RESEARCH | Egyptian Journal of Agricultural Research | Plant Pathology

A tea tree oil-based nanoemulsion for controlling root-knot nematodes *in vitro*

Marwa F. Gad[1]; Dina S.S. Ibrahim[2] and Abdel-Tawab H. Mossa[1]*

Address:

[1]*Pesticide Chemistry Department, Chemical Industries Research Institute, National Research Centre, 33 El Bohouth Street (former El Tahrir St.), P.O. 12622, Giza, Egypt*

[2]*Nematodes Diseases Research Department, Plant Pathology Research Institute, Agricultural Research Centre,* Giza, Egypt.

*Corresponding Author: **Abdel-Tawab Mossa**, e-mail: abdeltawab.mossa@yahoo.com; at.mossa@nrc.sci.eg

Received: 07-03-2024; **Accepted:** 23-05-2024; **Published:** 13-60-2024 **DOI:** 10.21608/EJAR.2024.275346.1525

ABSTRACT

This study aims to develop a sustainable and environmentally friendly nanoemulsion to control root-knot nematodes (RKN). A number of nano-formulations have been prepared using gum Arabic and tea tree oil. Thirteen samples were prepared using different ratios of tea tree oil, GA, and Tween 80, including 1:1:0, 1:2:0, 1:1:1, and 1:0:1. A stable nanoemulsion was produced after ten minutes of sonication. Various physicochemical and stability tests, such as centrifugation, thermodynamics, and heating, cooling, and freezing cycles, have been performed on tea tree oil-based nanoemulsion. Sample 2C, Sample 3C, Sample 4C, and Sample 5C are the four stable samples in which the droplet size distribution was determined. The nematicidal efficacy of these formulations was evaluated against hatchability and mortality juveniles of root-knot nematodes. Samples 2C, 3C, 4C, and 5C exhibited droplet sizes of 42.7 nm, 40.6 nm, 93.6 nm, and 144.8 nm, respectively. Nanoformulation 5C showed the highest nematicidal activity, with a significant increase of 23.9%. Samples 5C (23.9%) and 3C (10.70%) demonstrated pronounced toxic effects based on the toxicity index. samples 3C (19%), 5C (13%), and 4C (11%) led to decreased hatching rates of root-knot nematode eggs. These results demonstrated that nanoemulsion exhibited potent nematicidal activity against root-knot nematode, especially sample 5C, which contained equal amounts of tea tree oil, and gum Arabic. Furthermore, nanoemulsion formulations reduced the hatching rate of nematode eggs, suggesting their potential application in controlling plant parasitic nematodes. Therefore, this nanoemulsion can be considered as potential alternatives to synthetic insecticides for the control of plant -parasitic nematodes. This study provides a novel and environmentally friendly method to develop nanoemulsion formulations from natural oils with promising biological properties for sustainable nematode management strategies.

Keywords: Green-nematicidal, Nanoformulation, Tea tree oil, Gum Arabic, Root-knot nematodes

INTRODUCTION

As the global awareness of environmental issues grows, many sectors are looking for ways to adopt more sustainable practices and reduce their ecological footprint. One of these sectors is agriculture, which faces the challenge of balancing crop protection and biodiversity conservation (Allen *et al.,* 2010; Akkari and Bryant 2016; Ali and Erenstein 2017; Abbass *et al.,* 2022). In this context, green technologies offer a promising alternative to conventional chemical pesticides, which can have negative impacts on human health, soil quality, and non-target organisms (Nuruzzaman *et al.,* 2019; de Oliveira 2020; Machado, Pereira, and Sousa 2023). Green technologies are based on the use of biological agents, such as natural enemies, pathogens, or pheromones, to control pest populations in a more eco-friendly and cost-effective way (HE *et al.,* 2019; Baker, Green, and Loker 2020). These technologies can also enhance crop productivity, resilience, and diversity, as well as contribute to food security and rural development. Therefore, investing in green technologies for agricultural pest control is not only a matter of environmental responsibility, but also a strategic opportunity for innovation and competitiveness (Nuruzzaman *et al.*, 2019; de Oliveira 2020; Muhie 2022; Machado, Pereira, and Sousa 2023).

Root-knot nematodes (RKN) are the most common and economically important of all plant-parasitic nematodes (Abrantes *et al.,* 2022). They are common in regions with warm temperatures or brief winters, and they can infect more than 2000 different plant species globally, accounting for 5% of crop loss (Sasser *et al.,* 1985). Potatoes are considered one of the crops susceptible to infection by root-knot nematode. These nematodes can cause significant losses in potato productivity and yields, a problem that is not limited to a particular region. The loss extends across the tropics, subtropics and temperate regions (Jones *et al.,* 2013; Janati *et al.,* 2018; Lima *et al.,* 2018). RKN invades the root cells near the tip and induce the formation of specialized feeding structures called giant cells, which disrupt the normal development and function of the vascular system *(*Escobar *et al*., 2015). To prevent or reduce the damage caused by RKN, various strategies have been reconnoitered, such as breeding for resistance, applying chemical nematicides, and enhancing soil health and biological diversity (Singh *et al.,* 2017; Buys, 2022).

RKN species are a major threat to many crops, as they can infect and damage different plant varieties. Farmers often rely on synthetic nematicides to protect their crops from these nematodes. However, these nematicides have negative impacts on human health and the environment, and their application is subject to increasing regulations and prohibitions (Becker, 2014; Gad, 2014). Currently, there is increasing interest in investing in green technologies in many fields, agricultural pest control included. On the other hand, green nematicides are eco-friendly nematicides that originate from natural sources, such as plants or microorganisms. Green nematicides can provide effective and sustainable nematode management, without causing ecological or human harm. They are a promising alternative to synthetic nematicides, and their development is an important research area (Isman 2000a; ; Bernard, Egnin, and Bonsi 2017; Raveau, Fontaine, and Lounès-Hadj Sahraoui 2020a; Mesa-Valle *et al.,* 2020; Catani *et al.,* 2023a).

Melaleuca alternifolia (tea tree) oil (TTO) is an essential oil, extracted from the leaves and terminal branches of an Australian native plant by steam and vacuum distillation processes (Carson, Hammer, and Riley 2006; Huynh *et al.,* 2012). TTO has been widely used for a wide range of skin conditions such as bruises, insect bites, and skin infections (Digilio *et al.,* 2008; Athanassiou *et al.,* 2015; Liao *et al.* 2016; Budhiraja *et al.,* 1999; Isman 2000b; Catani *et al.,* 2023b). TTO contains more than 100 volatile components that are mostly oxygenated hydrocarbons, monocyclic monoterpenes, and bicyclic monoterpenes (Brophy *et al.*, 1989). There are 15 major components in melaleuca oil, including terpinen-4-ol (T4O), γ-terpinene, α-terpinene, 1,8-cineole, p-cymene, terpinolene, α-terpineol, α-pinene, sabinene, aromadendrene, ledene, δ-cadinene, limonene, globulol, and viridiflorol, among which T4O is the most abundant (Lam *et al.,* 2020).

Gum Arabic (GA) is a naturally produced from the hardened sap of two species of acacia: *Vachellia* (*Acacia*) *seyal* and *Acacia Senegal* (Dauqan *et al.,* 2013; Verma *et al.,* 2021). Arabic gum, gum Sudani, gum acacia, Indian gum, acacia gum, Senegal gum, and so forth are some more names for it. Gum is mostly collected from wild trees for industrial usage, primarily in Sudan (80%) (Jamal 1994; El Amin and Luukkanen 2006; Awad *et al.,* 2018). Gum Arabic is created chemically by the combination of polysaccharides, primarily galactose, and arabinose, with glycoproteins, which are proteins with a prosthetic group or co-factor of carbohydrates (Fong, and Lamport 1991; Dauqan and Abdullah 2013; Goodrum *et al.*, 2000). GA, also known as E414 or I414 in the US, is a water-soluble, edible stabilizing component that is widely used in the food and soft drink industries. Food and pharmaceutical industries use GA to stabilize and emulsify oil-in-water (O/W) emulsions (De Folter, Van Ruijven, and Velikov 2012; Montenegro *et al.,* 2012; Wei and Huang 2019).

Water and oil are two immiscible liquids that are typically utilized in commerce to create nanoemulsion of the water-in-oil (W/O) or oil-in-water (O/W) types (Borthakur *et al.,* 2016; Singh *et al.,* 2017). While O/W nanoemulsion consist of a tiny number of oil drops spread in an aqueous phase, W/O nanoemulsion contain small drops of water dispersed in an oil phase (Borthakur *et al.,* 2016; Jafari *et al.,* 2017). Nowadays, Tween 80, a synthetic nonionic surfactant and emulsifier polyoxyethylene sorbitan monooleate, is widely used to create new nanoformulation. This molecule belongs to the class of O/W emulsifiers because it is viscous and soluble in water, with a hydrophilic-lipophilic balance (HLB) value of 15 (Huston and Larson, 2014; Ziedan *et al.,* 2022; Hassan *et al.* 2023).

Nanoemulsion have previously been produced by spontaneous emulsification, a process that combines an organic phase, a lipophilic surfactant, and a solvent that is soluble in water (Beheshti, and Assadpour 2013; Mushtaq *et al.,* 2023). However, the environment and human health are negatively impacted by these synthetic surfactants and solvents (Badmus *et al.,* 2021). There is little knowledge available regarding the use of natural surfactants like GA in nematicide nanoemulsion formulations.

This study aims to develop a green and sustainable nanoemulsion for controlling root-knot nematodes. Different nanoformulation based on tea tree oil and Gum Arabic has been developed, and their potential nematicidal activity against nematodes has been assessed.

MATERIALS AND METHODS

Chemicals and regent:

Gum Acacia, Technical Powder (Gum Arabic) was purchased from Fisher Chemical, Fisher Scientific UK Ltd., England, and tea tree oil was obtained from Sigma-Aldrich Chemie GmbH, St. Louis, MO, USA. The source of Polysorbate 80 (Tween 80) was VWR International 201, Rue Carnot F-94126 Fontenay/Bois, France. Other chemicals and reagents have been obtained from local scientific suppliers in Cairo, Egypt, as well as Sigma-Aldrich.

Tea tree oil nanoemulsion preparation:

A new formulation of 2.5% tea tree oil-based nanoemulsion with or without GA at different ratios was developed, based on previous studies (Mohafrash, *et al.,* 2022; Mohamed, *et al.,* 2022) with some modifications. The formulations of tea tree oil, Tween 80, and GA were developed using the following ratios: 1:1:0, 1:2:0, 1:1:1, and 1:0:1. The emulsion was sonicated for 2.5, 5, and 10 minutes (Ultrasonic, Sonics & Materials, INC. 53 Church Hill RD., Newtown, CT, USA) using a 13 nm-diameter probe at 20 kHz frequency and 750 W of power after dropping of the organic phase under constant stirring at 600 rpm for 30 min. The sonicator probe was continuously over-cooled by keeping it on ice.

Tea tree-GA-nanoformulation stability studies:

On the tea tree oil-based nanoemulsion, several physicochemical experiments (such as centrifugation, thermodynamics, heating, cooling, and freeze cycles) were carried out. After each test, the stability of each sample was evaluated using standard procedures (Mukherjee, and Chandrasekaran 2013; Mossa *et al.,* 2018). After every test, all samples have been inspected and their stability evaluated immediately and after three months. The droplet size distribution of four stable samples was determined. After that, the samples were then stored in a laboratory environment for a maximum of three months to collect additional observations.

The droplet size of the stable nanoemulsion:

Using dynamic light scattering (DLS), the droplet size distribution of the stable nanoemulsion (Samples 2C, 3C, 4C, and 5C) was investigated. For droplet size analysis, the DLS parameters were 30 °C and a 632.8 nm line of a HeNe laser at a 90° angle.

Nematicidal activity study:

Extraction of Nematodes:

Using egg masses from *Meloidogyne incognita* (Kofoid & White) Chitwood, which had previously been identified as females (Taylor *et al.,* 1955), the highly susceptible host, *Coleus blumei*, was infected individually. The salted loamy sandy soil was used to fill plastic pots averaging 25 centimeters in diameter with coleus plants. Pots were stored outside on a sanitized bench in the greenhouse at Nematodes Diseases Research Department, the Plant Pathology Research Institute (PPRI), Agricultural Research Centre (ARC), Egypt. Two months later, the plants had been loaded up, and various coleus plants were infected by the diseased roots . The *M. incognita*-infected plants were removed from the soil and treated with a warm bath to extract the eggs. Cobb's method was used after the roots had been cut into 2-3 cm pieces and treated with 5% sodium hypochlorite (NaOCl) to dissolve the gelatinous matrix. The roots were then stirred vigorously for approximately 5 minutes. After using many sieves to remove the debris, the eggs were collected on a 500-mesh filter. After that, the egg suspension was transferred to Whatman No. 1 filter paper, put on a sieve, and incubated at 28°C on a Petri dish prepared for the appearance of second-stage juveniles. The recently hatched juveniles were used in the study.

***In vitro* nematicidal activity study:**

The effects of the nanoemulsion on the juvenile (J2) mortality and egg hatching of the *M. incognita* nematode were assessed using *in vitro* experiments. In order to evaluate hatching, one hundred eggs were placed individually in Petri dishes (6 cm d), and the egg masses were incubated at a concentration of 1.65% nanoemulsion for three and five days. Following that, percentages of egg-hatching inhibition were calculated and noted for each treatment.

Toxicity index = % of unhatched eggs of treatment / % of unhatched eggs of normal emulsion of tea tree oil [samples with a toxicity index (TI) of more than one are highly toxic]; % of Toxicity Increased (TI %) = [(toxicity index of treatment / toxicity index of normal emulsion of tea tree) X 100] - 100.

One hundred second-stage juveniles (J2) were individually placed in Petri dishes (6 cm d) and subjected to nanoemulsion at a concentration of 1.65% for varying exposure times (24, 48, and 72 hours) under the same conditions to conduct the mortality investigation. The control group was treated with distilled water. Every treatment was carried out three times. For every observation, a stereomicroscope was employed. After that, percentages of nematode mortality were calculated and recorded for each treatment.

Toxicity index = mortality of treatment/mortality of normal emulsion of tea tree oil [samples with a toxicity index (TI) of more than one are highly toxic]; % of Toxicity Increased (TI %) = [(toxicity index of treatment / toxicity index of normal emulsion of tea tree) X 100] - 100.

Statistical analysis:

Data were subjected to statistical analysis (ANOVA) (Gomez *et al.,* 1984), followed by Duncan's multiple range tests to compare means (Duncan, 1955).

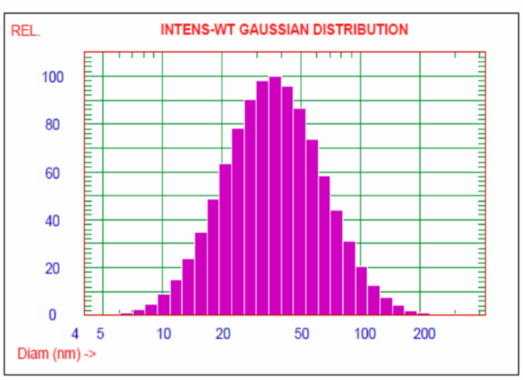
RESULTS

The nanoemulsion was prepared using tea tree oil, Arabic gum, and Tween 80 in varying proportions (as shown in Table 1 and Fig. 1). Different ratios—1:1:0, 1:2:0, 1:1:1, and 1:0:1-were employed for samples 1, 2 (A, B, C), 3 (A, B, C), 4 (A, B, C), and 5 (A, B, C). A total of thirteen samples were generated, with an oil percentage of 2.5%. The physicochemical properties and stability of the tea tree oil-based nanoemulsion, formulated with Arabic gum and Tween 80, were assessed (see Table 2). For the preparation of nanoemulsion, ultrasonic treatment was employed at varying exposure times of 2.5, 5, and 10 minutes. The results indicated that a sonication duration of 10 minutes was notably more effective, resulting in a stable nanoemulsion with high resistance to centrifugation, heating-cooling cycles, and freeze-thaw cycles. Specifically, samples 2C, 3C, 4C, and 5C (as listed in Table 3) exhibited droplet sizes of 42.7 nm, 40.6 nm, 93.6 nm, and 144.8 nm, respectively (as detailed in Table 3 and depicted in Fig. 2).

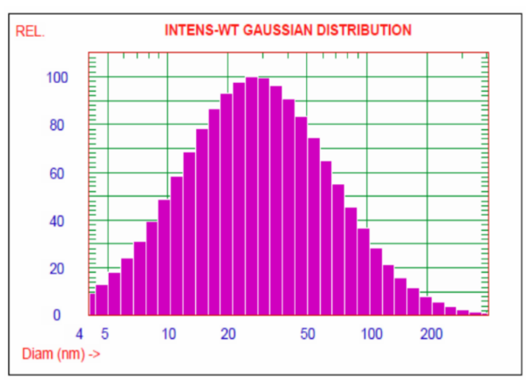
Nano formulations Normal

5C 4C 3C 2C 1C

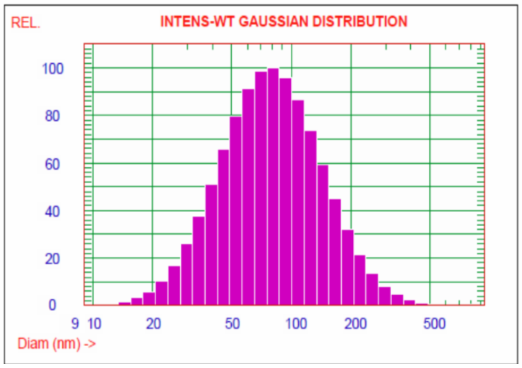
Fig. 1. Normal emulsion of tea tree oil (sample no. 1), tea tree oil + tween 80 at ratio 1:1 (sample no. 2C), at 1:2 (sample no. 3C), tea tree oil + tween 80+ gum Arabic at 1:1:1 (sample no. 4C), and tree oil + gum Arabic at 1:2 (sample no. 5C) after 10 minutes as sonication time.



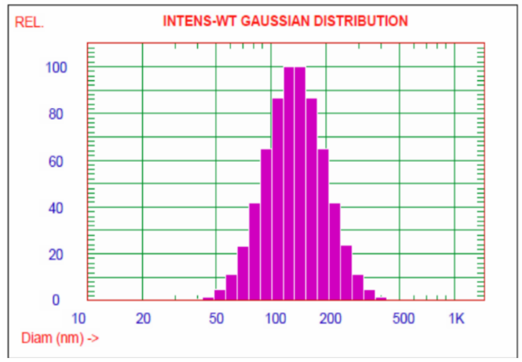
2C (droplet 42.7 nm)



3C (droplet 40.6 nm)



4C (droplet 93.6 nm)



5C (droplet 144.8 nm)

Fig. 2. Droplet size distribution of tea tree oil nanoemulsions samples number 2C, 3C, 4C and 5C.

Table 1. Content of gum Arabic, distilled water, tween 80, and tea tree oil in the nanoformulations (w/w).

Sample no.	**Ratio (oil: tween: gum Arabic)**	**Formulation content (g/100g)**			
		Tea tree oil	**Tween 80**	**Gum Arabic**	**distilled water**
1	1:1:0	2.5	2.5	---	95
2(A,B,C)	1:1:0	2.5	2.5	---	95
3(A,B,C)	1:2:0	2.5	5.0	---	92.5
4(A,B,C)	1:1:1	2.5	2.5	2.5	92.5
5(A,B,C)	1:0:1	2.5	---	5.0	92.5

Table 2. Physicochemical/stability studies and sonication time of the nanoformulations.

Sample no.	**Code**	**Sonication (min)**	**Physicochemical/stability studies**			**Stability result**
			Centrifugation	**Heating-Cooling cycle**	**Freeze-Thaw cycle**	
2	2A	2.5	-	-	-	x
	2B	5	-	+	+	x
	2C	10	+	+	+	√
3	3A	2.5	-	+	+	x
	3B	5	-	+	+	x
	3C	10	+	+	+	√
4	4A	2.5	-	+	+	x
	4B	5	-	+	+	x
	4C	10	+	+	+	√
5	5A	2.5	-	-	-	x
	5B	5	-	-	-	x
	5C	10	+	+	+	√

Table 3. Droplets size distribution of sample numbers 2C, 3C, 4C and 5C.

Items	**Sample no.**			
	2C	3C	4C	5C
Mean diameter (nm)	42.7	40.6	93.6	144.8
Variance (P.I.)	0.335	0.773	0.350	0.142
Cumulative Result (nm)				
25 % of distribution	< 24.4	<15.9	<52.7	104.5
90 % of distribution	< 75.8	<85.5	<167.6	218.4

The following sample numbers are included in the table: tea tree oil + tween 80 at ratio 1:1 (sample no. 2C), at 1:2 (sample no. 3C), tea tree oil + tween 80+ gum Arabic at 1:1:1 (sample no. 4C), and tree oil + gum Arabic at 1:2 (sample no. 5C). Note: The droplet size distribution is not applied to the normal emulsion (sample no. 1).

Nematicidal activity studies were conducted on stable nanoformulations (samples 2C, 3C, 4C, and 5C) using juveniles (J_2) of root-knot nematode. The exposure times were 24, 48, and 72 hours (as detailed in Table 4 and depicted in Fig. 3). Notably, nanoformulation 5C exhibited the highest nematicidal activity, showing a significant increase of 23.9%. Furthermore, based on the toxicity index and percentage of toxicity increase, samples

5C (23.9%) and 3C (10.70%) demonstrated the most pronounced toxic effects. Additionally, the impact of various nanoformulations on hatching root-knot nematode eggs was evaluated after three and five days of exposure (as described in Table 5 and shown in Fig. 3). The results indicated that samples 3C (19%), 5C (13%), and 4C (11%) led to decreased hatching rates.

Table 4. *In vitro* efficacy (% of mortality) of normal emulsion of tea tree oil and their different nanoformulations against juvenile (J2) of root-knot nematode at varying exposure times.

Treatments	Exposure time hours			Toxicity index after 72hs	% of toxicity increased
	24	48	72		
Control	1.66^{d}	3.66^{c}	10.33^{c}	----	----
1	36.66^{c}	57.66^{b}	68.33^{b}	1.000	0.00
2C	50.66^{b}	59.00^{b}	69.33^{b}	1.015	1.50
3C	57.00^{ab}	69.33^{ab}	75.66^{ab}	1.107	10.70
4C	45.66^{bc}	63.00^{b}	73.33^{b}	1.073	7.30
5C	68.00^{a}	77.33^{a}	84.66^{a}	1.239	23.90

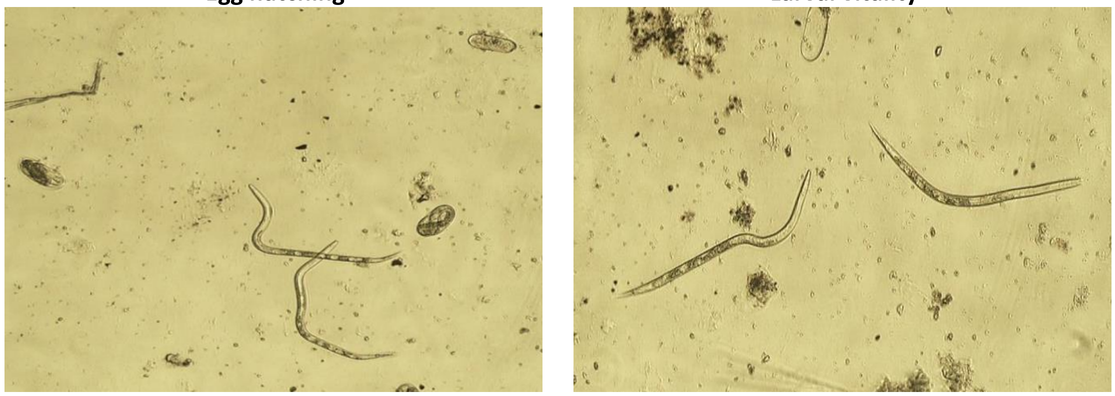
The following sample numbers are included in the table: normal emulsion of tea tree oil (sample no. 1), tea tree oil + tween 80 at ratio 1:1 (sample no. 2C), at 1:2 (sample no. 3C), tea tree oil + tween 80+ gum Arabic at 1:1:1 (sample no. 4C), and tree oil + gum Arabic at 1:2 (sample no. 5C). Toxicity index = mortality of treatment/mortality of normal emulsion of tea tree oil (samples with a toxicity index (TI) of more than one are highly toxic); % of Toxicity Increased (TI %) = [(toxicity index of treatment / toxicity index of normal emulsion of tea tree) X 100] - 100.

Table 5. *In vitro* efficacy on hatched eggs of normal emulsion of tea tree oil and their different nanoformulations against eggs of root-knot nematode at varying exposure times.

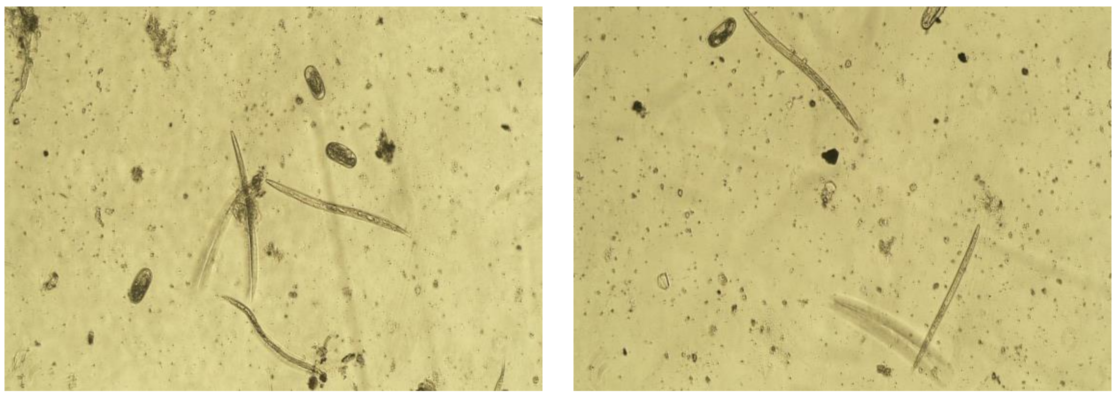
Treatments	hatched eggs		Unhatched eggs		Toxicity index after 5 days	% of toxicity increased
	exposure time days		exposure time days			
	3	5	3	5		
Control	72.00^{a}	92.66^{a}	28.00^{d}	7.34^{d}	---	----
1	29.00^{b}	52.66^{bc}	71.00^{c}	47.34^{c}	1.00	0.00
2C	21.33^{bc}	53.66^{b}	78.67^{ab}	46.34^{c}	0.98	- 2.00
3C	20.00^{bc}	43.66^{c}	80.00^{ab}	56.34^{a}	1.19	19.00
4C	15.66^{c}	47.33^{bc}	84.34^{a}	52.67^{b}	1.11	11.00
5C	24.66^{bc}	46.33^{bc}	75.34^{b}	53.67^{b}	1.13	13.00

The following sample numbers are included in the table: normal emulsion of tea tree oil (sample no. 1), tea tree oil + tween 80 at ratio 1:1 (sample no. 2C), at 1:2 (sample no. 3C), tea tree oil + tween 80+ gum Arabic at 1:1:1 (sample no. 4C), and tree oil + gum Arabic at 1:2 (sample no. 5C). Toxicity index = % of unhatched eggs of treatment / % of unhatched eggs of normal emulsion of tea tree oil (samples with a toxicity index (TI) of more than one are highly toxic); % of Toxicity Increased (TI %) = [(toxicity index of treatment / toxicity index of normal emulsion of tea tree) X 100] - 100.

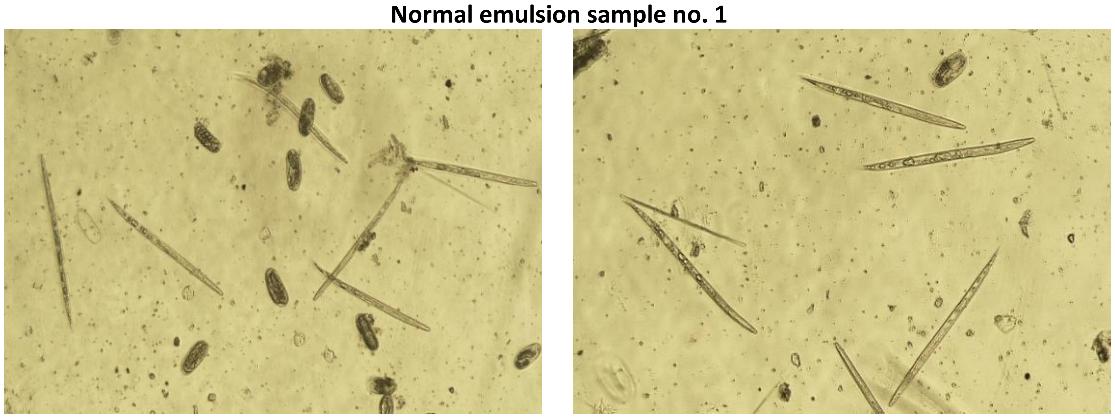
Egg hatching | **Larval vitality**



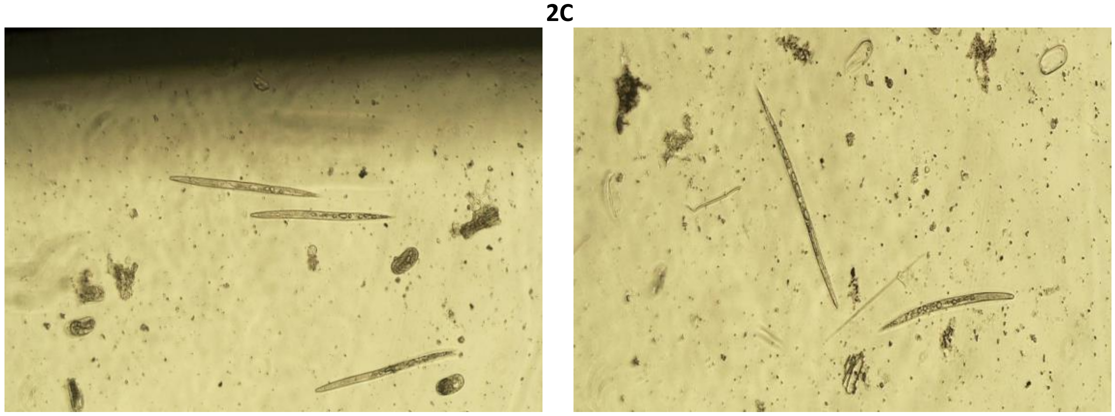
Control



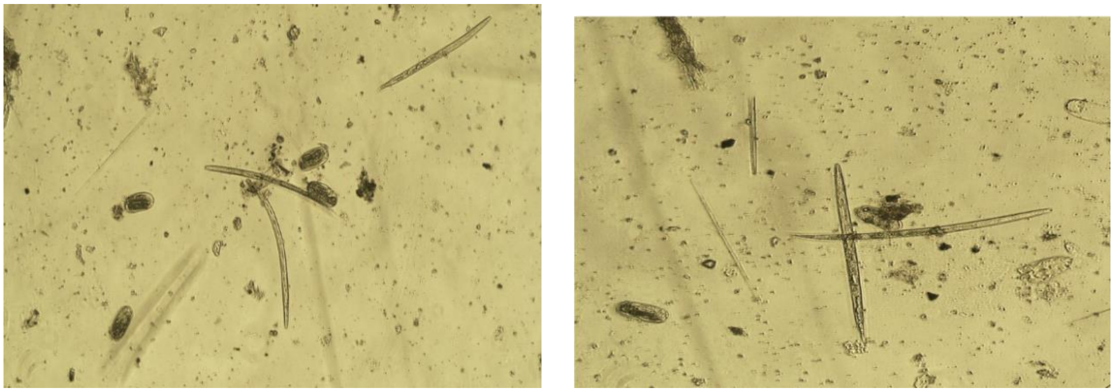
Normal emulsion sample no. 1



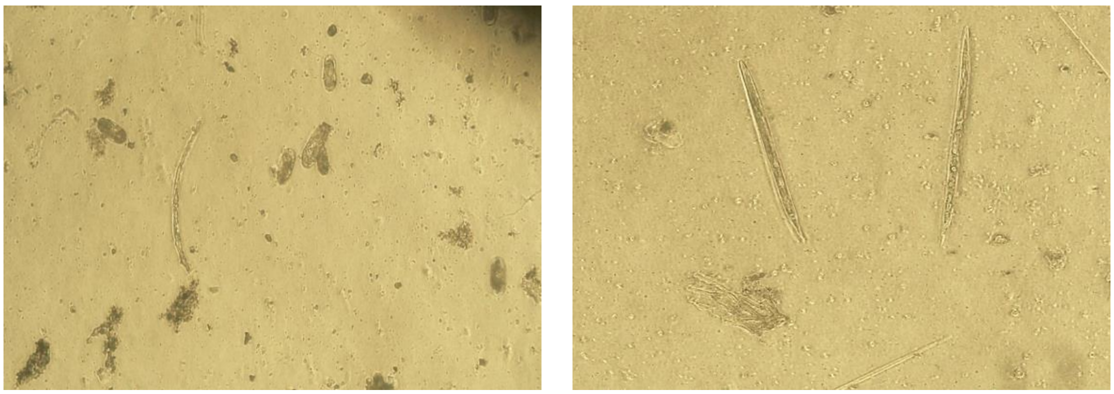
2C



3C



4C



5C

Fig. 3. Microscopic photograph of egg hatching and larval vitality of juvenile (J2) of root-knot nematode exposed to normal oil, 2C, 3C, 4C and 5 C nanoemulsions of tree oil at concentration of 1700 ppm.

DISCUSSION

In the current study, different amounts of tea tree oil, gum Arabic and Tween 80 were used to prepare nanoemulsion. Thirteen samples all of which were developed using a 2.5% concentration of tea tree oil. After 10 minutes of sonication. The results indicated that the most stable samples are 2C, 3C, 4C, and 5C, which include tea tree oil: Tween 80: Arabic gum at ratios of 1:1:0, 1:2:0, 1:1:1, and 1:0:1. The findings demonstrated a correlation between the droplet size of the nanoemulsion and the sonication time as well as the ratio of tea tree oil to tween 80. As the time of sonication and the tween 80 ratio increased, the droplet size decreased. Sample 2C showed a droplet size of 42.7 nm at 1:1 oil: tween 80 ratios, whereas sample 3C showed smaller droplets, averaging 40.6 nm at a 1:1 oil: tween 80 ratios. At a 1:1 ratio of gum Arabic to tea tree oil, the nanoformulation, with a droplet size of 144.8 nm, demonstrated remarkable stability. Similar results have been found in other studies on the effects of an oil-to-tween 80 ratio and ultrasonic exposure time. According to these studies, obtaining a small nanoemulsion droplet size and ultrasonic time have a direct connection (Anjali *et al.,* 2012; Mukherjee, and Chandrasekaran 2013; Mossa *,* 2018; Mohafrash *et. al.,* 2022). In this instance, it must be kept in mind that a small nanoemulsion droplet size may increase in diameter size as a result of increasing ultrasonic time or tween ratio (Modarres-Gheisari *et al.*, 2019). Therefore, obtaining the most suitable experimental conditions is necessary for obtaining the appropriate results.

Studies on the nematicidal activity of stable nanoformulations were carried out with juveniles (J_2) of root-knot nematode. There were three exposure periods: 24, 48, and 72 hours. For instance, the most active nematicidal compound was nanoformulation 5C. Samples 5C and 3C showed the most prominent toxic effects, with toxicity increases of 23.9% and 10.70%, respectively, based on the toxicity index and percentage of toxicity increase. The efficiency of plant oils and extracts in controlling root-knot nematode has been evaluated in several studies. According to the findings of these studies, oils have nematicidal activity and are potentially effective at controlling nematodes (Rady 2018; Catani *et al.,* 2023).

The findings demonstrated that when tea tree oil nanoemulsion treated eggs at a 1.65% concentration using various nanoformulations after three and five days of treatment, the percentage of egg inhibition decreased in comparison to the control and normal tea tree oil treatment. When comparing samples 3C, 5C, and 4C to tea tree oil, the effects of different nanoformulations on hatching root-knot nematode eggs showed that the previous sample had decreased hatching rates by 19%, 13%, and 11%, respectively. Studies have shown that some oils, such as tea tree oil, have an impact on the hatching process of root knot nematode eggs (Oka *et al*., 2000; Ibrahim, Traboulsi, and El-Haj 2006; Sarri *et al.,* 2024).

The current study's findings showed that the tea tree oil nanoemulsion was more effective than normal oil. The results also showed that the use of tween 80 at a ratio of 1: 2 enhanced the effectiveness of nanoemulsion-tea tree oil in sample 3C when compared to other treatments on hatching eggs (56.34% after 5 days). The increase in the percentage of non-hatching eggs after three days of exposure could be explained by the oil's nanoemulsion delaying the hatching process of the eggs. A tea tree oil nanoemulsion called Sample 5C, which was developed using gum Arabic as an emulsification agent, showed significant juvenile nematode (J2) mortality (84.66% after 72hrs). Tea tree oil shows nematicidal activity because it contains different active chemical components, among which are terpinen-4-ol, α-terpinene, 1,8 cineole, α-pinene, γ-terpinene, p-cymene, and α-pinene (Mee, and Riley 2002; Navarro *et al.*, 2008; de Groot and Schmidt 2016). Understandably, given their complex chemical constitution, an important portion of the biological activity in essential oils is also attributable to the synergistic or complimentary activities of many components (Gómez-Rincón *et al.,* 2014; Mossa 2016). The mechanism behind tea tree oil's nematicidal effects may involve inhibiting the movement and function of parasites due to its impact on acetylcholine and/or acetylcholinesterase function. Terpinen-4-ol and 1,8 cineole, both major components of tea tree oil, exhibit anticholinesterase activity (Mills *et al.,* 2004; Gómez-Rincón *et al.,* 2014). Because Arabic gum is used as a surfactant in tea tree nanoemulsion, it has a high nematicidal activity. This could be attributed to the good nanoformulation, which increases the bioactive components' contact area, stability, solubility, penetration, and bioavailability due to the lowest droplet size of the nanoformulation.

CONCLUSION

In conclusion, this study demonstrated the successful preparation and characterization of tea tree oil-based nanoemulsion using gum Arabic and Tween 80 as emulsifiers. The nanoemulsions were stable under various stress conditions and showed different droplet sizes depending on the ratio of the components. The nanoemulsion also exhibited potent nematicidal activity against root-knot nematode, especially sample 5C, which contained equal amounts of tea tree oil, and gum Arabic. Moreover, the nanoemulsion reduced the hatching rate of nematode eggs. Based on these results, there can be potential application of these nanoemulsions in controlling plant parasitic nematodes. This study provides a novel and eco-friendly approach for developing nanoemulsions from natural ingredients with promising biological properties.

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