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Insecticidal Activity of Selected Essential Oils and their Nano-Emulsions Against *Rhyzopertha dominica* (Fab.) (Bostrichidae: Coleoptera)

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Abstract: The present study evaluated the insecticidal efficiency of selected essential oils (clove, peppermint, eucalyptus, and ginger) and their nano-emulsions against the lesser grain borer, Rhyzopertha dominica, under laboratory conditions. Nano-emulsions were successfully prepared using an ultrasonic method, producing stable formulations with droplet sizes ranging from 106.48 to 146.73 nm. Toxicity bioassays revealed a clear concentration- and time-dependent mortality across all treatments, with nano-emulsions consistently outperforming their crude counterparts. Clove oil exhibited the highest toxicity, where nano-emulsification reduced the LC_{50} value from 1.110% to 0.701% after 24 h and from 0.534% to 0.262% after 72 h. After 72 h of exposure, the LC_{50} values for peppermint, eucalyptus, and ginger nano-emulsions reached 0.519%, 0.574%, and 1.446%, respectively, compared to 1.178%, 1.151%, and 2.144% for their bulk forms. Furthermore, long-term evaluation over 90 days showed that nano-emulsions caused a significant reduction in F_1 progeny production, reaching up to 93.48% for clove, 93.27% for eucalyptus, 87.55% for peppermint, and 82.63% for ginger nano-emulsions at a 2% concentration, thereby effectively preventing grain weight loss. Despite these strong insecticidal effects, seed germination percentages remained high (>90%) in most treatments, confirming low phytotoxicity and safety for stored grain protection. Overall, essential oil nano-emulsions represent highly effective and eco-friendly alternatives for R. dominica management

Key words: Essential oils; Nano-emulsions; *Rhyzopertha dominica*; Grain weight loss; Germination; LC_{50}

1. Introduction

Stored grains represent a fundamental component of global food security, serving as a primary source of nutrition for both humans and livestock. However, these commodities are highly susceptible to infestation by a wide range of insect pests during storage, leading to significant quantitative and qualitative losses in both developed and developing countries. These losses are not limited to direct feeding damage but also include contamination, reduced market value, and deterioration of seed viability **(Bharathi, *et al*., 2017 and Stathas *et al*., 2023)**. Among the most destructive pests, the lesser grain borer, *R. dominica*, is of particular concern due to its ability to infest intact grains and develop internally, which makes early detection and control extremely difficult **(Rees, 2004; Salman *et al*., 2019 and Awadalla, *et al*., 2023)**.

Chemical insecticides have long been the primary method for managing stored-product insects. While these compounds are generally effective, their repeated and indiscriminate use has resulted in several serious challenges, including the development of insect resistance, environmental pollution, and the accumulation of harmful residues in food products. These issues pose significant risks to human health and non-target organisms, and they have prompted increasing regulatory restrictions on pesticide use **(Arthur, 1996; Pimentel, 2005 and Muñoz-Bautista *et al*., 2025)**. Consequently, there is a growing need to explore alternative pest control strategies that are both effective and environmentally sustainable.

Plant-derived essential oils have emerged as promising candidates in this regard. These natural products are composed of complex mixtures of volatile compounds, particularly terpenoids and phenolic constituents, which exhibit a wide spectrum of biological activities against insect pests. Their modes of action include contact toxicity, fumigant activity, repellency, and interference with insect physiological processes **(Gupta *et al*., 2023; Bordoloi *et al*. 2024)**. Essential oils extracted from clove (*Syzygium aromaticum*), peppermint (*Mentha piperita*), eucalyptus (*Eucalyptus globulus*), and ginger (*Zingiber officinale*) have been widely reported to possess strong insecticidal properties and are considered potential alternatives to conventional insecticides **(Abdelrazik *et al*. 2018; Mohammadi *et al*., 2019).**

Despite these advantages, the practical application of essential oils remains constrained by several limitations. Their high volatility, susceptibility to oxidation, poor water solubility, and rapid degradation under environmental conditions significantly reduce their persistence and effectiveness in real storage systems **(Bakkali *et al*., 2008)**. These drawbacks limit their large-scale application and necessitate the development of improved delivery systems.

Recent advances in nanotechnology have provided innovative approaches to overcome these challenges. Among these, nano-emulsions have gained considerable attention as efficient carriers for essential oils. Nano-emulsions are thermodynamically stable systems characterized by very small droplet sizes, typically in the nanometer range, which enhance the dispersion, stability, and bioavailability of active compounds **(McClements, 2012; Mesbah *et al*. 2023; Preeti *et al*. 2023)**. The reduced droplet size increases the surface area and facilitates better interaction with the insect cuticle and respiratory system, thereby improving penetration and overall insecticidal efficacy **(Pavoni *et al*., 2019)**. Additionally, nano-formulations can provide controlled release and prolonged activity, further enhancing their effectiveness under storage conditions.

Although numerous studies have investigated the insecticidal activity of essential oils and, more recently, their nano-formulations, limited information is available regarding the comparative performance of crude and nano-emulsified oils against stored-product insects under the same experimental conditions. In particular, there is a lack of comprehensive studies evaluating their combined effects on acute toxicity, progeny suppression, and grain protection.

Therefore, the present study aimed to evaluate the insecticidal potential of selected essential oils and their nano-emulsions against *R. dominica* under laboratory conditions. The study specifically focused on comparing their effects on adult mortality, progeny production, and grain weight loss, in order to assess the potential of nano-formulations as effective and sustainable alternatives for the management of stored-product insect pests.

2. Materials and Methods

A laboratory strain of the lesser grain borer, *R. dominica*, was used throughout the present study. The insect culture was maintained under laboratory conditions at the Department of Economic Entomology, Faculty of Agriculture, Damanhour University, Egypt, for several successive generations without exposure to insecticides to ensure susceptibility and population uniformity. Adults aged 2–3 days were used in all experiments and were reared on clean, disinfested wheat grains at 27 ± 2°C and 65 ± 5% relative humidity.

Four essential oils were evaluated, namely clove oil from *Syzygium aromaticum*, peppermint oil from *Mentha piperita*, eucalyptus oil from *Eucalyptus globulus*, and ginger oil from *Zingiber officinale*. These oils are known to contain bioactive constituents such as eugenol, menthol, eucalyptol, and zingiberene, which have been widely reported to possess insecticidal and fumigant activity against stored-product insects **(Isman, 2020; Regnault-Roger *et al*., 2012; Garrido-Miranda *et al*., 2022).** Each oil was tested in both crude and nano-emulsified forms.

Nano-emulsions were prepared using a high-energy ultrasonic emulsification technique. Briefly, oil-in-water systems were formulated using 5% (v/v) essential oil as the active phase, 10% (v/v) Tween 80 as a non-ionic surfactant, and 85% (v/v) deionized water. Initially, the aqueous phase containing the surfactant was homogenized under continuous stirring, followed by the gradual addition of the oil phase under high-speed agitation to form a coarse emulsion. The pre-emulsion was then subjected to continuous stirring for approximately 30 minutes to ensure uniform dispersion.

Subsequently, the coarse emulsion was processed using an ultrasonic homogenizer (HD 2070, equipped with a 3 mm probe). Sonication was performed at approximately 75% amplitude and a frequency of 20 kHz for 15 minutes under pulsed conditions. The probe was centrally immersed in the emulsion to ensure uniform energy distribution. The process was carried out under cooling conditions to maintain the temperature below 25°C and prevent thermal degradation of volatile oil constituents. The formation of nano-sized droplets was achieved through the mechanism of Acoustic cavitation, which generates intense shear forces leading to droplet size reduction into the nanometric range.

The physicochemical properties of the prepared nano-emulsions were characterized to evaluate their stability and quality. Droplet size and polydispersity index (PDI) were determined using dynamic light scattering (DLS), while zeta potential was measured to assess electrostatic stability. In addition, pH values were measured using a calibrated digital pH meter, and viscosity was determined at room temperature using a viscometer. These parameters were used to assess the homogeneity, dispersion efficiency, and stability of the nano-emulsions.

For bioassay experiments, sterilized wheat grains were used as the substrate. Treatments were expressed as volume per weight (v/w), where crude oil concentrations (0.5, 1, 2, 3, and 4%) corresponded to 0.5, 1, 2, 3, and 4 mL of formulation per 100 g of grains, respectively. Similarly, nano-emulsion treatments (0.25, 0.5, 1, 1.5, and 2%) were applied as 0.25, 0.5, 1, 1.5, and 2 mL per 100 g of grains. In all cases, treatments were scaled proportionally to the experimental unit (20 g grains), ensuring accurate dosing.

Each treatment was applied by adding the required volume of formulation to the grains followed by thorough manual shaking to ensure uniform coating. Ten unsexed adults of *R. dominica* were introduced into each jar, and each treatment was replicated four times. Mortality was recorded after 24, 48, and 72 hours, and insects were considered dead when no movement was observed upon gentle probing.

Mortality data were corrected using Abbott's formula **(Abbott, 1925)**:

$$\text{Corrected mortality (\%)} = T-C/100-C\times 100$$

where T is mortality in treatment and C is mortality in control.

The relative efficiency of nano-emulsification was determined by comparing the LC_{50} values of nano-formulations with those of bulk oils using the following equation: Relative efficiency (%) = 1 − (LC_{50} nano / LC_{50} bulk) × 100.

To evaluate the long-term effects of treatments, adult insects were removed after the exposure period, and the treated grains were kept under the same laboratory conditions for 90 days, corresponding to approximately one full life cycle of the insect **(Hamza and Hamza, 2018)**. At the end of this period, the number of newly emerged adults (F_1 progeny) was counted for each treatment. The percentage reduction in progeny relative to control was calculated using the following equation:

$$\text{Reduction (\%)} = C-T/C\times 100$$

where C is the number of emerged adults in the control and T is the number in the treatment.

Grain weight loss was also determined to assess feeding damage. Grain samples were weighed before and after the experimental period, and percentage weight loss was calculated as:

$$\text{Weight loss (\%)} = \text{Initial weight} - \text{Final weight/Initial weight} \times 100$$

Data obtained from mortality, progeny production, and weight loss were subjected to probit analysis following the method described by Finney (1971). The analysis was performed using LdP-Line software (Ehab Software, Cairo, Egypt) to estimate LC_{50}, values along with their 95% confidence limits. In addition, the toxicity index (TI) was determined following the method described by Sun (1950) to compare the relative toxicity among the tested treatments. The index was calculated using the following equation:

$$TI = A/B\times 100$$

where A is the LC_{50} value of the most toxic treatment, while B represents the LC_{50} value of the treatment under comparison.

The germination test

The germination test was conducted following the method of Qi and Burkholder (1981). A total of 80 wheat grains were randomly taken from each treatment and control, and divided into four replicates (20 grains each). The grains were placed in Petri dishes lined with moistened cotton and incubated under laboratory conditions.

After four days, the number of germinated and non-germinated grains was recorded, and germination percentage was calculated for each replicate using the formula: Germination (%) = (number of germinated grains / total grains) × 100.

3. Results

The physicochemical properties of the prepared nano-emulsions

The physicochemical properties of the prepared nano-emulsions are presented in Table (1). The obtained results confirmed the successful formation of nano-sized droplets for all tested essential oils, with droplet sizes ranging from 106.48 to 146.73 nm. The smallest droplet size was recorded for clove oil nano-emulsion (106.48 ± 3.32 nm), followed by ginger (118.43 ± 2.01 nm), peppermint (132.58 ± 3.72 nm), while eucalyptus oil exhibited the largest droplet size (146.73 ± 2.46 nm).

The pH values of all nano-emulsions were found to be slightly acidic to near neutral, ranging between 5.92 and 6.42. Ginger oil showed the lowest pH value (5.92), whereas eucalyptus oil recorded the highest (6.42), indicating that all formulations were within an acceptable range for stability and application.

Zeta potential values ranged from −16.8 to −26.3 mV, reflecting moderate to good stability of the nano-emulsions. The highest negative charge was observed in peppermint oil (−26.3 mV), followed by clove (−22.8 mV), ginger (−19.6 mV), and eucalyptus (−16.8 mV), suggesting differences in electrostatic stabilization among the formulations.

Regarding viscosity, the nano-emulsions exhibited relatively low to moderate values ranging from 14.6 to 22.7 mPa·s. Clove oil showed the highest viscosity (22.7 mPa·s), while eucalyptus oil had the lowest value (14.6 mPa·s), indicating variation in flow properties among the tested formulations.

The polydispersity index (PDI) values ranged between 0.20 and 0.325, indicating a relatively narrow size distribution and acceptable homogeneity of the nano-emulsions. The lowest PDI was recorded for eucalyptus oil (0.20 ± 0.02), followed by clove (0.22 ± 0.01), ginger (0.28 ± 0.01), whereas peppermint oil showed the highest value (0.325 ± 0.01), suggesting comparatively broader size distribution.

Overall, the obtained results demonstrate that all prepared nano-emulsions possessed suitable physicochemical characteristics, including nanoscale droplet size, acceptable stability, and uniform dispersion, confirming the efficiency of the ultrasonication method in producing stable nano-formulations.

Table (1). Physicochemical characteristics of the prepared nano-emulsions of the tested essential oils

Oil	Droplet size (nm)	pH	Zeta potential (mV)	Viscosity (mpa.s)	PDI ± SE
Clove	106.48±3.32	6.11	-22.8	22.7	0.22±0.01
Peppermint	132.58±3.72	6.31	-26.3	17.8	0.325±0.01
Eucalyptus	146.73±2.46	6.42	-16.8	14.6	0.2±0.02
Ginger	118.43±2.01	5.92	-19.6	18.9	0.28±0.01

The toxicity of crude and nano-formulated essential oils against *R. dominica* after 24 hours of exposure is presented in Table (2). The obtained LC_{50} values clearly indicated considerable variation in the toxic potency among the tested oils in both bulk and nano forms.

Among the crude oils, clove oil exhibited the highest toxicity, recording the lowest LC_{50} value (1.11%), and was therefore considered the standard reference with a toxicity index of 100%. This was followed by eucalyptus oil (LC_{50} = 1.604%; TI = 69.2%), peppermint oil (LC_{50} = 2.06%; TI = 53.88%), while ginger oil showed the lowest toxicity with the highest LC_{50} value (4.618%; TI = 24.04%).

A similar trend was observed for nano-emulsions; however, all oils demonstrated enhanced toxicity compared to their bulk counterparts. Clove nano-emulsion remained the most potent treatment, with an LC_{50} value of 0.701% and a toxicity index of 100%. Peppermint ranked second (LC_{50} = 1.158%; TI = 60.54%), followed by eucalyptus (LC_{50} = 1.311%; TI = 53.47%), whereas ginger nano-emulsion showed the lowest toxicity (LC_{50} = 2.927%; TI = 24.05%).

The relative efficiency (RE) values further confirmed the improvement in toxicity due to nano-formulation. Peppermint oil showed the highest enhancement (RE = 0.44), followed by clove and ginger oils (RE = 0.37 for both), while eucalyptus oil exhibited the lowest increase in efficacy (RE = 0.18).

In general, nano-emulsification resulted in a marked reduction in LC_{50} values and a corresponding increase in toxicity across all tested oils, indicating the effectiveness of nano-formulation in improving insecticidal performance.

Table (2). Toxicity of crude and nano-emulsified essential oils against *R. dominica* (adults) after 24 hours of exposure, expressed as LC_{50}, confidence limits, slope, chi-square, toxicity index (TI), and relative efficiency (RE)

Oil	Bulk formulation						Nano-emulsions						RE
	LC_{50}	C.L.		Slope±SE	X2	TI	LC_{50}	C.L.		Slope±SE	X2	TI	
		Lower	Upper					Lower	Upper				
Clove	**1.11**	0.83	1.38	2.16±0.31	0.91	100	**0.70**	0.512	0.916	1.59±0.29	3.97	100	0.37
Peppermint	**2.06**	1.62	2.73	1.76±0.31	0.52	53.88	**1.16**	0.948	1.465	2.213±0.34	0.94	60.54	0.44
Eucalyptus	**1.60**	1.31	1.94	2.37±0.33	1.72	69.2	**1.31**	0.987	1.993	1.504±0.30	0.85	53.47	0.18
Ginger	**4.62**	3.35	8.72	1.77±0.37	0.36	24.04	**2.93**	2.034	6.632	1.893±0.43	0.21	24.05	0.37

C.L.= Confidence limits; X2= Chi-square; TI= Toxicity Index; RE

The toxicity of the tested essential oils

The toxicity of the tested essential oils increased markedly after 48 hours of exposure, as evidenced by the general decline in LC_{50} values for both bulk and nano-formulations (Table 3). This reduction indicates a clear time-dependent enhancement in toxicity against *R. dominica*.

For bulk formulations, clove oil continued to exhibit the highest toxicity, with the lowest LC_{50} value (0.827%), and thus retained the highest toxicity index (100%). Eucalyptus oil ranked second (LC_{50} = 1.257%; TI = 65.79%), followed by peppermint oil (LC_{50} = 1.577%; TI = 52.44%), while ginger oil remained the least toxic treatment (LC_{50} = 3.059%; TI = 27.03%). The slope values across treatments were relatively moderate, indicating a consistent dose–response relationship.

A pronounced enhancement in toxicity was observed for nano-emulsions compared to their corresponding bulk oils. Clove nano-emulsion again showed the greatest efficacy, with an LC_{50} value of 0.495%, maintaining its position as the most toxic treatment (TI = 100%). Peppermint nano-emulsion exhibited a substantial improvement, with LC_{50} reduced to 0.774% and an increased toxicity index (63.95%), followed by eucalyptus (LC_{50} = 0.83%; TI = 59.64%). In contrast, ginger nano-emulsion remained the least effective despite improvement (LC_{50} = 2.405%; TI = 20.58%).

Notably, the magnitude of improvement due to nano-formulation varied among the tested oils. Peppermint oil showed the greatest relative enhancement in toxicity (RE = 0.51), suggesting a higher responsiveness to nano-emulsification, whereas eucalyptus (RE = 0.34) and clove (RE = 0.40) exhibited moderate improvement. Ginger oil showed the lowest increase (RE = 0.21), indicating comparatively limited enhancement upon nano-conversion.

Overall, the results at 48 hours confirm that both exposure time and nano-formulation play a significant role in increasing toxicity. The consistent reduction in LC_{50} values and the corresponding increase in toxicity indices highlight the superior performance of nano-emulsions over bulk oils, with clove oil maintaining its dominance across all treatments.

Table (3). Toxicity of crude and nano-emulsified essential oils against *R. dominica* (adults) after 48 hours of exposure, expressed as LC_{50}, confidence limits, slope, chi-square, and toxicity index (TI)

Oil	Bulk formulation						Nano-emulsions						
	LC_{50}	C.L.		Slope±SE	X2	TI	LC_{50}	C.L.		Slope±SE	X2	TI	
		Lower	Upper					Lower	Upper				
Clove	**0.83**	0.59	1.05	2.09±0.32	3.26	100	**0.50**	0.33	0.65	1.64±0.295	1.43	100	0.4
Peppermint	**1.58**	1.22	2.01	1.82±0.3	1.28	52.44	**0.77**	0.62	0.95	2.21±0.317	1.58	63.95	0.51
Eucalyptus	**1.26**	1.09	1.64	2.35±0.32	4.79	65.79	**0.83**	0.62	1.11	1.54±0.293	1.16	59.64	0.34
Ginger	**3.06**	2.28	4.98	1.51±0.31	1.47	27.03	**2.41**	1.69	5.02	1.62±0.354	1.20	20.58	0.21

C.L.= Confidence limits; X2= Chi-square; TI= Toxicity Index; RE

After 72 hours of exposure, a pronounced increase in toxicity was observed across all treatments, as reflected by the substantial decline in LC_{50} values compared to earlier exposure periods (Table 4). This trend confirms the cumulative toxic effect of the tested oils over time against *R. dominica*.

Clove oil consistently maintained its position as the most toxic treatment in both bulk and nano forms throughout the experimental period. At 72 hours, the LC_{50} value of crude clove oil decreased markedly to 0.534%, while its nano-emulsion exhibited a significantly lower LC_{50} value of 0.262%, indicating a strong enhancement in toxicity. Accordingly, clove oil remained the reference standard with the highest toxicity index (100%) in both formulations.

Peppermint and eucalyptus oils showed comparable toxicity levels at this exposure interval. In bulk form, their LC_{50} values were 1.178% and 1.151%, respectively, with relatively close toxicity indices (45.33% and 46.39%). Upon nano-formulation, both oils exhibited clear improvements, with LC_{50} values decreasing to 0.519% for peppermint and 0.574% for eucalyptus. However, peppermint nano-emulsion slightly surpassed eucalyptus in toxicity, as reflected by its higher toxicity index (50.48%).

Ginger oil remained the least toxic treatment, despite a noticeable reduction in LC_{50} values over time. The LC_{50} of crude ginger oil reached 2.144%, while its nano-emulsion showed improved toxicity (LC_{50} = 1.446%), although still significantly lower than the other tested oils.

The relative efficiency (RE) values at 72 hours revealed a more pronounced enhancement due to nano-emulsification compared to earlier time intervals. Peppermint oil showed the highest improvement (RE = 0.56), followed closely by clove (RE = 0.51) and eucalyptus (RE = 0.50), whereas ginger oil exhibited the lowest relative enhancement (RE = 0.33). These findings indicate that the effectiveness of nano-formulation becomes more evident with prolonged exposure.

Overall, the results demonstrate a clear time-dependent increase in toxicity and confirm that nano-emulsions significantly enhance the insecticidal activity of essential oils. The consistent superiority of clove oil, along with the marked improvement in peppermint and eucalyptus oils upon nano-conversion, highlights the potential of nano-formulations as effective alternatives for the control of stored-product insects.

Table (4). Toxicity of crude and nano-emulsified essential oils against *R. dominica* (adults) after 72 hours of exposure, expressed as LC_{50}, confidence limits, slope, chi-square, toxicity index (TI), and relative efficiency (RE)

Oil	Bulk formulation						Nano-emulsions						RE
	LC_{50}	C.L.		Slope±SE	X2	TI	LC_{50}	C.L.		Slope±SE	X2	TI	
		Lower	Upper					Lower	Upper				
Clove	**0.53**	0.28	0.74	1.80±0.39	1.59	100	**0.26**	0.15	0.36	1.87±0.32	0.76	100	0.51
Peppermint	**1.18**	0.86	1.50	1.78±0.298	1.39	45.33	**0.52**	0.40	0.63	2.3±0.323	3.72	50.48	0.56
Eucalyptus	**1.15**	0.91	1.39	2.41±0.324	7.26	46.39	**0.57**	0.39	0.76	1.51±0.29	1.82	45.64	0.50
Ginger	**2.14**	1.67	2.89	1.72±0.306	1.54	24.91	**1.45**	1.04	2.53	1.32±0.299	1.87	18.12	0.33

C.L.= Confidence limits; X2= Chi-square; TI= Toxicity Index; RE

Effect of crude and nano-emulsified essential oils on progeny production of *R. dominica*

The effect of crude and nano-emulsified essential oils on progeny production of *R. dominica* is presented in Table (5). The results clearly demonstrated a concentration-dependent reduction in the number of emerged adults across all tested oils, with a more pronounced effect observed in nano-emulsions compared to their corresponding bulk formulations.

Among the tested oils, clove oil exhibited the highest inhibitory effect on progeny production. In its crude form, the mean number of progeny decreased from 37 individuals at 0.5% to 8.5 individuals at 4%, corresponding to a reduction ranging from 29.29% to 83.88%. A substantially greater effect was observed for the nano-emulsion, where progeny numbers declined from 36 at 0.25% to only 3.5 individuals at 2%, achieving a maximum reduction of 93.48%. This indicates a strong enhancement in reproductive suppression following nano-formulation.

Peppermint oil showed a moderate effect compared to clove oil. The crude formulation reduced progeny numbers from 47.25 at 0.5% to 12.25 at 4%, with reduction percentages increasing from 10% to 76.64%. The nano-emulsion demonstrated improved efficacy, reducing progeny from 40.5 at 0.25% to 6.5 individuals at 2%, with a maximum reduction of 87.55%.

A similar trend was observed for eucalyptus oil, where the crude form reduced progeny from 46 to 10 individuals across the tested concentrations, corresponding to a reduction of 12.49% to 80.87%. In contrast, the nano-emulsion achieved a stronger effect, decreasing progeny from 39.25 to 3.5 individuals, with reduction percentages reaching up to 93.27%, indicating a high level of reproductive suppression.

Ginger oil exhibited the lowest effect among the tested oils. In its crude form, progeny reduction ranged from only 2.89% at the lowest concentration to 69.37% at the highest concentration. However, nano-formulation improved its performance, with reduction percentages increasing from 15.19% to 82.63%, although it remained less effective compared to the other oils.

Overall, the results confirm that all tested essential oils significantly reduced progeny production in a concentration-dependent manner. Moreover, nano-emulsification markedly enhanced the efficacy of all oils, resulting in greater suppression of progeny compared to crude formulations, with clove oil consistently showing the highest activity, followed by eucalyptus and peppermint, while ginger oil exhibited the lowest effect.

Table (5). Effect of crude and nano-emulsified essential oils on progeny production of *R. dominica* and percentage reduction relative to control

Oil	Bulk			Nanoemulsion		
	Conc.	Mean number of progeny	Reduction in progeny [%]	Conc.	Mean number of progeny	Reduction in progeny [%]
Clove	0.5	37±2.58d	29.29±9.04jk	0.25	36±3.37d	31.14±10.18j
	1	28.75±2.22f	45.4±2.41h	0.5	27.25±3.2fg	48.25±5.16gh
	2	17.25±1.71i	67.23±1.28d	1	13±0.82ij	75.3±0.76de
	3	14±1.83ij	73.38±3.44bcd	1.5	8.25±1.26ki	84.42±0.93bc
	4	8.5±1.29k	83.88±2.08a	2	3.5±1.29m	93.48±1.92a
Peppermint	0.5	47.25±2.5bc	10±7.26im	0.25	40.5±1.91c	22.93±4.66k
	1	33±2.16e	37.3±2.54i	0.5	30±3.37ef	43.18±2.25hi
	2	27.75±3.59fg	46.84±9.54gh	1	24±2.94g	54.16±7.05g
	3	21.75±3.77h	58.98±3.88ef	1.5	15.75±4.78hi	70.52±6.84ef
	4	12.25±1.23jk	76.64±3.06abc	2	6.5±1.29im	87.55±2.82ab
Eucalyptus	0.5	46±2.45c	12.49±5.28i	0.25	39.25±2.63cd	25.42±3.08jk
	1	35.75±3.2de	32.09±4.73ijk	0.5	30.25±2.63ef	42.53±3.87hi
	2	24±1.83gh	54.12±6.43fg	1	17.5±2.38h	66.47±6.54f
	3	17.5±1.91i	66.83±1.6de	1.5	10.75±1.71jk	79.71±1.38cd
	4	10±0.82k	80.87±2.73ab	2	3.5±1.29m	93.27±2.6a
Ginger	0.5	51±1.83ab	2.89±6.33m	0.25	44.5±1.29b	15.19±6.85l
	1	38.5±3.79d	26.1±13.23k	0.5	31.75±4.03e	38.98±12.32i
	2	34.75±2.75de	34±3.23ij	1	28.5±3ef	45.92±3.64hi
	3	24.25±2.75gh	54.04±2.86fg	1.5	19±2.94h	64.12±2.85f
	4	16.25±2.87i	69.37±2.56cd	2	9.25±2.62jki	82.63±3.53bcd
Control		52.75±4.92a	-		52.75±4.92a	-
F values		105.6938	76.874279		104.59382	85.1612
LSD		3.75652	7.886229		3.9558	7.58649

Effect of crude and nano-emulsified essential oils on grain weight loss

The percentage weight loss of wheat grains after 90 days of storage is presented in Table (6). The results clearly demonstrated that all tested essential oils significantly reduced grain loss compared to the untreated control, which recorded the highest loss (30.13%). The reduction in weight loss was concentration-dependent and more pronounced in nano-emulsions than in crude formulations across all tested oils.

Clove oil exhibited the highest protective effect among all treatments. In its crude form, weight loss decreased from 14.63% at 0.5% concentration to 3.75% at 4%. However, the nano-emulsion showed superior performance, reducing weight loss from 11.75% at 0.25% to only 1.88% at 2%. This substantial reduction indicates the strong ability of nano-clove formulation to protect wheat grains from insect damage.

Peppermint oil showed a moderate reduction in grain loss compared to clove oil. The crude formulation reduced weight loss from 21.88% to 14.13% across the tested concentrations, whereas the nano-emulsion further decreased loss from 19.63% to 11.38%. Although the nano-form improved efficacy, peppermint remained less effective than clove oil.

Eucalyptus oil demonstrated a clear improvement in reducing grain loss with increasing concentration. The crude form reduced weight loss from 19% to 8.87%, while the nano-emulsion achieved a greater reduction from 16.75% to 7%. The difference between crude and nano formulations was evident, with nano-emulsion consistently providing better protection.

Ginger oil showed the lowest efficacy among the tested oils. In the crude form, weight loss decreased from 28.75% to 14.13%, while nano-formulation improved the outcome, reducing loss from 26.63% to 11.5%. Despite this improvement, ginger oil remained less effective compared to the other oils in minimizing grain damage.

Overall, nano-emulsions exhibited superior performance over crude oils in reducing grain weight loss for all tested essential oils. The enhanced efficacy of nano-formulations was particularly evident in clove oil, followed by eucalyptus and peppermint, while ginger oil showed the least protective effect. These results highlight the significant role of nano-emulsification in improving grain protection against insect infestation.

Table (6). Effect of crude and nano-emulsified essential oils on percentage weight loss of wheat grainsinfested with *R. dominica* after 90 days of storage

Oil	Bulk			Nanoemulsion		
	Conc.	Wheat loss	Germination %	Conc.	Wheat loss	Germination %
Clove	0.5	14.63±1.44	100±0a	0.25	11.75±1.04	100±0a
	1	9.25±0.87	100±0a	0.5	7.38±1.11	100±0a
	2	4.75±0.96	100±0a	1	3.13±0.85	98.75±2.5ab
	3	4.5±0.71	97.5±2.89abc	1.5	2.88±0.85	97.5±2.89abc
	4	3.75±0.65	95±4.08c	2	1.88±0.48	93.75±2.5d
Peppermint	0.5	21.88±1.11	100±0a	0.25	19.63±0.48	100±0a
	1	19±0.91	98.75±2.5ab	0.5	16.75±0.65	100±0a
	2	16.5±0.91	100±0a	1	14.25±0.65	98.75±2.5ab
	3	14.63±0.85	98.75±2.5ab	1.5	12.75±0.87	96.25±4.79bcd
	4	14.13±0.85	96.25±4.79bc	2	11.38±0.75	95±4.08cd
Eucalyptus	0.5	19±1.47	100±0a	0.25	16.75±1.04	100±0a
	1	14.63±1.11	100±0a	0.5	12.25±1.32	100±0a
	2	11.75±1.19	100±0a	1	9.75±1.32	98.75±2.5ab
	3	9.25±1.32	98.75±2.5ab	1.5	7.375±1.18	97.5±2.89abc
	4	8.87±1.25	96.25±2.5bc	2	7±1.08	95±0cd
Ginger	0.5	28.75±1.04	100±0a	0.25	26.63±0.85	100±0a
	1	21.63±0.85	100±0a	0.5	19.5±0.58	100±0a
	2	19.25±0.65	100±0a	1	16.5±0.71	100±0a
	3	16.63±1.25	100±0a	1.5	14.25±1.04	98.75±2.5ab
	4	14.13±0.85	98.75±2.5ab	2	11.5±0.41	97.5±2.89abc
Control		30.13±0.75	100±0a	Control	30.13±0.75	100±0a
			LSD=2.74356			LSD=3.01858
			F=2.471			F=3.41086

The effect of crude and nano-emulsified essential oils on seed germination of wheat is presented in Table (6). The results indicated that germination percentage was generally high across all treatments, with most values remaining close to the control (100%), suggesting that the tested oils had relatively low phytotoxic effects under the experimental conditions. In the case of crude oils, clove oil showed a slight reduction in germination at higher concentrations, decreasing to 95% at 4%, whereas peppermint and eucalyptus showed moderate reductions (96.25%) at the same concentration. Ginger oil exhibited the least effect, maintaining germination rates of 100% up to 3% and slightly decreasing to 98.75% at 4%.

For nano-emulsions, a similar trend was observed; however, a slightly greater reduction in germination was detected at higher concentrations compared to crude formulations. Clove nano-emulsion showed the highest inhibitory effect, reducing germination to 93.75% at 2%, followed by peppermint and eucalyptus (95%), while ginger nano-emulsion remained the least phytotoxic, maintaining relatively high germination (97.5%). Despite these reductions, the differences among treatments were relatively small, and germination remained above 90% in all cases. Overall, the results suggest that nano-formulations slightly enhanced the negative effect on germination compared to crude oils, but without causing severe impairment of seed viability.

4. Discussion

The obtained physicochemical properties of the prepared nano-emulsions clearly confirmed the successful formation of stable formulations with droplet sizes within the nanometric range. Droplet size, PDI, and zeta potential are considered critical parameters influencing the stability and biological performance of nano-emulsions. The relatively small droplet sizes (106–146 nm) and low PDI values observed in the present study indicate uniform dispersion and good homogeneity, which are essential for enhancing the bioactivity of essential oils. Similar findings were reported by **McClements (2012)**, who emphasized that reducing droplet size increases surface area and improves the interaction between active compounds and target organisms. Moreover, the moderately negative zeta potential values obtained (−16.8 to −26.3 mV) suggest acceptable physical stability of the nano-emulsions, which contributes to preventing droplet aggregation and maintaining dispersion over time.

The enhanced toxicity observed for nano-emulsions compared to crude oils across all exposure periods (24, 48, and 72 h) can be attributed to the improved physicochemical characteristics of the nano-formulations. The reduction in LC_{50} values with increasing exposure time reflects a cumulative toxic effect, which has been widely reported for essential oils due to their multiple modes of action, including disruption of the insect nervous system and interference with respiration **(Isman, 2006)**. The superior performance of nano-emulsions is mainly related to their smaller droplet size, which facilitates better penetration through the insect cuticle and increases the bioavailability of active compounds. This is consistent with findings by **Pavoni (2019)**, who reported that nano-formulations significantly enhance the insecticidal efficiency of plant-derived compounds.

Among the tested oils, clove oil consistently exhibited the highest toxicity, which can be explained by its high content of eugenol, a phenolic compound known for its strong insecticidal activity. Eugenol has been reported to act as a neurotoxin affecting octopamine receptors and disrupting enzyme systems in insects **(Regnault-Roger *et al*., 2012).** In contrast, ginger oil showed the lowest toxicity, which may be attributed to differences in its chemical composition and lower concentration of highly active constituents. The observed variation among oils highlights the importance of chemical composition in determining insecticidal activity, as previously demonstrated by **Bakkali (2008)**.

The results of progeny reduction further support the toxic effects observed in adult mortality. The significant decrease in progeny production with increasing concentration indicates that the tested oils not only affect adult survival but also interfere with reproduction and development. This may be due to ovicidal effects or disruption of larval development inside the grain. The stronger suppression observed in nano-emulsions suggests improved penetration into grains and greater accessibility to immature stages of *R. dominica*. Similar results were reported by **Isman (2006)**, who indicated that essential oils can inhibit reproduction and reduce population growth in stored-product insects.

The reduction in grain weight loss observed in the present study is a direct consequence of decreased insect activity and progeny development. Treatments that achieved higher mortality and greater progeny suppression, particularly clove and eucalyptus oils, resulted in significantly lower grain damage. The superior performance of nano-emulsions in reducing weight loss confirms their higher efficiency in protecting stored grains. These findings are in agreement with previous studies indicating that plant oils and their formulations can effectively reduce feeding damage and preserve grain quality during storage **(Arthur, 1996)**.

Despite the strong insecticidal activity observed, the impact on seed germination remained relatively limited. Germination percentages remained above 90% in most treatments, indicating low phytotoxicity of the tested oils under the experimental conditions. However, a slight reduction in germination at higher concentrations, particularly in nano-formulations, suggests that increased bioavailability of active compounds may also affect seed physiological processes. This effect could be related to the interference of essential oil components with enzymatic activities involved in germination or with membrane integrity. Similar observations were reported by **Bakkali (2008)**, who noted that essential oils may exhibit phytotoxic effects at higher concentrations due to their bioactive constituents.

Overall, the present findings demonstrate a clear relationship between nano-formulation, increased insecticidal activity, and improved grain protection, with only minor effects on seed viability. The enhanced performance of nano-emulsions highlights their potential as effective and environmentally safe alternatives to conventional insecticides in stored grain protection systems. At the same time, the slight reduction in germination at higher concentrations suggests the need to optimize application rates to achieve a balance between maximum insect control and minimal impact on seed quality.

5. Conclusion

The results demonstrated that nano-emulsification significantly enhanced the insecticidal activity of essential oils against *R. dominica*. Clove oil showed the highest efficacy in terms of toxicity, progeny reduction, and grain protection. Nano-formulations provided superior performance compared to crude oils while maintaining high germination rates. These findings highlight the potential of nano-emulsified essential oils as safe and effective alternatives for stored grain pest management.

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