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Bioactivity of essential oils in nanoemulsions formulation against *Varroa Mite* and their selectivity for *Apis mellifera* under laboratory and colony conditions

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Abstract

The development of innovative, effective, and safe varocides based on essential oil nanoemulsions (NEO) will assist to mitigate the negative effects of synthetic acaricides. To use ultrasonic emulsification to synthesize a nanoemulsion of essential oils and test their biological activity against the varroa mite and selectivity for *Apis mellifera*. When bioassayed with surface contact toxicity and exposure time of 4 hours, the LC₅₀ values of all investigated nanoemulsions were (1.06, 3.11, 1.66, and 1.47 $\mu\text{g}/\text{cm}^2$) of NEO of Garlic, Camphor, Chamomile, and Jojoba, respectively, with no significant variations between them. The LC₅₀ of NEO oils were (0.610.23, 1.020.22, 0.710.24, and 0.640.24 $\mu\text{g}/\text{cm}^2$) as exposure time increased to 24 hours of exposure, The data showed that all Nano-emulsion oils (NEO) are good selective after 24 hours of exposure. The maximum selective ratio was on Chamomile treatment followed by Jojoba (452.27 and 427.65%). The average reduction infestation percentage of all NEO ranged between 72.56 and 78.96% against *V. destructor* without any significant differences between them. Their degree of selectivity was good selective against bee workers. All tested nanoemulsion oils have no detrimental effects on honey bees and colonies. NEO can be part of an IPM program to control varroa mites and is considered an ideal way to control this mite. These materials are therefore environmentally safe, selective for bees, easy to use, available, and economical.

Keywords: *Varroa destructor*, nanoemulsion oils, *Apis mellifera*, selectivity

INTRODUCTION

Varroa infestation can cause up to 25% loss of body weight, severe wing deformation, and shortened worker and drone lifespans. This spleen-infected colony suffers a drastic reduction in workers bee number and, if left untreated, will eventually lose (Hussein et al., 2016).

Due to the heavy use of synthetic acaricides, residues have accumulated in bee products (Hussein et al., 2002; Hussein and Mostafa, 2009; Bargańska et al., 2016; Calatayud-Vernich et al., 2016; Chiesa et al., 2016; Hussein et al., 2016; Porrini et al., 2016; Sattler et al., 2016; Calatayud-Vernich et al., 2017; Nai et al., 2017; Eshbah et al., 2018; Kast, 2021; Végh et al., 2023). The buildup of miticide resistance in *V. destructor* populations and the contamination spectrum of beehive products make to create of new treatment strategies that minimize the potential for rapid resistance development and residue build-up very much needed. The natural product is selective and has little or no adverse effect on non-target species and can be used as a component of *V. destructor* control. (Calo et al., 2015; Benelli et al., 2018; Eshbah et al., 2018).

Natural substances are effective against many pests, including mites, insects, nematodes, weeds, and fungi (Mossa et al., 2018). They have several and different modes of action and a variety of sites of action (Mossa, 2016).

Utilizing nanotechnology can increase effectiveness or decrease environmental contamination. Nanotechnology has a broad range of possible applications and advantages. Use of modern insecticide formulations based on nanomaterials to control pests. (Prasad et al., 2014; Ragaie and Sabry, 2014; El Azim and Balah, 2016; Gupta et al, 2018). Nano formulations have a higher surface area ratio that means more of the total volume of pesticide is in contact with the pests, allows for a reduction in pesticide dose required.

The aim of this study is to provide information on the effects of four nanoemulsion formulations of essential oils on varroa mites and their selectivity for *A. mellifera*, and to determine which nanoemulsion NEO maximizes Varroa control and improves bee colony and hive growth, and minimize the impact on clean up environment at minimal cost.

MATERIALS AND METHODS

2. 1. Preparation of Nanoemulsion

Garlic, camphor, chamomile and jojoba essential oils were obtained from (El Hawag Natural Oil Extraction and Packaging Company, Egypt), Tween 20 (polyoxyethylene (20) sorbitan monolaurate) and deionized water were bought from El Gomheria Chemical Company. Nanoemulsion (NEO) was made by mixing one volume oil with one volume hexane (co-surfactant) and one volume surfactants (Tween 20 and Tween 80) to a final volume of 100 mL by adding deionized water and vortexing several times. The resulting formulation was sonicated in an ultrasonic bath for 2 hours and stored under laboratory conditions for testing. (Ghotbi et al., 2014; El Azim and Balah, 2016)

2.2. Chemical and Physical tests of prepared NEO formulation:

a-Thermal stability

Stability was tested according to (El Azim and Balah, 2016; Sinha et al., 2016; Dakhli et al, 2023) thermal and mechanical stresses. "Preparations (25 ml) were stored at elevated temperatures (40 ± 2 C°, 25 ± 2 C° and 4 ± 2 C°) and centrifuged at 2000 rpm. speed at various intervals (20, 40, 60, and 120 min.). then visually inspected (phase separation). And observe any physical changes such as loss of clarity, carbon essence, cloudiness, etc. Samples were also monitored for the loss of the aqueous phase, which is an essential part of emulsion stability.

B- Centrifugation stress test:

Following the same procedure as the previous thermal tests, the sample was centrifuged at 2000 rpm. at various intervals (2, 4, 6, 8, 10 and 20 minutes.) and then visually inspected (phase separation).

C. pH values

The pH of the preparation not only affects the stability of the emulsions, but also changes the solubility and bioavailability of the ingredients at the point of penetration by nanoemulsion. The pH values of samples were measured with a pH meter at a temperature of 25 ± 2 °C.

D. Electrical conductivity

Electrical conductivity was determined by an EC Meter (Orion 150 A from Thermo Electron Corporation, USA) at room temperature. The transparency of the formulations was determined by measuring the transmission percentage in scanning mode with purified water taken as a blank using UV-VIS spectrophotometers (Thermo, Nicolet evolution 300) according to (Date and Nagarsenker, 2008)

E. Droplets size

The droplet size of the preparations was measured with a droplet size analyzer “(PSS NICOMP, N3000, Dynamic light scattering, Particle Size Systems, Inc. Santa Barbara, California, USA)”, and the measurement was performed without diluting the composition. 0.1 mL of nanoemulsion is dispersed in 50 mL (500-fold dilution) of double-distilled water in a volumetric flask and gently mixed by inverting the flask.

2.3 .Toxicological studies on nano emulsion oils

Thin film method (contact toxicity) used on adult workers of *A. mellifera* and varroa mite to test the selective effects of essential oil nano formulations tested as contact poisons. Four concentrations were prepared for each NEO; three replicates and the relative acute toxicity of each product was determined by recording mortality four and 24 h after each treatment. This time it was chosen because it was believed that a rapid determination of the effects of the products (Milani, 2001). Mites were transferred to a Petri dish and examined under a stereo microscope. Adult of Varroa mites that not moved when examined with a fine brush were considered dead. For each concentration, ten adult bee workers and Five adult Varroa mites were used in each replicate. For the thin film test, one ml of each concentration was taken and placed in a Petri dish. The Petri dishes were rotated horizontally at room temperature for 2-3 h until the solvents had completely evaporated and the compound residues homogeneously coated the inner surface of the dishes before they were presented to the insects. The same technique was used for adult's bee workers. Average mortality was estimated post 4 hours and 24 hours from treatment. Corrected mortality was estimated using the Abbott equation (Abbott, 1925). Probit analysis was done according to (Finney, 1971). The variance of LC₅₀

between compounds was determined by comparing the 95% confidence limits, quiet square (x^2), used to determine the statistical significance of response heterogeneity. The oil selective ratio (SR) of the nanoemulsion was calculated as

Selective ratio % =

$$\left\{ \frac{LC50 \text{ of nano emulsion oils against } Apis mellifera}{LC50 \text{ of NEO against varroa}} \right\} * 100$$

2.4. Experimental honeybee colonies

15 Hives were selected from colonies of a private apiary located in Shosha 7 village, Minya Governorate, which were heavily infested with mites and not treated with acaricides for at least one year and were used as a source of mites. The experimental colonies were divided to 5 groups, each with 3 colonies as replicates. The average colony strength studied was 8 combs covered by bees housed in a Langstroth hive. The experiment was repeated twice in 2022. season and treatment averages were calculated.

2.5.1. Evaluation the tested materials

Infestation percentage in live bees was determined through randomly collection of about 50 bees /colony in a jar partially filled with water and few drops of detergent (Liquid soap). The bees were shaken for thirty seconds, then filtered through muslin (10 mesh/ inch) to remove the bees, and the passed mite counted (Komeili, 1988). This procedure was applied before application and fourteen days post application, and the treatment was repeated 30 days later.

Infestation in brood was determined as follows. 100 capped brood cell per colony were selected randomly. The cell was removed with forceps and the pupae were removed to examine for mites (Marcangel et al., 1992). The number of dead mites was counted by covering the bottom of each brood chamber with sticky white paper coated with a thin layer of petroleum jelly. Evaluation of the efficiency of nanoemulsion oils on Varroa mite by calculating the mite reduction Percentage (Henderson and Tilton, 1955) equation.

$$\text{Reduction \%} = \left(1 - \left(\frac{Ta * Cb}{Tb * Ca} \right) \right) * 100$$

Where: T_a is % infestation post-treatment; T_b is % infestation pre-treatment; C_b is % infestation pre-treatment for the control. C_a is % infestation of mite post-treatment for the control.

Reduction %	degree of selectivity	Reduction % in Bees	degree of selectivity
Less than 25%	good selective	25-49%	selective
49-79 %	medium selective	79-89 %	slab selective

2.5.3. Evaluation of side effect of NEO in individuals of treated bee colonies

2.5.3.1. The daily spawning rate of queens

Combs of treated colonies was checked every four days for two consecutive days. The daily laying rate was calculated by subtracting the first day's total number of eggs from the next day's total number of eggs. Count the oocytes according to the described method (Kefuss, 1978).

2.5.3.2. Pupation percentage.

The pupation% was calculated by marking the area of capped cells (100 cells) in each experimental hive with a transparent sheet. (Hassan and Aly, 1998). The marked areas were re-examined 5 days later to determine the number of cells that reached the pupae stage and larval viability was estimated with the following formula: Percent pupation was measured twice for each treatment period.

$$\text{Pupation \%} = \frac{\text{No. of capped cells (pupaed)}}{\text{The original No. of cells having larvae}} \times 100$$

2.5.3.3. Worker longevity

On the second day after treatment, half of the comb cage was used to cage a compact area (4 x 6 inches per colony) of chick combs sealed from the treated colonies. After 12 days, the cage area and nest were inspected and the bees were removed from the colony along with the cargo from half the hive. The plucked hives removed bees that were not covered with brush. 100 hatched bees were carefully penned one by one through a hole in the cage and marked on the chest with a pot containing a specific color of fast-drying paint. All marked bees and their hives re-introduced into their respective colonies using the colonization method. (Grout, 1960). Tested colonies were inspected at

2.5.2. Determination of selectivity degree

To determine the selectivity of different treatments against bee colony individuals, the mortality % in bee population were calculated and Metcalf Scheme (Metcalf, 1972) was adopted as follows.

intervals of four days until there were no marked workers. Life longevity was calculated as the average between the rest day on which the bees were observed and the next observation day. (Terada et al., 1975).

2.5.3.4. Estimation of Honey Production

The honey yield of the experimental hives was estimated by measuring the open and closed honey compartments in June and September and converted to weight according to using the following formula: (Shawer et al., 1986).

$$\text{Honey yield in kg} = \frac{\text{Area of honey (square inches)} \times 10.64}{1000}$$

10.64 = grams of honey /square inch based on the averages calculated from uncapped and capped honey from combs of various thicknesses.

2.6. Statistical analysis

Collected data were subjected to analysis of variance according to (Mead et al., 1993). Statistical analysis was performed using Costat software with (F-test). Differences between means were calculated by Duncan's test.

RESULTS AND DISCUSSION

3.1. Chemical tests and Physical of Nanoemulsions tested formulations.

Size of droplets of Nano particles (Nm), pH values, Electrical conductivity, formulation and transparency, were determined and are shown in Table 1.

3.1.1. Particle size of formulated emulsions:

Particle size is an important factor affecting the characterization of camphor, chamomile, garlic and jojoba oil formulations. Sizes of nanoe

particle were 10.4 ± 0.59 , 66.5 ± 0.4 , 64.1 ± 0.39 , and 90.7 ± 0.75 nm for camphor, jojoba, chamomile, and garlic. Each nanoemulsion formulation loaded with NEO exposed to sonication for 2 hours showed minimal dispersion. In the final step, the diameter of the hydrodynamic droplets was reduced to stabilize and improve the dispersion quality.

3.1.2. pH values

NEO had higher pH ranged from 6.71 to 7.4

3.1.3. Electrical conductivity

EC values ranged from 0.101 to 0.132 m mol/cm (nano EO). These data explain the high steady-state formation of the continuous aqueous phase (Table 1). Nano emulsion (NEO) featured excellent transparency and high (UV/VIS) transmittance compared to Mac-E, which was milky white and showed lower transmittance of 91.32-35.0% for Nano-E. as shown in Table 1.

3.1.4. Mechanical Stability of Formulation:

Prepared emulsion (Nano-E) was subjected to stability evaluation by centrifugation at 2000 rpm for 20, 40, 60 and 120 minutes. (Table 2) while thermal stability at 4 ± 1 , 25 ± 1 , and 40 ± 1 °C was achieved after 1, 10, 20 and 30 days. Results showed the stability of Nano-E to pellets after centrifugation for 20, 40, and 60 minutes. However, more trace precipitation was recorded at 60 and 120 minutes. The thermal stability of Nano-E was stable at 4 ± 1 , 25 ± 1 , and 40 ± 1 °C for 30 days (Table 2). At laboratory temperature, it was stable for more than 5 months with no aggregation or separation. The selection of the appropriate mixture (type and amount) of oil, water, and surfactants used in nanoemulsions (nanoe) was a key point in controlling the suitability of the prepared formulations to perform their functions. The sizes of the formulated nanoemulsions ranged from 1 to 100 nm, close to standard nanoe. These results supported by (Bouchemal et al., 2004; Anton and Vandamme, 2011; Ghotbi et al., 2014).

Table 1: The acaricidal efficacy of different concentrations of abamectin (vertimec 1.8 EC) as reference acaricide and *Thuja orientalis* plant extract after one, two, and three days of treatment.

Days	Vertimec 1.8% EC				<i>T. orientalis</i> extract			
	LC ₅₀	Slope	X ²	Toxicity index ^a	LC ₅₀	Slope	X ²	Toxicity index
1	1.029	0.976 ± 0.251	16.76	1	8.354	893.0 ± 0.335	3.017	0.537
2	2.747	0.453 ± 0.243	1.123	0.374	6.310	0.428 ± 0.344	0.647	0.711
3	1.657	0.596 ± 0.244	0.760	0.621	4.488	0.429 ± 0.234	3.517	1

Notes: Toxicity ratio^a is calculated as the least LC₅₀ value for baseline toxicity / the compounds' LC₅₀ value.

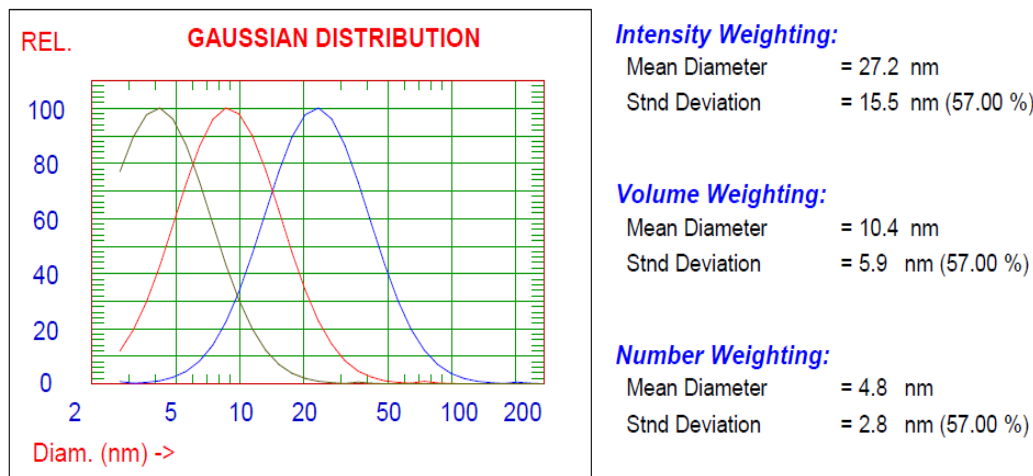


Fig 1: Emulsions distribution analysis (particle size of Camphor oil)

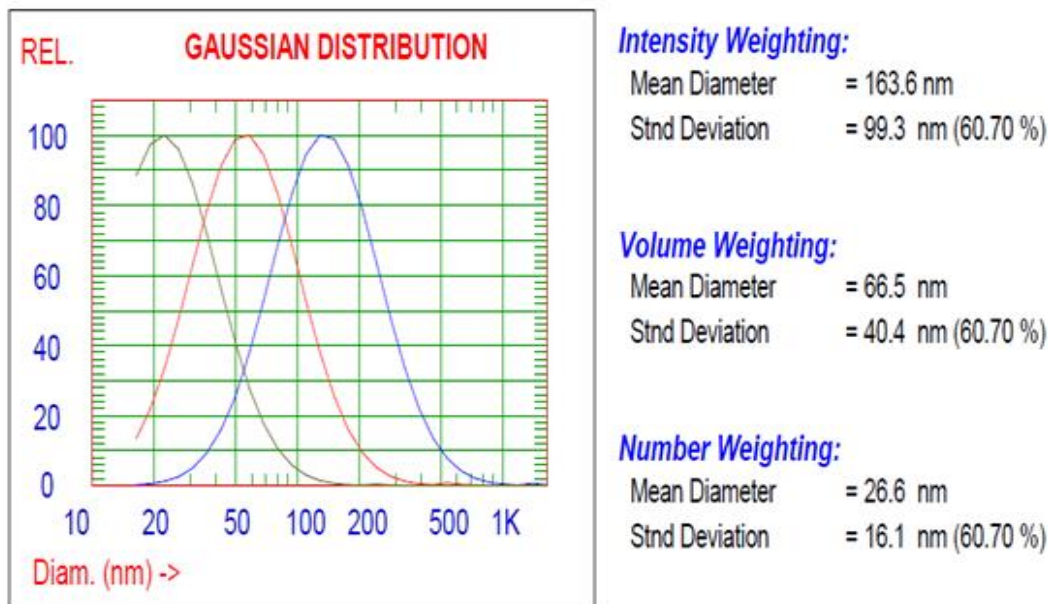


Fig 2: Emulsions distribution analysis (particle size of Chamomile oil)

3.2. Toxicity of Nanoemulsion oils (NEO) against adult of *Varroa destructor*, and its host, *Apis mellifera* Using thin layer technique.

3.2.1. Bioassay study of Nanoemulsion

oils (NEO) prepared with the tested oils against adult of *Varroa destructor* Using thin layer technique.

When the nanoemulsion oils (NEO) were biologically analyzed by the thin-layer technique, data in table (3) are consistent with nanoemulsion oils tested. indicates toxicity against *Varroa* mites. the 4-hour LC_{50} values were 3.11, 1.66, and 1.47 $\mu\text{g}/\text{cm}^2$) for garlic, chamomile and jojoba. No significant differences were observed between LC_{50} values. Garlic, chamomile and jojoba Nano-E were the most toxic oils and camphor NOE was the least toxic oil with a marked difference when comparing its LC_{50} to the other NEOs tested at 4 hours exposure time. The nanoemulsion oil (NEO) toxicity of all oils tested increased with increasing exposure time as indicated by the decrease in LC_{50} values. There was a difference between them after 24 hours of exposure. These results indicate that nanoemulsion oils showed the highest efficacy against the parasite *V. destructor*. This indicates that the oil contains inhibitory allelochemicals, but its effect

depends on the properties and composition of the oil. Nanoemulsion formulations showed faster release of active ingredients after application due to unique surface properties that allow to penetrate the parasites skin. The size of the formulated nanoemulsion was close to the standard nanoemulsion size of 1-100 nm (Casanova et al., 2005; Campolo et al., 2017) to fulfill their role. The nano-E size of the jojoba nano-E formulation did not exceed 90.7 ± 0.75 nm, but ranged from 66.5 ± 0.40 for chamomile to 10.4 ± 0.59 for other nano-E oils. These results are supported by (Mossa, 2016). Garlic oil, chamomile, jojoba, and camphor essential oil nanoemulsion were effective against adult *V. destructor*. These results have been previously reported for terpinene, p-cymene, carvacrol, 1,8-cineole, p-cymene, and α -terpinene (Ebert et al., 2007; Anton and Vandamme, 2011; Chiesa et al., 2016; Bahreini et al., 2020), α -pinene, 1,8-cineole, borneol, carvacrol, p-cymene and β -pinene (Mossa, 2016; Calatayud-Vernich et al., 2017; Bahreini et al., 2020; Campolo et al., 2020; Lucia et al., 2020; Awad et al., 2022; Giunti et al., 2022) It affected the development of many insects and mites.

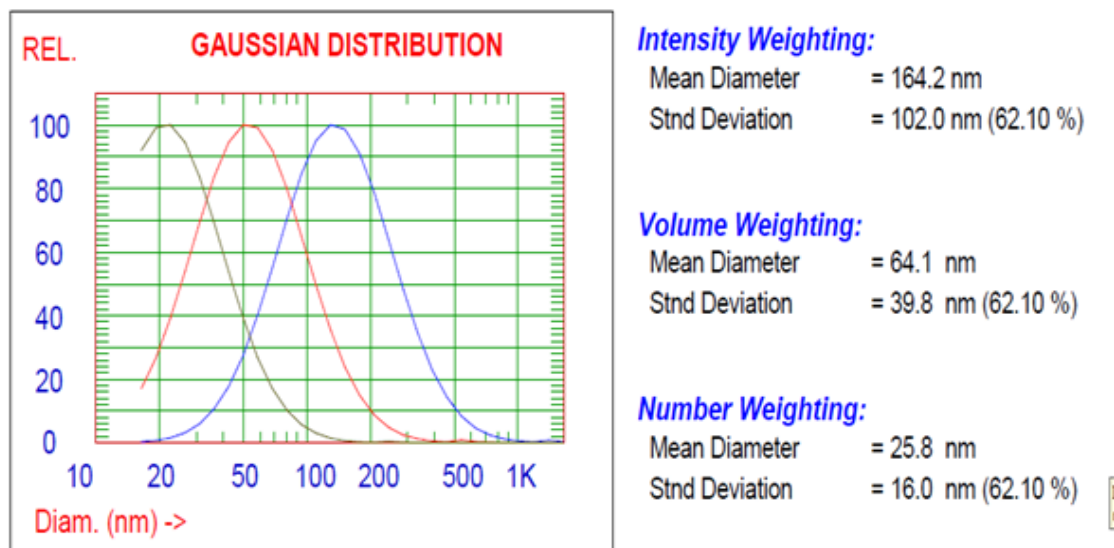


Fig 3: Emulsions distribution analysis (particle size of Garlic oil)

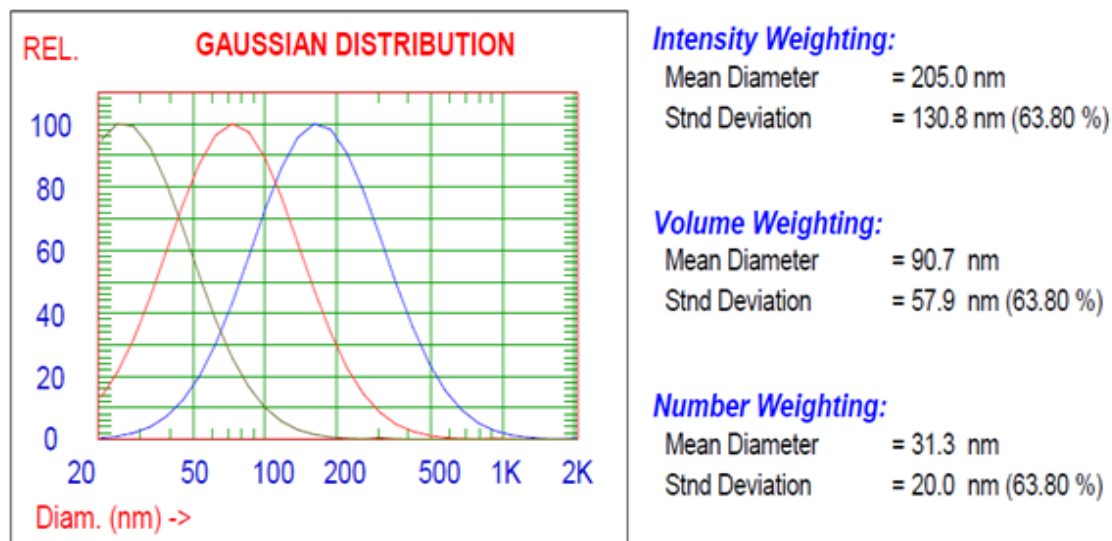


Fig 4: Emulsions distribution analysis (particle size of Jojoba oil)

Table 2. Separating phases of the emulsion after centrifugation at 2000 rpm

Centrifugation Time min.	Camphor Nano-E	Chamomile Nano-E	Garlic Nano-E	Jojoba Nano-E
20	-	-	-	-
40	-	-	-	-
60	-	-	-	-
120	2.5	2.5	2.75	3.75

Table 3: Toxicity of Nano- emulsion oils (NEO) against adult of *Varroa destructor* Using thin layer technique.

Nano Emulsion Oils	LC ₅₀ ±se µg/cm ²	fiducial limits		Slope	X ²	Time
		upper	lower			
Garlic	1.06±0.22	1.72	0.78	1.87	4.93	After 4 hrs.
Camphor	3.110±0.22	4.92	1.96	1.84	3.93	
Chamomile	1.66±0.18	2.75	1.19	1.79	2.43	
Jojoba	1.47±0.27	2.13	0.85	2.14	1.16	
Garlic	0.91±0.26	1.04	0.35	2.18	2.84	After 24 hrs.
Camphor	1.02±0.22	1.54	0.66	1.26	0.52	
Chamomile	0.0.88±0.29	1.07	0.47	1.54	3.08	
Jojoba	0.94±0.29	1.04	0.38	1.34	1.13	

Ibrahim (2017) showed that plant oils contain ingredients from flavonoids that which play a role as toxic and lethal substances for pests. They mentioned that flavonoids were antioxidant which superoxide free radical, antiaging cell which enhancing bee growth. (Eder et al., 2021) indicated that Garlic-based products have been previously studied as eco-friendly nematocides, and acaricides and their active ingredients. We could arrange the superior NEO oils in descending order as follows, Garlic, Jojoba, Chamomile, Camphor as shown in Table (3). This statement is in partially agreement with (Ibrahim, 2017; Campolo et al., 2020; Lucia et al., 2020; Eder et al., 2021; Bava et al., 2023; Mounghthipmalai et al., 2023)

3.2.2. Toxicity of Nano- emulsion oils (NEO) against *A. mellifera* using thin layer technique.

Data in Table 4 show the effect of Nanoemulsion (NEO) of different oils when determined with thin layer technique against bee workers after four and 24hrs. After 4hrs data were not enough to calculate the LC₅₀. The values of LC₅₀ after 24 hours were (2.91±0.50, 3.18±0.58, 3.98±0.48 and 4.02±0.51, of Garlic, Camphor, Chamomile and Jojoba respectively. No significantly differences between LC₅₀ values of all NEO were observed. NEO of Garlic and Camphor followed with Chamomile were the most highly toxic Nano- E oils. Jojoba nano emulsion oil gave the least toxic one. Our results are in agreement with other researchers (Ebert et al., 2007; Sattler et al., 2016; da Silva et al., 2020; Giunti et al., 2022; Dakhli et al., 2023). They indicated that essential oils are volatile

mixtures of hydrocarbons with different functional groups. They showed that essential oils have great potential as an element in integrated pest management, especially in greenhouses and other closed systems. The superiority of garlic nanoemulsion results has already been confirmed by various scientists.(Prasad et al., 2014; Hussein et al., 2016; Mossa, 2016; Sattler et al., 2016; Mossa et al., 2018) They stated that garlic extract has many effects due to its hormonal (auxin-like) properties that play important roles in the lateral expansion and elongation of cells.

3.2.3. Selective toxicity of Nano- emulsion oils (Nano-E.) against worker of *A. mellifera*

Table (5) showed that all tested Nano-emulsion oils are good selective after 24 hours of exposure. The maximum selective ratio was on Chamomile treatment followed by Jojoba (452.27 and 427.65 %). With regarding to selectivity of these oils against workers of *A. mellifera* may be due to the layer of wax surrounded with worker cuticle.

Due to its small size of particle and low surface tension, nano formulations are effective against adult mites, enhancing the penetration and uptake of the active ingredient of essential oils into the body of mites, and promoting the penetration of insecticides. It exterminates mites and plays an important role in its acaricidal effect. ((Koul, 2019; Pavoni et al., 2019). Our results in agreement with other studies on the nanoemulsion based on EOs as effective and good selective insecticides (Mossa, 2016; Mossa et al., 2018; Koul, 2019).

EO has been shown to have antibacterial, insecticidal, and acaricidal effects on a variety of

organisms (Isman, 2006). The increased toxicity of nanoemulsions could be due to the increased surface area of emulsion droplets as a result of nanoemulsion formation. This effect increases biological activity and makes nanoemulsion oils more acaricidal than regular emulsions. Previous studies have suggested that the insecticidal activity of garlic EO may be due to its main components such as diallyltrisulfide, allylmethyltrisulfide and dimethyltrisulfide (Koul, 2004; Swidan, 2007). EO, extracts and their components from garlic and other plants have been reported to exhibit acaricidal activity against several insectivorous mites (Pontes et al., 2007; Laborda et al., 2013); Moreover (Saad et al., 2006; El-Zemity et al., 2010; Ribeiro et al., 2014; Sugumar et al., 2014) nanoemulsions of EO have been shown to affect the chemical and physical properties of nanodroplets. It is highly toxic and has biological effects. Differences in

susceptibility between mites and worker bees may be due to their search for microenvironments to live, feed and reproduce. In this regard, the low sensitivity of *V. destructor* may be due to exposure to environmental stress. We created a new nanoformulation of his EO using water as a solvent. Acute oral toxicity of this formulation was studied in male rats and several biochemical parameters were also measured in serum. B. Albumin, total protein, AST and ALT (Mossa et al., 2018). When used to treat rats it is considered non-toxic to mammals. Also, the stability of the garlic oil nanoemulsion, had high acaricidal activity, and absence of toxic organic solvents make this formulation a potential acaricidal product. Our results suggest the possibility of developing suitable natural nano-acaricides from essential oils.

Table 4: Toxicity of the tested nano oils against worker of *A. mellifera* after 24 hrs. of exposure.

Nano Emulsion Oils	LC50±se µg/cm ²	fiducial limits		Slope	X ²
		Upper	lower		
Garlic	2.91±0.5	4.06	2.16	2.62	127
Camphor	3.18±0.58	4.24	2.36	2.21	1.29
Chamomile	3.98±0.48	4.29	2.41	3.35	1.01
Jojoba	4.02±0.51	4.25	3.22	3.02	2.2

Table 5: Selective toxicity of nanoemulsion oils (NEO) against *A. mellifera*

Nano-E Oils	Lc ₅₀ ±Se µg/cm ² on adult of <i>V. destructor</i>	Lc ₅₀ ±Se µg/cm ² on workers of <i>A. mellifera</i>	Selective ratio after 24 h
Garlic Nano-E	0.91±0.26	2.91±0.5	319.78 %
Camphor Nano-E	1.02±0.22	3.18±0.58	311.76%
Chamomile Nano-E	0.88±0.29	3.98±0.48	452.27%
Jojoba Nano-E	0.94±0.29	4.02±0.51	427.65%

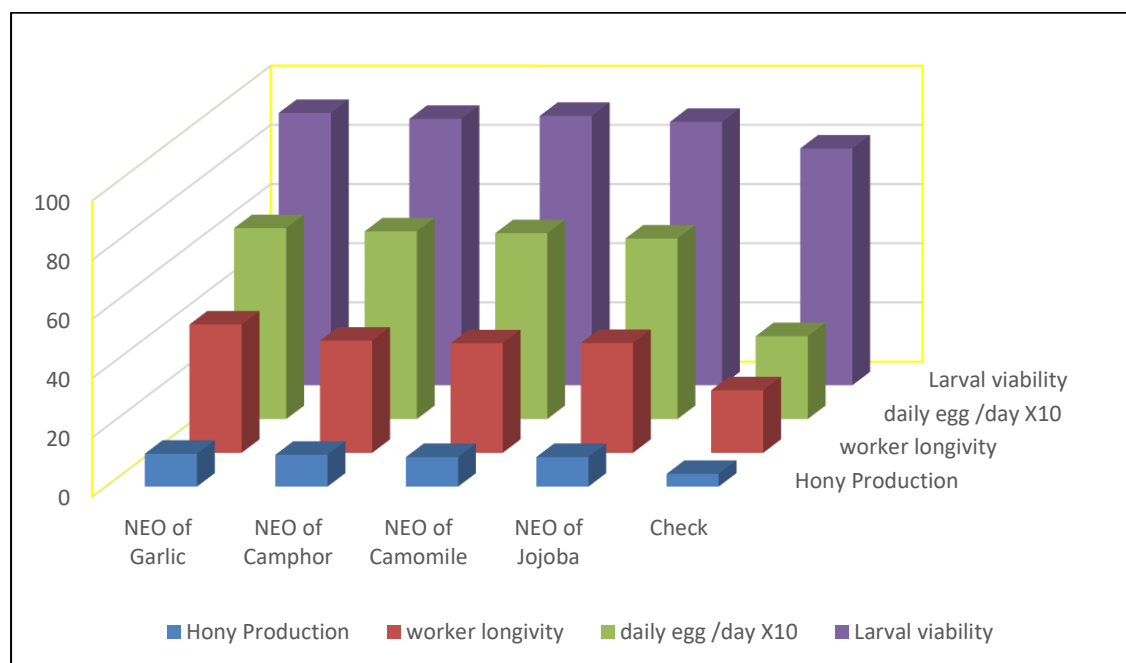
3.3. Evaluation of NEO of tested oils at bee hive

3.3.1. Efficiency of NEO against *V. destructor* and *A. mellifera*

The results in Table 6 showed that treatment of infected colonies with doses of tested natural plant product NEO (20 ml/colony) reduced varroa infestation to varying degrees. The mean reduction percentage on adult bees ranged from (94.67 to 92.22%) was recorded when NEO was sprayed with Chamomile (94.67%), followed by Garlic and Camphor (93.88% and 93,65% reduction, respectively), and jojoba (92.22%). At the same time, treatment of infected colonies with these NEOs resulted in significant differences in larval infestation. The average number of dead varroa mites was measured when colonies were sprayed twice with Jojoba-derived NEO (83.63%) and then with camphor (81.88%). The difference between the rates of reduction of mite infestation with his NEO oil treatments tested was striking.

Table 6. Effect of spraying of NEO of tested oils with 20 ml/ colony on their infestation level of the parasitic mite, *Varroa destructor* after 14 days post treatments (General Average)

Treatments	1 st spray % Reduction of infestation			2 nd spray % Reduction of infestation			Aveg . of % Reduction of infestation			G. Avg. Red.%
	Adult	brood	fallen varroa mite	Adult	brood	fallen varroa mite	Adult bees	brood	Fallen mite	
Garlic	92.27	79.08	46.67	95.48	61.40	76.21	93.88	70.24	61.41	75.18
Camphor	95.62	77.08	40.67	91.67	86.67	82.00	93.65	81.88	61.33	78.96
Chamo-mile	96.01	69.44	32.83	93.33	91.67	56.72	94.67	80.55	44.78	73.33
Jojoba	94.11	84.64	40.00	90.33	82.62	43.46	92.22	83.63	41.83	72.56

**Fig 1. Effect of spraying, of NEO on daily rate of egg laid, larvae viability %, worker longevity and productivity of honey bees for season of 2022**

3.3. Selectivity of NEO of tested oils on larval viability, worker longevity, daily egg laying and honey production

As shown in Figure 1, colonies treated with 20 ml NEO had different effects on daily egg retrieval rates. With an average of two sprays, her daily egg laying rates of the queen bee of the colonies treated with garlic and camphor, chamomile and jojoba are 645.00, 634.00, 628 and 610.00 eggs/day respectively. I understand. Compare to the queen bee egg count in an untreated colony (280.00 eggs per day). The highest percentage of larval survival (92.00%) was recorded in black seed treated colonies, with no significant difference in efficacy between treatments, but a significant effect between treatments and controls. Larval survival in untreated colonies was (82.50%). The life expectancy of workers in garlic-treated and camphor-treated colonies was 43, 37 and 34.90 days, respectively, compared to 21.13 days for workers in control colonies. Regarding the honey production of the experimental bees, the data show that the highest honey production rate (11.1 kg/colony) was achieved by colonies treated with NEO from garlic oil, followed by colonies treated with NEO from camphor and chamomile. indicated that it was Significant difference (10.75 kg/colony vs. 10.1 kg/colony).

Studying the mechanism of action of essential oils and natural chemicals is important for tick control as it can provide useful information on the most appropriate formulations and methods of administration. Volatile compounds consisting of alkanes, alcohols, aldehydes, terpenoids, especially monoterpenoids, found in many plant extracts and essential oils have gassing properties (Ariana et al., 2002; Al-Waili et al., 2012) Essential oils are used as antibacterial agents, acaricides, and insecticides, as well as to repel insects and mites, and to protect stored produce. They represent an effective alternative to synthetic pesticides with no adverse environmental (Isman and Machial, 2006; Isman, 2006). Additionally, interest in natural products such as essential oils has seen a resurgence in the last decade. This is mainly due to the fumigating and contact-killing effects of essential oils and the long history of use, which makes regulatory approval mechanisms for research less stringent (Isman, 2006).

Generally, new replacements for control *V. destructiva* agents are needed because of the rapid and widespread populations of pyrethroid and organophosphate-tolerant mites and the potential contamination of beehive products with these chemicals (Hussein and Mostafa, 2009). NEO essential oils serve as an alternative to traditional treatments. From our results, we can conclude that treatment of varroa-infested colonies with different properties of the NEO showed good selectivity for honeybees. Future studies may support this effect by focusing on characterizing dose-response relationships between components and mite / bee virulence and effects on mite behavior.

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