castaneum*, respectively. The corresponding values after 72 hrs were 0.8 and 6.9, 0.9 and 37.2 and 0.8 and 33.4 µl /ml for the three tested insect species, respectively.

Results of fumigant toxicity revealed that the mortality increased with increasing concentrations and extending exposure time. Adults of *S. oryzae* were more susceptible to all treatments of oil vapor followed by *T. castaneum* adults while adults of *R. dominica* was the least susceptible one.

### 3.2.3 The repellency activity

The results of repellent effect of eucalyptus oil NE on the three tested insect species were concentration dependent (Fig. 2). Different concentrations of eucalyptusoil NE(10, 5, 2.5 and 1.25 µl /ml) were repellency to *S. oryzae* adults by 95.0, 81.7, 73.3 and 51.7%, respectively. The highest concentration of 10 µl /ml showed 80% repellency against *R. dominica* adults, while it was 46.7% repellency at a lower concentration of 1.25 µl /ml. Also, the repellency for *T. casteneum* beetle was obvious when compared to the highest and the lowest concentration one (85% and 56.7% respectively).

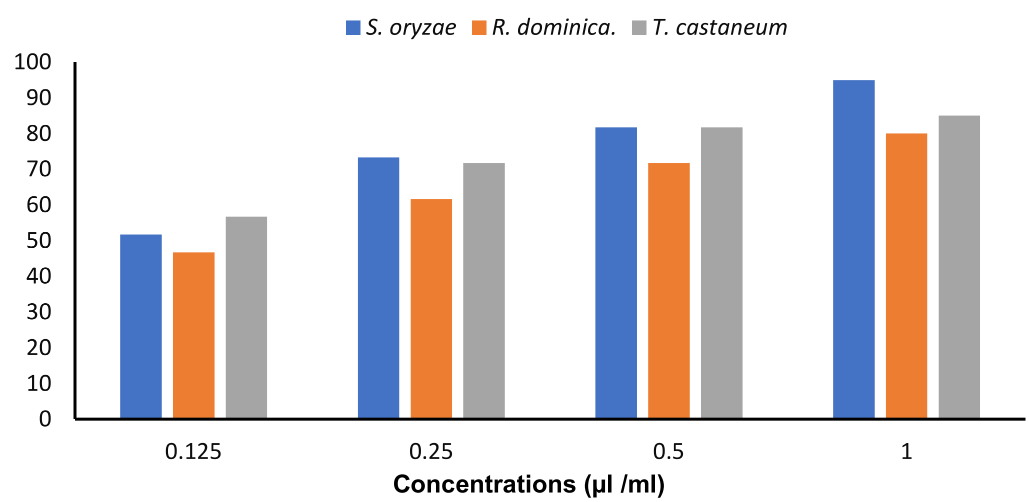


**Fig. 2. Percentage adult repellency of eucalyptus oil nanoemulsion on three stored grain insects**

**Table 3. Fumigant effect of eucalyptus oil nanoemulsion against three stored grain insects**

| Conc .µL/ml | % adult mortality ± S.E at indicated periods (hours) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 24 | | | 48 | | | 72 | | |
| | ***S. o.*** | ***R. d.*** | ***T. c.*** | ***S. o.*** | ***R. d.*** | ***T. c.*** | ***S. o.*** | ***R. d.*** | ***T. c.*** |
| 10 | 95.0±0.58 | 70.0±1.15 | 71.7±0.67 | 96.7±0.33 | 80.0±0.58 | 80.0±0.58 | 98.3±0.33 | 85.0±0.58 | 85.0±0.58 |
| 5 | 81.7±0.88 | 61.7±0.88 | 66.7±0.67 | 86.7±0.33 | 71.7±0.88 | 73.3±0.33 | 93.3±0.33 | 78.3±0.88 | 81.7±0.33 |
| 2.5 | 73.3±0.88 | 58.3±0.88 | 63.3±0.33 | 76.7±0.88 | 61.7±0.67 | 65.0±0.58 | 85.0±0.58 | 68.3±0.33 | 71.7±0.33 |
| 1.25 | 51.7±0.88 | 45.0±0.58 | 45.0±1.15 | 53.3±0.88 | 46.7±0.67 | 48.3±1.20 | 60.00±1.15 | 55.0±0.58 | 56.7±0.67 |
| control | 00.0±0.00 | 00.0±0.00 | 00.0±0.00 | 00.0±0.00 | 00.0±0.00 | 00.0±0.00 | 00.0±0.00 | 00.0±0.00 | 00.0±0.00 |

*S. o. = S. oryzae. R. d. =R. dominica. T. c. = T. castaneum. S.E. =Standard Error*

**Table 4. Lethal concentrations from eucalyptus oil nanoemulsion vapour to three stored grain insects at various exposure periods**

| Insect species | Period (hrs) | Lethal concentrations (and their 95% confidence limits) | | Slope ± S.E | R |
|---|---|---|---|---|---|
| | | $LC_{50}$ | $LC_{95}$ | | |
| *S. oryzae* | 24 | 1.2(0.8- 1.5) | 11.9(8.4-21.1) | 1.62± 0.22 | 0.99 |
| | 48 | 1.1(0. 8-1.4) | 8.6(6.4-14.0) | 1.84± 0.25 | 0.99 |
| | 72 | 0.8(0.5-1.1) | 6.9(5.0-12.4) | 1.80± 0.30 | 0.99 |
| *R. dominica* | 48 | 1.4(0.7-2.0) | 58.0(25.2-359.8) | 1.02 ±0.20 | 0.99 |
| | 72 | 0.9(0.4- 1.4) | 37.2(17.6-200.8) | 1.02 ±0.21 | 0.99 |
| *T. castaneum* | 48 | 1.2(0.6-1.8) | 59.6(24.8-443.0) | 0.97 ±0.20 | 0.99 |
| | 72 | 0.8(0.3-1.2) | 33.4(15.9-186.3) | 0.99 ±0.21 | 0.98 |

R = Correlation coefficient of regression line.  S.E. = Standard error of regression

The NE-formulation more effective than original EO because of small particle size, low surface tension will help in spreading, increase penetration and uptake of active components of EO into the insect body and accelerates the insecticide penetration through the insect cuticle and plays a crucial role in the insecticidal activity [13,35,36]. Also, the insecticidal activity of eucalyptus oil based nanoemulsion formulation may be due to the presence of 1,8-cineol that found as a major component [13,37]. Our results agree with several studies on the nanoemulsion based on EOs as effective insecticides [38-40].

## 4. CONCLUSION

A stable nanoemulsion formulation using eucalyptus oil and biodegradable non-ionic surfactant by spontaneous emulsification method and characterized by droplet size, polydispersity index, pH, refractive index, density, surface tension, and viscosity. The prepared nanoemulsion formulation having a small particle size 8.57 nm with PDI 0.28 and the insecticidal activity of the prepared nanoemulsion was higher than the original oil against the three tested stored grain insects. Thus, it holds a promise as an alternative to chemical insecticides to reduce pesticide load in the environment and food chain. Further studies are needed on the mechanism of action of nanoemulsion formulation on different target insects, as well as toxicological evaluation on non-target species.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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