



Chemical Constituents and Formulation of Castor Fruit Extracts and Their Nematicidal Activity on Root-knot nematode (*Meloidogyne incognita*)

Hosny M. Raddy¹, Mohamed H. A. El-Sawah² and Heba H. Fahmy^{2*}

¹ Department of Plant Protection, Faculty of Agriculture, Al-Azhar University, Egypt

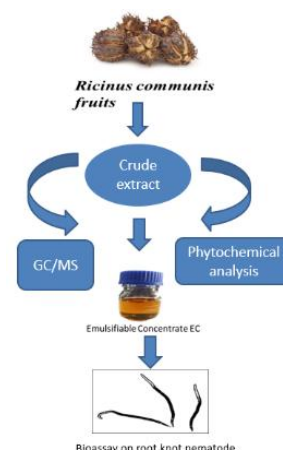
² Department of Pesticides Formulation Research, Central Agricultural Pesticides Laboratory, Agricultural Research Center, Giza, Egypt



Abstract

In this study, dried fruits of the castor plant (*Ricinus communis*) were extracted using different solvents: n-hexane, ethyl acetate, and ethanol, and then formulated as an emulsifiable concentrate (EC). Additionally, the various secondary metabolites and chemical constituents of the extracts were identified through phytochemical screening tests and Gas Chromatography-Mass Spectrometry (GC-MS) analysis. Furthermore, the nematicidal activity of the tested extracts and their ECs was evaluated against the root-knot nematode (*M. incognita*) under both laboratory and greenhouse conditions. Results of the phytochemical screening showed that most of the extracts possessed steroids, terpenoids, saponins, alkaloids, flavonoids, phenols, and tannins. Thirty-one compounds were identified in n-hexane extract, the main constituents were 10E, 12Z-Octadecadienoic acid (12.26%), Ricinoleic acid (25.14 %), Tetracosane (4.05%), Stigmasterol (3.17%), Gamma.-Sitosterol (5.78%) and Lupeol (10.54%), and sixteen compounds for ethanolic extracts were linoleic acid ethyl ester (1.73%), (E)-9-Octadecanoic acid ethyl ester (2.78%), 9-Octadecanoic acid, 12-hydroxy- ethyl ester, [R-(Z)]- (4.43%), Ricinine (1.25%) and Ricinoleic acid (32.94%). Most of the prepared formulations had stable values during different storage conditions. The bioassay results indicated that the most significant nematicidal toxicity on the second stage juveniles (J2) of the nematode was achieved through the treatment with the EC formulation of the n-hexane extract (LC50= 15.31 mg/L), followed by the EC of the ethanol extract (LC50=26.81 mg/L), and the EC of the ethyl acetate extract (LC50=33.94 mg/L) after 48 hours of exposure. Additionally, in the greenhouse experiment, all treatments significantly ($P \leq 0.05$) reduced nematode infection and improved plant growth parameters of eggplant plants compared to the untreated treatment.

Keywords: *Ricinus communis* L. fruits; phytochemical analysis; emulsifiable concentrates (ECs); physicochemical properties; *Meloidogyne incognita*, nematicidal activity



1. Introduction

Root-knot nematode, genus *Meloidogyne*, is a major pest that attacks numerous cultivated crops. Responsible for 90% of the damage caused by plant-parasitic nematodes [1], this pest was widely distributed in cultivated areas of Egypt, particularly in newly reclaimed areas with light sandy soils. It causes significant damage to vegetable crops, including eggplants [2]. Various strategies have been employed to manage root-knot nematodes in infested areas, including crop rotation, organic amendments, development of nematode-resistant varieties, biological control, and chemical nematicides [3, 4]. However, due to health and environmental risks, nematicides, the most rapid and efficient method to control this pest, have been withdrawn from the market [5]. Currently, there is a global trend towards eco-friendly and effective management practices to combat nematode infestation for sustainable agriculture [6]. One promising approach to combat root-knot nematodes is the use and development of plant-based nematicides.

The Euphorbiaceae family is the largest and most diverse in the plant kingdom, comprising approximately 340 genera and around 8,000 species [7]. It has been reported that many phytochemicals from Euphorbiaceae plants exhibit insecticidal, larvicidal, and ovicidal actions against various insects [8, 9]. The castor plant (*Ricinus communis* L.) is one of several species in this family that are considered medicinal plants, grown worldwide due to their tolerance to various weather conditions. Recently, a total of 83 different compounds have been identified from various parts of *R. communis*, including

*Corresponding author e-mail: heba.hajjal@gmail.com; (Heba H. Fahmy).

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alkaloids, terpenoids, flavonoids, benzoic acid derivatives, coumarins, and fatty acids. These substances have shown cytotoxic, insecticidal, anti-inflammatory, antioxidant, anti-asthmatic, and other effects [10]. Additionally, the nematocidal effect of plant extracts from this plant has been reported in many previous studies [11-13].

Degradation and volatilization of bioactive compounds are the main factors that reduce the effectiveness of plant-based products in the field. Consequently, the potential compatibility of certain plant materials for agricultural use is underestimated due to the loss of bioactive substances. One way to avoid these disadvantages is to develop suitable formulations that enhance the durability of these bioactive botanicals. Various techniques have been employed to overcome these problems, including encapsulation, nanoemulsion, nanoparticle synthesis, and emulsified concentrate formulations, to enhance solubility, persistence and effectiveness [14, 15].

One of the most common pesticide formulations is emulsifiable concentrate (EC), which offers advantages like high storage stability and relatively high biological activity. Typically, ECs are oily liquid formulations with optical transparency, made by dissolving the active ingredient in organic solvents (such as xylene, toluene, or benzene), which may also contain surfactants and other additives. These systems are then diluted with water before application, resulting in the spontaneous formation of an oil-in-water emulsion that contains the active ingredients within oil droplets [16].

Therefore, the objectives of the present work were 1) to determine the chemical compositions of the three extracts (ethanol, ethyl acetate, and hexane) of *R. communis* fruits by Gas Chromatography-Mass Spectrometry (GC-MS). 2) Formulate these extracts as EC formulations and determine their physicochemical properties, characterization, and stability. 3) Evaluate the nematocidal activities of these three extracts and their ECs against *M. incognita* in laboratory and greenhouse experiments.

2. Materials and Methods

2.1. Chemicals

All the different solvents, such as hexane, ethyl acetate, ethanol, dimethyl-formamide (DMF), dimethyl sulfoxide (DMSO), acetone, xylene, and cyclohexanone were supplied by El-Nasr Pharmaceutical Chemicals Company (ADWIC), Egypt. The following reagents: sulfuric acid, hydrochloric acid, sodium hydroxide, ferric chloride, iodine, and potassium iodide were supplied by Merck & Co., Inc. Emulsifiers: Rodacal 60/BE and Alkamuls RC were supplied by Rhodia. Tween 20, 80, and Span 80 were supplied by Qualikems Fine Chem Pvt. Ltd. INDIA, and Distilled Water used in all preparation steps was delivered by Water Distillator LABCONCO water PROT. MPS LABCONCO Corporation, Kansas City, Missouri 64132-USA.

2.2. Preparation of plant sample collection and extraction procedure

The ripe dried fruits (capsules and seeds) of *R. communis* were gathered during August 2022 from different regions in the EL-Qalubia government and were identified and authenticated by the Egyptian National Botanical Institute, Dokki, Giza, Egypt. The fruits were washed with tap water and dried in the laboratory, then ground into small pieces with the help of an electrical grinder and stored in dark conditions.

The crude extracts of *R. communis* fruits were obtained using the maceration method described by [17] by soaking 100 g of crushed fruits in sequentially different solvents: n-hexane, ethyl acetate, and ethanol for 7 days in a glass bottle, and then the bottle was shaken for one hour each day at 150 rpm speed. The plant suspensions were filtered through filter paper, and then the filtrates were concentrated by evaporating the solvent with a rotary evaporator at a temperature of 40°C. The obtained extracts were weighed and stored in air-tight bottles in the refrigerator at 4°C until use.

2.3. Identification of the chemical constituents and qualitative analysis of castor fruit extracts

2.3.1. Phytochemical screening assay

Phytochemical screening can be carried out on crude extracts using the proper tests to determine the type of phytochemicals present in the crude extracts as described [18-21].

2.3.1.1. Test for steroids (Salkowaskitest)

In a test tube, 2 mL of chloroform was mixed with 2g of each crude extract, and then 1 mL of concentrated sulfuric acid was added slowly. Once sulfuric acid reached the mixture surface, two layers of red and greenish-yellow fluorescent color formed, indicating the presence of steroids.

2.3.1.2. Test for terpenoids (Salkowski test)

2g of each crude extract was dissolved in 2 ml of chloroform, and then 3 ml of concentrated sulfuric acid was added. The appearance of reddish-brown coloration indicated the presence of terpenoids.

2.3.1.3. Test for saponins (Foam test)

5 mL of distilled water was mixed with 2 g of each crude extract in a test tube, and then the mixture was vigorously shaken. The observed stable foam of at least 2cm in length indicates the presence of saponins.

2.3.1.4. Test for alkaloids (Wagner's test)

A few drops of Wagner's reagent were added to 2 g of each crude extract. The formation of a reddish-brown precipitate indicated the presence of alkaloids.

2.3.1.5. Test for Flavonoids (Alkaline reagent test)

In a test tube, 2 g of each crude extract was treated with a few drops of 20 % sodium hydroxide solution. An intense yellow color, which becomes colorless with the addition of diluted acid, indicated the presence of flavonoids.

2.3.1.6. Test for Tannins (Braemer's test)

2g of each crude extract was treated with 2 ml of 10 % ferric chloride solution. Appearances of a blue or greenish color indicate the presence of tannins.

2.3.1.7. Test for phenols (Ferric chloride test)

In a test tube, 2g of each crude extract was treated with 0.5 ml of 5 % ferric chloride solution. A blue or black coloration indicated the presence of phenols.

2.3.2. Gas chromatography-mass spectrometry (GC-MS) analysis

The chemical constituents of castor fruit extracts were analyzed by an Agilent GC-MS system gas chromatograph (7890B) fitted with a mass spectrometer detector (5977A) using an HP-5MS column (30 m x 0.25 mm internal diameter and 0.25 m film thickness). The experimental conditions are as follows: Carrier gas: helium with a flow rate of 1.0 ml/min, and a split ratio of 1:30. Temperature program: 40 °C for 1 min; rising at 4 °C /min to 150 °C and held for 6 min; rising at 4 °C/min to 210 °C and held for 1 min. Identification of castor fruit extract components was performed by comparing the spectrum fragmentation pattern with those stored in the Wiley and NIST Mass Spectral Library data and retention time.

2.4. Preparation of castor fruit extracts as 20 % emulsifiable concentrates (ECs) formulations

Emulsifiable concentrate (ECs) formulations of each crude extract were formulated by following the method described by [22] using a simple mixing procedure. Castor fruit extracts (20 g) were dissolved in appropriate organic solvents, then were mixed with a blend of two non-ionic surfactants in several ratios at different hydrophile-lipophile balance (HLB) into a beaker using a magnetic hot plate stirrer model Terrey Pines Scientific", USA, at approximate 150 rpm for 5 minutes then mixed with the solvent to make up the complete homogenized volume (100% w/w) of a clear EC formulations.

2.5. Physicochemical characteristics of the prepared emulsifiable concentrates (ECs) formulations under different storage conditions**2.5.1. The storage stability test**

Storage stability was tested according to the official CIPAC standard method. Briefly, two samples of each EC crude extract formulation were separately packed in a glass bottle, one stored at 0 °C for 7 days and the other one at 54 °C for 14 days [23]. After storage, the samples were cooled to room temperature, and the volume of separated material was then recorded. The physicochemical parameters were then determined.

2.5.2. Surface tension

The surface tension of each EC crude extract formulation was measured according to [24] by using a Force tensiometer sigma 700 USA du Nouy method with a platinum-Wilhelmy plate. The instrument was calibrated before testing, and then samples were placed in a cleaned vessel to be measured. The corrected value was recorded as (dyne/cm³).

2.5.3. Free acidity or alkalinity

The acidity or alkalinity of the EC formulations was determined by titration with either standardized acid solution or standardized alkali solution using electrometric end point determination according to [25]. About 10 gs of sample were weighed into a beaker then distilled water was added (100 ml) and stirred to homogenize. Then the sample solution was titrated electrometrically to pH 7 at room temperature.

2.5.4. Density and specific gravity

The density and specific gravity of the EC formulations were measured according to [26] by Rodolph Densitometer DDM 2910 attached to the autosampler. Using the autosampler, a small volume (about 1-2 ml) of each sample was introduced into the clean and dry sample tube of the instrument. Ensure that the sample tube is properly filled and that no gas bubbles are present. After the instrument displays a steady reading, density and specific gravity are recorded.

2.5.5. Refractive index

The refractive index of the EC formulations was determined according to [27] at 25°C ± 0.1 using ATAGO Refractometer DR-AI. One or two drops of the sample were placed on the lower prism face, then the prism assembly was closed and locked. Waited for about 3 minutes, as the temperature equilibrium time, and then looked through the eyepiece to observe the field consisting of a light and dark portion. The instrument was adjusted so that the boundary between the light and dark portions of the field was as sharp as possible; the refractive index value was then recorded from the instrument scale.

2.5.6. Viscosity

The viscosity of the EC formulations was measured according to [28] with a digital rotational viscometer "Brookfield DV II+ PRO" (Brookfield, USA). The temperature was kept at 25°C during the measurement using a water bath (Model: TC-502, USA). The measurement process is carried out using a preset command with the software attached to the viscometer, by selecting the proper spindle and rotational speed, it gives the reading directly in centipoise (cP).

2.5.7. Flash point

The flash point of EC formulations was measured according to ASTM D3828 [29] by using (Kolchler Rapid Flash Tester, Closed Cup, USA), at room temperature (about 25 °C), the sample to be tested (about 2ml) was transferred to the filling cup orifice by the apparatus syringe, then the test timer and heating source were started. This measuring method depends on accelerated heating of the closed cup containing the sample, into which the device flame was instantaneously inserted over the surface of the test specimen until a flash spark occurred. The temperature was then recorded as a flash point (or no flash) at the test range temperature.

2.6. Physical properties of spray solutions of the prepared emulsifiable concentrates (ECs) formulations under different storage conditions

2.6.1. Emulsion stability and re-emulsification

The emulsion stability of the spray solution was measured according to [30] using spray solutions prepared in a graduated cylinder (100 ml) with a glass stopper under a dilution rate of 5% v/v for both WHO standard soft and hard water [31]. The cylinder was then stoppered and inverted 30 times subsequently, and the observation of any free oil or cream separation amount in the cylinder was recorded, then, the cylinder was allowed to stand undisturbed in a water bath ($30 \pm 2^\circ\text{C}$), for various time intervals (initial time, 0.5 h and 24 h for re-emulsification).

2.6.2. Persistent foam

The Persistent foam is a measurement for the amount of foam likely to appear in the spray tank after dilution of the product with water. About 5 mL of the formulation was added to hard water (95 mL) in a measuring cylinder. The cylinder was inverted 30 times and then left on the bench for 5 minutes. After that, the volume of foam was recorded according to [32].

2.6.3. pH

The pH value of spray solutions was measured according to [33] by using a pH Meter (Model: Jenway 3510, UK). The pH electrode was first calibrated before testing by using a buffered solution of pH 4, 7, and 10 (Park, Scientific Limited, Northampton, UK). One gram of the formulation was weighed into a beaker of 100 ml of distilled water and stirred to completely mix. Then, the electrode was dipped in the sample solution and left for 5 min without disturbance at room temperature to allow the pH value to stabilize, and then the pH value was recorded.

2.6.4. Conductivity

The conductivity of spray solutions was measured according to [34] using the Conductivity and Salinity meter “Thermo Orion model 115A+, USA”. Before the measurement, the conductometer was calibrated with a 0.01 M KCl solution. The measurements were made at room temperature ($25^\circ\text{C} \pm 2$). One gram of the formulation was weighed into 100 ml of distilled water in a beaker and vigorously stirred to complete mixing. The electrode was then immersed in a dilution beaker and left for 1-2min to allow the conductivity value (μS) to stabilize.

2.6.5. Surface tension

It was determined according to [24] as mentioned before.

2.7. Nematicidal activity of *R. communis* fruit crude extracts and their emulsifiable concentrate (ECs) formulations

2.7.1. Nematode inoculum

To obtain nematode inoculum, southern root-knot nematodes were isolated from infested tomato (*Solanum lycopersicum* L.) roots collected from an open field in the El-Tahadi region of Beheira Governorate, Egypt. Using the female perineal patterning method, this species was identified as *Meloidogyne incognita* [35]. The inoculum was cultivated on eggplant (*Solanum melongena* L.) seedlings. For every experiment, the inoculum is obtained from infected plants. The mature egg masses from the source cultures were placed in sieves lined with tissue paper, measuring 9 cm in diameter and having 1 mm pores. To hatch second-stage juveniles (J2s), these sieves were then placed in petri dishes filled with distilled water and incubated at 27°C . Additional population of J2s was raised in the same manner to obtain the necessary inoculum for the experiments [36].

2.7.2. Laboratory experiment

The nematicidal activity of ethanol, ethyl acetate, and hexane extracts of *R. communis* fruits, as well as their emulsifiable concentrate (EC) formulation (represented by six treatments), was tested on second-stage nematode juveniles (J2s) under laboratory conditions. Each treatment was prepared in six concentrations, ranging from 10 to 500 ppm, based on the concentration of crude extracts or their 20% EC using distilled water. Then, 1 mL of nematode suspension (approximately 100 ± 10 M. *incognita* J2) was added to each glass vial (10 mL in volume capacity) and mixed with diluted solutions of the examined extracts to achieve the required concentrations. Each treatment and the control were replicated three times, and all treatments were incubated at ($27 \pm 2^\circ\text{C}$) and observed after 48 hours to evaluate their nematicidal activity. J2s were considered dead if they displayed a motionless straight posture and did not react to being touched by a small probe [37]. Mortality was recorded in 1 milliliter under 100 times magnification over the designated duration. The following equation was used to calculate the mortality percentages:

$$\text{Mortality (\%)} = (\text{Dead juveniles}) / (\text{Total number of juveniles}) \times 100$$

According to Finney [38], the obtained data were expressed as toxicity lines, and the LC_{50} values (the concentration at which 50% of the nematodes were killed) were determined by the probit analysis software program.

2.7.3. Pots experiment

The experiment aimed to evaluate the effectiveness of *R. communis* fruit extracts (ethanol, ethyl acetate, and n-hexane) and their emulsifiable concentrate (20% EC) formulation in controlling *M. incognita* on tomato plants in clay pots under greenhouse conditions at the Department of Plant Protection, Faculty of Agriculture, Al-Azhar University. The experiment began by transplanting a 4-week-old eggplant seedling into each pot (20 cm in diameter) filled with 2 kg of clay loam soil (1: 1 sand to clay w/w) and watering them regularly. A week later, freshly hatched second-stage juveniles (J2s) were introduced to each pot (1000 larvae/pot) by pipetting 10 ml of the inoculum suspension into three holes around the root system. After inoculation, the holes were covered with moist soil. Immediately after inoculation, the plants were treated with the tested extracts and their EC formulation at a final concentration of 1000 ppm through soil drenching. Untreated pots were inoculated with J2s and functioned as controls. Every treatment was arranged in a completely randomized block design, replicated five times, and maintained at $30\pm5^{\circ}\text{C}$ in the greenhouse. Water was added as needed. The plants in each pot were carefully removed after 50 days of inoculation, and 250g of soil was removed from each pot and soaked for 30 minutes while being gently agitated in a container halfway full of tap water. Following their extraction using 200 and 400 mesh sieves, the J2s were tallied. Following a thorough washing to remove any remaining soil, measurements of the plants' fresh root weight, fresh shoot weight, and shoot length were taken. The number of egg masses and galls in each root system was also counted using a stereomicroscope. The percentage decrease in the nematode parameter is computed using the following formula:

$$\text{Reduction (\%)} = (a-b)/a \times 100$$

a = number of (Galls r/Root) or (Juveniles / 250 g soil) in control

b = number of (Galls r/Root) or (Juveniles / 250 g soil) in treatment

2.8. Statistical analysis.

ANOVA with the Costat software [39] was used to analyze the data, and Duncan's [40] multiple range tests was used to identify significant differences between the treatments at a probability level of $P < 0.05$. The log-probit software tool Ldp Line® model "Ehabsoft" was used to estimate the LC_{50} values and depict the toxicity lines [41].

3. Results

3.1. Preliminary phytochemical screening of the *R. communis* fruit extracts

Phytochemical screening of n-hexane, ethylacetate, and ethanolic fruit extracts of *R. communis* L. revealed the presence of steroids, terpenoids, saponins, alkaloids, flavonoids, tannins, and phenols in ethanolic extract. In contrast, the n-hexane and ethyl acetate extracts showed the presence of steroids, terpenoids, saponins, and the absence of alkaloids, flavonoids, tannins, and phenols (Table 1).

Table 1: Phytochemical screening of *R. communis* L. fruit extracts.

Phytochemical compounds	n-hexane extract	ethyl acetate extract	ethanolic extract
Steroids	+	+	+
Terpenoids	+	+	+
Saponins	+	+	+
Alkaloids	-	-	+
Flavonoids	-	-	+
Tannins	-	-	+
Phenols	-	-	+

(+) indicates the presence and (–) indicates the absence of phytochemicals compounds

3.2. Chemical compositions of the *R. communis* L. fruit extracts

Results of the GC-MS analysis of n-hexane and ethanolic fruit extracts of *R. communis* L. are shown in Tables 2 and 3. Thirty-one compounds were identified in the n-hexane extract, with 10E 12Z-Octadecadienoic acid (12.26%), Ricinoleic acid (25.14 %), Tetracosane (4.05%), Stigmasterol (3.17 %), Gamma-Sitosterol (5.78%), and Lupeol (10.54%) being the main constituents (Table 2). Additionally, the ethanolic extract identified sixteen compounds, with most of them being linoleic acid ethyl ester (1.73%), (E)-9-Octadecanonic acid ethyl ester (2.78%), 9- Octadecanonic acid, 12-hydroxy- ethyl ester, [R-(Z)]- (4.43%), Ricinine (1.25%) and Ricinoleic acid (32.94%) (Table 3).

Table 2: Chemical compositions of n-hexane castor fruit extract.

No peak	Components	Retention Time (min.)	Area (%)
1	Benzene , (1-butyl hexyl)-	14.924	1.09
2	Benzene , (1- propyl heptyl)-	14.999	0.88
3	Benzene , (1-ethyl octyl)-	15.182	0.81
4	Benzene , (1-methyl nonyl)-	15.485	0.82
5	Benzene , (1-butyl heptyl)-	15.931	3.41
6	Benzene , (1-propyl octyl)-	16.029	1.73
7	Benzene , (1-ethyl nonyl)-	16.217	1.52
8	Benzene ,(1-methyl decyl)-	16.509	1.63
9	Benzene , (1-pentyl heptyl)-	16.864	2.01
10	Benzene , (1-butyl octyl)-	16.910	2.16
11	Benzene , (1- propyl nonyl)-	17.013	1.51
12	Benzene ,(1-ethyl decyl)-	17.202	1.40
13	Benzene ,(1-methyl undecyl)-	17.499	1.45
14	Benzene , (1-pentyl octyl)-	17.785	2.52
15	Benzene , (1- butyl nonyl)-	17.848	1.65
16	Benzene ,(1- propyl decyl)-	17.957	1.24
17	Benzene , (1-ethyl undecyl)-	18.146	1.05
18	Benzene ,(1-methyl dodecyl)-	18.438	1.06
19	13-Hexyloxacyclotridec-10-en-2-one	19.691	0.56
20	n-Hexadecanoic acid	19.788	0.87
21	10E, 12Z-Octadecadienoic acid	21.585	12.26
22	Phthalic acid, di(2-propyl pentyl) ester	24.978	1.96
23	Ricinoleic acid	25.384	25.14
24	9-octadecenoic acid, 12-hydroxy-	26.111	0.68
25	Squalene	27.541	2.92
26	Tetracosane	27.541	4.05
27	Hexadecane, 1-iodo-	28.983	1.43
28	Oxirane, tetradecyl-	30.288	2.63
29	Stigmasterol	30.677	3.17
30	Gamma.-Sitosterol	31.272	5.78
31	Lupeol	31.730	10.54

Table 3: Chemical compositions of ethanolic castor fruit extract

No peak	Components	Retention Time (min.)	Area (%)
1	4-Phenylphenol	11.829	0.06
2	Piperidine,2-pentyl-	13.494	0.02
3	Hexadecanoic acid, ethyl ester	19.136	0.97
4	n- Hexadecanonic acid	19.771	0.47
5	Linoleic acid ethyl ester	20.538	1.73
6	(E)-9-Octadecanoic acid, ethyl ester	20.584	2.78
7	Octadecanoic acid, ethyl ester	20.835	0.62
8	9,12- Octadecanonic acid (Z,Z)-	21.591	4.43
9	9- Octadecanoic acid, 12-hydroxy-ethyl ester, [R-(Z)]-	22.615	48.89

10	Ricinine	23.061	1.25
11	Bis (2-ethylhexyl)phthalate	25.018	1.18
12	Ricinoleic acid	25.613	32.94
13	Difluoro(methylamino)phosphine sulfide	27.753	1.22
14	Stigmasterol	30.746	0.04
15	Beta.-Sitosterol	31.301	0.13
16	Lupeol	31.776	0.26

3.3. Physicochemical characteristics of the prepared emulsifiable concentrates (ECs) formulations.

Data in Table 4 demonstrated the physicochemical properties of the prepared formulations as 20% emulsifiable concentrates (ECs) pre- and after accelerated hot storage at 54°C for 14 days. The results indicated that the formulated C2H gives the lowest acidity values, 0.284 ± 0.012 and 0.234 ± 0.010 as H_2SO_4 at initial and after hot storage, respectively. On the other hand, the density and specific gravity values ranged from 1.0679 ± 0.0009 and 1.0699 ± 0.0009 to 1.0787 ± 0.0009 and 1.0819 ± 0.0009 , respectively, but slight changes occurred after hot storage. The formulated C2E recorded the lowest surface tension value, 26.293 ± 0.024 dyne/cm³ at initial time, while all formulations showed a slight decrease in surface tension values after hot storage. The viscosity of formulated C2H gave the highest values, 5.12 ± 0.118 and 5.21 ± 0.119 cP at initial and after storage, respectively, while slight changes were observed after hot storage. The refractive index of all formulations was in the range of 1.4607 ± 0.0461 to 1.4885 ± 0.0461 , with no changes occurring after hot storage. The flash point before and after hot storage for all formulations was $35 \pm 0.12^\circ\text{C}$. Additionally, all prepared formulations were stored in cold storage at 0°C for 7 days, as no separation or sedimentation was observed.

Table 4: physicochemical properties of castor fruits extract 20% EC formulations under different storage conditions.

Formulation names	C2H 20% EC		C2E 20% EC		C2T 20% EC	
Physical Properties	Initial	Hot storage	Initial	Hot storage	Initial	Hot Storage
Acidity as H_2SO_4	$0.284 \pm 0.012^*$	0.234 ± 0.010	0.737 ± 0.031	0.824 ± 0.035	0.788 ± 0.033	0.689 ± 0.029
Density (g/cm ³)	1.0787 ± 0.0009	1.0724 ± 0.0009	1.0677 ± 0.0009	1.0718 ± 0.0009	1.0679 ± 0.0009	1.0709 ± 0.0009
Specific gravity	1.0819 ± 0.0009	1.0756 ± 0.0009	1.0709 ± 0.0009	1.0750 ± 0.0009	1.0699 ± 0.0009	1.0738 ± 0.0009
Surface tension(dyne/cm)	27.215 ± 0.024	26.726 ± 0.024	26.794 ± 0.024	26.293 ± 0.024	26.915 ± 0.024	26.614 ± 0.024
Viscosity(cP)	5.12 ± 0.118	5.21 ± 0.119	4.41 ± 0.118	4.65 ± 0.122	3.58 ± 0.128	3.48 ± 0.123
Refractive index	1.4884 ± 0.0461	1.4882 ± 0.0461	1.4883 ± 0.0461	1.4885 ± 0.0461	1.4607 ± 0.0461	1.4597 ± 0.0461
Flash point($^\circ\text{C}$)	35 ± 0.12	35 ± 0.12	35 ± 0.12	35 ± 0.12	35 ± 0.12	35 ± 0.12

C2H= n-hexane extract C2E= ethyl acetate extract C2T= ethanol extract EC= Emulsifiable Concentrate (cP) = centipoise
Initial = instantly prepared samples at room temperature, Hot = storage at 54°C and* (mean $\pm$ standard error)

3.4. Physical properties of spray solutions of the prepared emulsifiable concentrates (ECs) formulations under different storage conditions

The obtained data in Table 5 illustrate the physical properties of soft and hard water spray solutions of castor fruit extracts 20% EC formulations under different storage conditions. All prepared formulations were passed in emulsion stability and foam tests pre- and post-accelerated hot storage. For the conductivity test, formulated C2T gave the highest values of 879 and 233 μS after hot storage in hard and soft water, respectively. While the surface tension of the formulated C2H recorded the lowest value of 26.559 ± 0.287 dyne/cm in hard water at the initial time, with slight changes occurring after accelerated hot storage in both hard and soft water. The tabulated results also showed that all prepared formulations are acidic. The lowest pH value was 3.91 ± 0.028 for the soft water spray solution of C2E after hot storage, but the highest pH value was 5.89 ± 0.028 for the soft water spray solution of C2H after hot storage.

Table 5: physical properties of spray solutions of castor fruit extract 20% EC formulations under different storage conditions.

Formulation Name	C2H 20% EC				C2E 20% EC				C2T 20% EC			
Physical Properties	Initial		Hot Storage		Initial		Hot Storage		Initial		Hot Storage	
	H.W	S.W	H.W	S.W	H.W	S.W	H.W	S.W	H.W	S.W	H.W	S.W
Foam	-	-	-	-	1	1.5	1	1	1	2	1	1.5
Emulsion stability (ml. cream sep.)	0.1	0.1	No Sep.	0.1	No Sep.	No Sep.	0.1	No Sep.	No Sep.	No Sep.	0.1ml	No Sep.
Conductivity (μ S)	832	169.1	830	170.5	871	210	870	210	874	222	879	233
Surface tension (dyne/cm ³)	26.55 9 $\pm$ 0.2 87	27.638 $\pm$ 0.190	27.818 $\pm$ 0.024	27.898 $\pm$ 0.036	27.406 $\pm$ 0.029	27.352 $\pm$ 0.026	27.347 $\pm$ 0.027	27.368 $\pm$ 0.027	27.540 $\pm$ 0.057	27.699 $\pm$ 0.032	27.647 $\pm$ 0.046	27.694 $\pm$ 0.027
pH	5.10 $\pm$ 0.028	5.40 $\pm$ 0.028	5.51 $\pm$ 0.028	5.89 $\pm$ 0.028	4.02 $\pm$ 0.028	3.94 $\pm$ 0.028	3.99 $\pm$ 0.028	3.91 $\pm$ 0.028	4.35 $\pm$ 0.028	4.28 $\pm$ 0.028	4.42 $\pm$ 0.028	4.38 $\pm$ 0.028

C2H= n-hexane extract C2E= ethyl acetate extract C2T= ethanol extract

3.5. Nematicidal activity of *R. communis* fruit crude extracts and their emulsifiable concentrate (ECs) formulations

3.5.1. Laboratory experiment

The nematicidal toxicity of ethanol, ethyl acetate, and hexane extracts of *R. communis* fruits, as well as their emulsifiable concentrate (EC) formulations, was tested on J2 of the root-knot nematodes under laboratory conditions Table 6. The results indicated that, in general, the EC formulations of the three extracts (n-hexane, ethyl acetate, and ethanol) exhibited greater toxicity action towards nematode larvae than their crude extracts from the same extraction solvent. The EC formulation of hexane extract showed the highest toxicity (LC_{50} = 15.31 mg/L) on J2 of nematodes, followed by the EC of ethanol extract (LC_{50} =26.81 mg/L) and the EC of ethyl acetate extract (LC_{50} =33.94 mg/L) after 48 hours of exposure. In contrast, the crude extracts had the least toxicity on nematodes, with LC_{50} values of 47.41, 87.64, and 249.76 mg/L for ethanol, hexane, and ethyl acetate crude extracts, respectively.

Table 6: Toxicity of three extracts (ethanol, ethyl acetate, and n-hexane) of *Ricinus communis* fruit and their emulsifiable concentration (ECs) against second-stage juvenile (J2) of root-knot nematode after 48 hours of exposure.

Treatments		LC ₅₀ ^a mg/L	95% confidence limits		Slope $\pm$ SE ^b
Extraction solvents	Formulation		Lower	Upper	
Ethanol	Crude	47.41	27.59	76.66	2.01 $\pm$ 0.16
	EC ^c	26.81	23.25	30.66	2.50 $\pm$ 0.23
Ethyl acetate	Crude	249.76	181.12	514.77	1.77 $\pm$ 0.16
	EC	33.94	20.83	50.1	2.29 $\pm$ 0.20
n-Hexane	Crude	87.64	74.69	103.44	1.63 $\pm$ 0.13
	EC	15.31	12.58	18.01	2.39 $\pm$ 0.32

^a the concentration causing 50 % mortality.

^b Slope of the concentration-mortality regression line $\pm$ standard error.

^c EC Emulsifiable concentrate.

3.5.2. Pot experiment

Data presented in Table 7 shows the nematicidal activity of ethanol, ethyl acetate, and hexane extracts of *R. communis* seeds and their emulsifiable concentrate (EC) formulation against *M. incognita* infecting eggplant roots under greenhouse conditions. The results indicated that all treatments significantly ($P \leq 0.05$) reduced nematode infection, as seen in gall number, egg masses on the root and the number of nematodes in the soil compared with the untreated control. Additionally, the EC formulation of the three extracts (hexane, ethyl acetate, and ethanol) showed higher efficiency in nematode criteria than their crude extracts of the same extraction solvent. The treatment with ethanol extract as EC and as crude achieved the highest reduction percent in root-galling (95.97 and 93.95 % respectively), eggmasses (97.37 and 94 % respectively), and nematodes in soil (90 and 76.67 % respectively). In contrast, the crude ethyl acetate extract was the least effective treatment in reducing root galling (41.94%), egg masses (46.05%), and nematodes in the soil (26.67%). The remaining treatments showed intermediate efficiency against the nematode.

Table 7: Effect of *Ricinus communis* fruit extracts (ethanol, ethyl acetate, and hexane) and their Emulsifiable concentrate (ECs) formulation on root-knot disease incidence in eggplant growing in pots.

Treatments		Galls No.** /Root	%Reduction	Egg-masses No. /Root	%Reduction	Juveniles No./250g soil	%Reduction
Ethanol	Crude	5.00e	93.95	1.33c	94.74	23.33c	76.67
	EC*	3.33e	95.97	0.67c	97.37	10.00c	90.00
Ethyl acetate	Crude	48.00b	41.94	13.67b	46.05	73.33b	26.67
	EC	16.33cd	80.24	4.00c	84.21	53.33b	46.67
n-hexane	Crude	25.00c	69.76	7.33bc	71.05	66.67b	33.33
	EC	8.33de	89.92	3.00c	88.16	30.00c	70.00
Untreated treatment		82.67a	0.00	25.33a	0.00	100.00a	0.00
LSD at 5%		10.64		8.36		23.24	

Numbers followed by the same letters within a column do not differ according to Duncan's multiple range test at $P \leq 0.05$.

*EC= Emulsifiable Concentrate

**No.= average number of the three replicates

The impact of treatments on the growth parameters of eggplant infected by *M. incognita* was presented in Table 8. The results showed that all treatments significantly improved eggplant growth criteria, as evidenced by the fresh weight of shoots and roots, as well as the length of both systems, compared to those of the untreated control plants. Remarkably, the EC formulation of hexane extract of *R. communis* was superior in the weight of the shoot and root of eggplants (10.36 and 9.10 g, respectively). Additionally, the other treatments reasonably enhanced plant growth parameters, with no significant difference observed between them.

Table 8: Effect of *Ricinus communis* fruit extracts (ethanol, ethyl acetate, and n-hexane) and their Emulsifiable concentrate (ECs) formulation on the plant growth of eggplant infected with root-knot nematode.

Treatments		Weight (g)		Length(cm)	
		Shoot	Root	shoot	Root
Ethanol	Crude	7.40b	7.43ab	24.33a	21.67a
	EC*	8.21b	6.96b	24.07a	21.33a
Ethyl acetate	Crude	6.84b	7.53ab	23.00ab	21.00a
	EC	8.46b	7.00b	23.67a	20.00a
n-hexane	Crude	7.74b	7.80ab	25.67a	22.66a
	EC	10.63a	9.10a	24.67a	19.66a
Untreated treatment		4.87c	4.93c	19.67b	15.00b
LSD at 5%		1.91	1.70	3.42	2.91

Numbers followed by the same letters within a column do not differ according to Duncan's multiple range test at $P \leq 0.05$.

*EC= Emulsifiable Concentrate

4. Discussion

Qualitative phytochemical analysis tests of n-hexane, ethyl acetate, and ethanolic fruit extracts of *R. communis* revealed the presence of numerous secondary metabolites, which play an essential role in the biological activities of medicinal plants. According to the current results obtained in Table 1, steroids, terpenoids, saponins, alkaloids, flavonoids, tannins, and phenols were present in the ethanolic extract, whereas n-hexane and ethyl acetate extracts showed the presence of steroids, terpenoids, and saponins but the absence of alkaloids, flavonoids, tannins, and phenols. These results are consistent with previous studies by [42-44].

On the other hand, GC-MS spectra of n-hexane and ethanolic fruit extracts of *R. communis* in Tables 2 and 3 revealed different chemical compounds responsible for biological properties. These main constituents in the n-hexane extract include 10E, 12Z-Octadecadienoic acid, Ricinoleic acid, Tetracosane, Stigmasterol, Gamma-Sitosterol, and Lupeol. In contrast the main components in the ethanolic extract are linoleic acid ethyl ester, (E)-9-Octadecanonic acid ethyl ester, 9-Octadecanonic

acid, 12-hydroxy- ethyl ester, [R-(Z)-], Ricinine and Ricinoleic acid. These findings align with those of authors [45-47] who found ricinoleic acid to be the most abundant component of castor seeds. [48] mentioned that fatty acid esters and the crystalline alkaloid ricinine are the main constituents of *R. communis* seeds and fruit extracts. Additionally, [49, 50] reported that triterpenoid lupeol was isolated from the coat of the castor bean. [51, 52] reported the presence of alkaloid ricinine in seeds of *R. communis*. [53] reported that phytosterols, specifically stigmasterol (35.80 %) and γ -sitosterol (44.77 %), were the main compounds of *R. communis*. [54] reported that the most abundant compound in castor seeds was 9, 12-Octadecadienoic acid (Z, Z)-methyl ester.

The current data show the physicochemical properties of the prepared EC formulations and their spray solutions. The viscosity of formulated C2H had the highest values, which is beneficial for the deposition of droplets on leaves, prevents droplet bounce, and improves efficacy [55]. The flash point for all formulations was 35 ± 0.12 . According to WHO specifications, liquid formulations must have a flash point of at least 22.8°C for safety during production, storage, transport, and handling [56]. The refractive index, which indicates purity, was in the range of 1.4607 ± 0.0461 to 1.4885 ± 0.0461 . For all formulations showing optical transparency. Additionally, all prepared formulations passed emulsion stability and foam tests in hard and soft water spray solutions. The volume of any cream layer did not exceed 2 mL, and the foam layers did not exceed 5 mL [32]. Conductivity values ranged from 830 to 879 μS in hard water and 169.1 to 233 μS in soft water. Changes in electrical conductivity may affect droplet electrification. Potentially increasing spray deposition on targets for improved biological efficacy [57].

The pH of the spray solution plays an important role in the stability and effectiveness of pesticides. The obtained results showed that the pH of soft water and hard water spray solutions has an acidic character, our results are constituent with [58, 59], who stated that the safe pH range for spray solutions from 4.5 to 7.0 PH affects the absorption of spray solutions through the cuticle and leaf surfaces as well as the phytotoxicity. Another important physical parameter of pesticide formulations is surface tension. The obtained results showed that the surface tension for all spray solutions ranged from 26.559 ± 0.287 to 27.898 ± 0.036 dyn/cm. Lower surface tension causes a decrease in droplet size as noted by [60], which allows for easier spreading on the plant surface, and increases the ratio between droplet coverage area and the amount of spray liquid required. This, in turn, reduces spray application rates as mentioned by [61] and improves the efficiency of pesticides, as discussed by [62].

According to our results, the tested extracts of *R. communis* fruit, in both crude and EC formulations, displayed nematocidal activity against root-knot nematodes, *M. incognita*. These findings are supported by several reports indicating that species of *R. communis* possess nematocidal activities against plant parasitic nematodes [63-66]. [67] Also observed that the aqueous extract of castor seeds significantly reduced egg hatching and increased larval mortality of *M. incognita*. Similarly, previous studies by [13] demonstrated that the water extract of castor beans had toxic effects on eggs and juveniles of nematodes, with juvenile mortality increasing as extract concentration increased. Additionally, [68] found that castor oil exhibited the most effective nematocidal effects against root-knot nematodes on Kiwifruit.

Our findings also showed that all the tested extracts of *R. communis* fruits remarkably reduced root-galling and other criteria of root-knot nematode, while also increasing plant growth parameters of eggplants compared to untreated plants in the pot experiments. Some previous reports support these findings. For example, [69] found that aqueous extracts of *R. communis* seeds reduced nematode criteria, including the number of galls and egg masses on tomato roots, as well as the number of juveniles in roots and soil, compared to nematocidal and non-treated plants. This resulted in higher increases in the lengths and weights of shoots as well as the numbers and weights of fruits on tomatoes. Similarly, research by [70] revealed that the extract of *R. communis* was a more effective gall suppressant and dry mass promoter for tomatoes than garlic and marigold. In line with this pattern, [71] used an alcoholic extract of the leaf and seed of castor bean against *M. incognita* on cucumber, which led to a reduced number of galls and decreased nematode population in the soil while also promoting the longitudinal growth of cucumbers. It has been reported that the oil cake of *R. communis* seeds can suppress plant parasitic nematodes when mixed into the soil [72].

According to the results of the phytochemical screening test, the tested extracts of *R. communis* contain a variety of secondary metabolites, including steroids, terpenoids, saponins, alkaloids, flavonoids, tannins, and phenols. These compounds may possess nematocidal activity, which is consistent with findings from [73], who observed the nematocidal potential of methanolic extracts of *R. communis* against *M. incognita*. They attributed this action to the presence of terpenoids, steroids, tannin, protein, coumarin, saponins, phenol, carbohydrate, and flavonoids revealed by the phytochemical screening of the tested extract. In another study, [65] discovered that nematode J2 became immobile and died after exposure to an aqueous extract of dried castor-oil plant leaves. They suggested that the nematodes' reaction to the product could be due to the activity of one or more secondary compounds with nematostatic and/or nematocidal properties present in the water extract of castor oil leaves, which may impair the functioning of the vital organs of the nematodes. This nematotoxic potential could be attributed to phenolic compounds [74], alkaloids, and terpenoids, whose nematocidal activity has been indicated by [75]. Additionally, [65] proposed that exposure of nematodes to water extracts from dried leaves of castor oil plants results in a change in the

pigmentation of their cuticles and organs. This change may be explained by the extract diffusing through the cuticle and accumulating in the nematode organs. Indeed, extracts from castor oil plant leaves contain certain oxygenated compounds with lipophilic features that enable them to dissolve cytoplasmic membranes of nematode cells, and their functional groups interfere with the structure of enzyme proteins [76].

The obtained results also demonstrate that the great nematocidal efficacy of n-hexane and ethanolic fruit extracts of *R. communis* may be due to the presence of major constituents containing active substances including 10E, 12Z-Octadecadienoic acid (12.26 %), Ricinoleic acid (25.14 %), Tetracosane (4.05 %), Stigmasterol (3.17 %), Gamma.-Sitosterol (5.78 %) and Lupeol (10.54 %) in the n-hexane extract. In the ethanolic extract, the identified compounds include linoleic acid ethyl ester (1.73 %), (E)-9-Octadecanoic acid ethyl ester (2.78 %), 9-Octadecanoic acid, 12-hydroxy-ethyl ester, [R-(Z)]-(4.43 %), Ricinine (1.25%) and Ricinoleic acid (32.94 %), as analyzed by GC/MS and listed in Table 2 and 3. This finding aligns with [77] who reported that fatty acid esters act as surfactants with activity against insect larvae and nematodes. [78] found that butyric, caprylic, capric, lauric, myristic, palmitic, and oleic acids significantly reduce *M. incognita* multiplication. [79] also reported that fatty acids reduce galls and egg masses, suppress egg hatching, and repel larvae and J2s. Additionally, Ricineloidic, ricinoleic, and 12, 13-epoxy-trans-9-octadecenoic acids were confirmed as nematocidal by [80]. Previous reports have shown 9, 12-Octadecadienoic acid, methyl ester, and Octadecanoic acid, 2, 3-dihydroxy propyl ester possesses nematocidal activity [81, 82]. Hexadecanoic acid possesses various biological activities, including antioxidant, hypocholesterolemic, nematocidal, and pesticide properties [83, 84]. [85] Indicated that ricinine, a toxin compound belonging to a piperidine alkaloid isolated from castor seed coat extract, is present in 1-5 %.

From the results obtained, it was observed that preparing *R. communis* extracts as an EC formulation played a significant role in enhancing their effectiveness against root-knot nematodes. These findings may be attributed to the physicochemical properties of the EC formulation as mentioned above. A few previous studies have focused on the bioactivity of formulated botanicals. For example, [86] discussed in a review article the potential use of plant-based extracts, essential oils, and isolated active compounds as formulations against plant pathogens. Additionally, it was found that the EC formulation of *R. communis* extracts was superior in nematocidal potency compared to their crude extracts. This superiority may be due to the presence of adjuvants and surfactant substances, which enhance the physicochemical properties of this formulation and subsequently improve their biological activity.

5. Conclusion

Based on the results obtained, we can conclude that the extracts from *R. communis* fruits can be utilized as an effective alternative for controlling root-knot nematodes, offering promising green nematocides. The application of *R. communis* extracts significantly reduced root galling and egg masses of *M. incognita* on eggplants by approximately 90 % and enhanced plant growth parameters to varying degrees. Therefore, formulating these extracts as EC is a significant advancement in improving their efficacy against root-knot nematodes.

Conflicts of interest

There are no conflicts to declare.

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