Journal of Plant Protection and Pathology

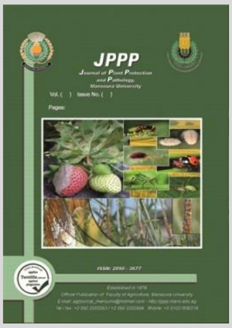
Journal homepage & Available online at: www.jppp.journals.ekb.eg

Eco-Friendly Formulations of Oil in Water Emulsion (EW) from *Melia azedarach* Fruit Extracts and their Nematicidal Activity against Root-Knot Nematode *(Meloidogyne incognita)* on Tomato Plants under Greenhouse Conditions

Cross Mark

Heba H. Fahmy[1]; H. M. Raddy[2], A. F. Aly[1] and Zakia K. Elkhiat[1]*

[1]Pest. Form. Res. Dept., Central Agri. Pest. Lab., Agricultural Research Centre (ARC), Dokki, Giza, Egypt.
[2]Department of plant protection, Faculty of agriculture, Al-Azhar University, Egypt



ABSTRACT

The present study aims to formulate, and evaluate the nematicidal activity of hexane, ethyl acetate, and ethanol extracts of green and ripe fruits of *Melia azedarach* (known as zanzalacht) against *Meloidogyne incognita* second-stage juveniles (J2s) under laboratory and greenhouse conditions on tomato plant. The most nematicidal activity was achieved by the treatment with ethanol extract of ripe fruits (LC_{50}/48h = 206.43 ppm). Chemical components of ripe fruit ethanol extract were identified by GC-MS spectrum to possess fatty acid ethyl ester (28.44%), 5-hydroxyfurfural (13.59%), 2(3H) furanone (6.44%), and vinyl acrylic acid (6.35%) as main components. The tested extracts were formulated as emulsion oil in water (EW), and their physicochemical properties were assessed under different storage conditions. The nematicidal activity of formulated extracts was evaluated and compared with that of Vydate® 24% SL in a greenhouse experiment. Moreover, the results of the pot experiments demonstrated that all treatments significantly reduced root-knot disease and suppressed nematode development when compared with the untreated tomato plant. The obtained results also showed that no significant differences were observed between the treatments of *M.azedarach* extracts and Vydate® 24% SL, where the percentage of reduction in galls/root was 80.00 and 78.18%, for reference nematicide and ripe hexane extract (RH 10%EW), respectively. All treatments increased shoot and root weights and lengths compared with the untreated control. It could be concluded that the potential use of *M. azedarach* extracts is a safe and substantial alternative to conventional nematicides within integrated root-knot nematode management under field conditions.

Keywords: *Melia azedarach* fruits, (GC-MS) Gas chromatography–mass spectrometry analysis, oil in water emulsion (EW), root-knot nematode, *Meloidogyne incognita*, tomato plant.

INTRODUCTION

The root-knot nematode, genus *Meloidogyne*, is one of the most common pests and diseases affecting tomato roots, and other economically important crops, resulting in significant yield losses (Xiao *et al.*, 2016 ;Padilla-Hurtado *et al.*, 2022). Root-knot nematode destroy crops annually more than $100 billion around the world (Ralmi *et al.*, 2016)

Egyptian root-knot nematode (*Meloidogyne* spp.) provide a significant problem for all vegetable crops (Ibrahim, 2011). When these nematodes enter and establish a feeding site in the roots, galls develop, causing the cells surrounding the stylet to enlarge. As a result, the plant is less able to absorb nutrients and water. (Karssen *et al.*, 2013). For this reason, it is necessary to track the population densities of root-knot nematode in cultivated crops (Chitwood, 2002). Crop rotation, organic amendments, nematode-resistant cultivar development, biological control (using antagonistic plants), and the use of chemical nematicides are some of the management techniques used to control root-knot nematode in affected areas (Xie *et al.*, 2016; Jordan, 2018; Win *et al.*, 2020) .

Although synthetic nematicides were the most effective and immediate control tool, they were reported to be hazardous to human health, environmental plants and animals, as well as agriculturally important soil flora and fauna, and they were quite expensive (Guan *et al.*, 2014; Nicolopoulou-Stamati *et al.*, 2016; Navarrete *et al.*, 2018; Alfy *et al.*, 2020;). This, combined with rising demand for organically grown foods, sparked a search for alternative pest management approaches and botanical pesticides as more appealing alternatives to chemical pesticides as well as safer and equally effective to chemically synthesized counterparts (Isman, 2006; Dawar *et al.*, 2008;).

The sustainable agriculture program is largely implemented through the use of environmentally friendly botanical nematicides. Furthermore, rather than traditional pesticide formulations, there is an increasing need for the use of ecologically benign water-based formulations such as oil-in-water emulsions, aqueous suspension concentrates, aqueous capsule suspensions, and so on (Campos *et al.*, 2019)

Oil-in-water (O/W) emulsions can be used as effective pesticide delivery systems in agriculture. This formulation type, according to reports, improves field performance by increasing bioactive component penetration efficacy to the target site, resulting in a lower application rate. It is also less flammable, less toxic, safer to transport and store, and less hazardous. (Mulqueen, 2003; Tadros, 1995, 2018;Ismail *et al.*,

* **Corresponding author.**
E-mail address: zakiaelkhiat@hotmail.com
DOI: 10.21608/jppp.2023.175370.1113

2010; Li *et al.,* 2020;). *Melia azedarach,* also known as Chinaberry, Indian lilac, or white cedar, is a Meliaceae family avenue tree found all over the world (Ascher *et al.,* 2002; Ntalli, *et al.,* 2010 a,b)

M. azedarach extracts have insecticidal (Akhtar *et al.,* 2008; Rachokarn *et al.,* 2008), antifungal (Carpinella *et al.,* 2003) and nematicidal properties (Cavoski et al., 2012; Ntalli *et al.,* 2018, 2020). *M. azedarach* extracts are biologically active due to the presence of alkaloids, tannins, saponins, steriods, diterpenes, and triterpenes (Chitwood, 2002). *M. azedarach* also contains hexadecanoic, acetic, and hexanoic acids, as well as furfural, 5-hydroxymethyl, and furfurol. Furfural is the most effective bionematicidal compound against *M. incognita* (Aoudia *et al.,* 2012).

The purpose of the shared study was to evaluate the nematicidal activity of crude *M. azedarach* fruits with several solvent extracts (hexane, ethyl acetate and ethanol), *in vitro* and *in vivo* against second stage juveniles (J2s) of the root knot nematode, *M. incognita* to develop sustainable and environmentally affable plant protection techniques to decrease yield loss carried on by the root-knot nematode and to enhance tomato plant health. We qualitatively and spectroscopically identify the common presence of specific phytochemical terpenoids, alkaloids, and steroids in elementary extract Preparation an emulsion oil in water (EW) formula from these crudes and then check their stability was also carried out.

MATERIALS AND METHODS

Solvents: Sigma Chemicals Co. provided the following solvents: hexane, ethyl acetate, ethanol, dimethylformamide (DMF), formaldehyde, and xylene were all given by Sigma Chemicals Co. (St. Louis, MO, USA).

Reagents: Merck & Co., Inc. supplied sulphuric acid, hydrochloric acid, sodium hydroxide, ferric chloride, iodine, and potassium iodide as reagents.

Emulsifiers: The following emulsifiers were supplied by Shoura Company for Chemicals: polyoxyethylene sorbitan monooleate (Tween 80), sorbitan fatty acid esters (Span 80), dodecylbenzene sulfonate (60/BE), Alkamuls RC (ethoxylated castor oil), and propylene glycol.

Nematicide use: Du Pont Egypt supplied the commercial nematicide oxamyl (Vydate 24% SL), which is used at the recommended dosage (3 L/fed).

Collection and identification of plants

The ripe and green *M. azedarach* fruits, collected from El-Qalubia between January and August 2020, were recognized by the Egyptian National Botanical Institute, Dokki, Giza, Egypt. The fruits were cleansed with tap water to remove dirt and dust before being dried for 20 days in the shade at 30–35oC. After being ground into small pieces in a specialized laboratory mill, the fruits were stored in a dry location until processing.(Harborne *et al.,* 1975; Hussein and Al-Marzoqi, 2020).

Extraction of *M. azedarach* fruits

The extraction was carried out using the method described by Abegunde and Ayodele-Oduola (2015) with some modifications, in which 100 g of crushed fruits were soaked in various organic solvents for 7 days, including hexane, ethyl acetate, and ethyl alcohol. The crude extract was filtered before being soaked in such solvents through Whatman filter paper (No.1). Plant extracts were concentrated to a constant weight in a rotary evaporator, and the yields were estimated as dry weights. The crude extracts were all stored at room temperature in dark glass vials.

Qualitative phytochemical tests

The green and ripe fruit extracts of *M.azedarach* were tested qualitatively for steroids, alkaloids, phenols, flavonoids, saponins, tannins, and terpenoids as characterised by characteristic colour change, (Harborne, 1998; Wagner and Ulrich-Merzenich (2009); Malar *et al.,* 2020). Each plant extract was examined independently using a specialised chemical reagent and following precise techniques as shown in Table (1).

Table 1. Qualitative phytochemical screening methods

Test	Test name	Observation
Terpenoids	Salkowaski reaction	Reddish-brown coloration
Alkaloids	Wagner's test	Reddish- brown precipitate
Flavonoids	Alkaline reagent test	Yellow color turned to colorless with HCL
Tannins	Braymer's test	Blue greenish color
Steroids	Salkowaski reaction	Red color for upper layer
Saponins	Foam test	Formation of foam

Gas chromatography–mass spectrometry (GC-MS) analysis of the crude extract of *M. azedarach* fruits.

An Agilent GC-MS system gas chromatograph (7890B) equipped with a mass spectrometer detector (5977A) was used to identify chemical constituents of crude plant extract using an HP-5MS column (30 m x 0.25 mm internal diameter and 0.25 m film thickness) and helium as the carrier gas at a flow rate of 1.0 ml/min. Different constituents were identified by comparing the spectrum fragmentation pattern to those stored in Wiley and NIST Mass Spectral Library data.

Formulation of crude plant extracts as emulsion oil-in-water (10%EW).

The phase inversion method was used to create an oil in water emulsion (Zhang 2014; Kumar *et al.,* 2015). Under the high shear effect of heating and stirring with a magnetic stirrer with a hot plate from Terrey Pines Scientific, USA, the oil phase was mixed with the water phase. Oil phase containing 10% crude extract by weight was dissolved in 5-8% immiscible organic solvent and about 10% blend emulsifier with an HLB value of 10–14 was thoroughly blended. Following that, the aqueous phase was gently added to the oil phase at a steady rate of agitation, and the resulting formulation was agitated for 30–40 minutes to produce a crude oil–10% EW formulation.

The physicochemical properties of the developed formulation (10%EW) and its spray solutions under various storage conditions.

Physicochemical properties were used to assess the quality and stability of the created formulations and spray solutions before and after storage. According to CIPAC MT 39.3 (1999) cold storage was used by storing 100 ml of the produced formulation in a sealed glass bottle in a refrigerator at 0 °C for 7 days. Whereas accelerated heat storage was carried out in accordance with CIPAC MT 46.3(1999) by placing the formulation sample (100 ml) in a sealed glass bottle in an oven at 54±2 °C for 14 days. The containers were retrieved from the refrigerator and oven at the end of the storage intervals and allowed to cool at room temperature. Following that, the volume and type of separation were recorded, followed by the physicochemical parameters.

Viscosity: Measured in centipoise (cp.) for formulation and spray solution (ASTM D 2196–15, 2015) using a Brookfield

DV2T Rheometer (Brookfield, USA) and a (TC-555, USA) water bath to manage test temperature.

Surface Tension: The surface tension of the produced formulation and its spray solution was measured by dyne/cm (ASTM D–1331–14,2014) on a "Force Tensiomate Sigma 700 USA" with a whilemy plate as a probe.

Density and Specific Gravity: The density gm/cm^3 and specific gravity were determined using a Rudolph Densitometer DDM-2910, USA, in accordance with (ASTM-D–4052-16,2016) standards.

pH measurement: pH was measured for both the formulation and the spray solution using a Jenway pH metre (3510-UK) and a HANNA pH electrode, as specified CIPAC MT 75.3(1999).

Free acidity or alkalinity: The free acidity or alkalinity of produced formulations was determined potentiometrically using a HANNA 901 automatic titrator, meeting the requirements of the CIPAC MT 191 (2005) test technique.

Emulsion stability: This test was performed visually on a 100 ml cylinder containing a 5% spray solution prepared by mixing 5 ml of the tested formulation with 95 ml of WHO (world health organization) standard hard water after 10 inversions and examined for any amount of free oil or creamy layer (ml) separated on the top or bottom of the emulsion after 30 minutes CIPAC MT 36.3(2003).

Electrical Conductivity: The conductivity of spray solutions was measured by "Thermo Orion model 115A+, USA" and determined by electrical conductivity unit micro-Siemens (µs) (CIPAC MT 32, 1995).

Preparation of nematode inoculums

Root-knot nematode, *M. incognita*, was cultured from naturally infected tomato (*Solanum lycopersicum*) roots gathered from the fields. Individual egg masses were extracted from root tissues and placed in a small glass capsule containing fresh water. The female was kept in a 4% formaldehyde solution for species identification using the criteria stated by Hartman and Sasser (1985).

A pure stock culture of *M. incognita* was created by planting tomato seedlings in a 25 cm diameter clay pot filled with previously steam sterilised sandy loam soil and injected separately with egg mass. Inoculated pots were kept in greenhouses and irrigated on a regular basis. The inoculum was grown on seedlings of eggplant *(Solanum melongena)*.The experiment was based on infected eggplant roots.

To obtain second stage juveniles (J2s) for the studies, mature egg masses were allowed to hatch using modified Baermann technique and the hatched juveniles were collected daily(Whitehead and Hemming ,1965).

Nematicidal activity of the prepared crude plant extracts as oil in water emulsion (EW) formulation

Laboratory experiment

The laboratory experiment was carried out to estimate the half lethal concentration (LC50) value of tested extracts against newly hatched J2s after 48 hours. As previously stated, six extract preparations (ethanol, ethyl acetate, and hexane extracts of *M. azedarach* (green and ripe fruits) were examined as 10% emulsion oil in water (EW) formulations. Each of them was diluted with distilled water to meet test concentrations ranging from 50 to 1000 ppm (six concentrations at least). A suspension of recently hatched J2s (100 juveniles) was placed in Petri dish (9cm diameter) containing 1ml of each concentration of the tested extracts. Three replicates were used for each concentration, and the control treatment was distilled water. After 48 hours, the mortality rate was estimated using the Abbott's formula (Abbott, 1925). Nematodes were considered dead if they did not move when probed with a fine needle (Abbasi *et al.,* 2008). The collected data were expressed as toxicity lines, and the LC50 values were calculated using probit analytic software (Finney, 1952).

Greenhouse experiment

The pot experiment was carried out in a greenhouse at Department of Plant Protection, Faculty of Agriculture, Al-Azhar University. Tomato seedlings (cv. 86) were transplanted at four weeks old into clay pots (15cm in diameter) filled with 1 kg of steam sterilised soil (1:1) (sand: clay). After one week, the plants were infected with nematode J2s (Raddy *et al.*, 2018) where, 10ml of nematode suspension containing roughly 1000 freshly hatched J2s were pipetted into each pot by gently pouring the J2s suspension into holes surrounding the root system. The holes were plugged by pressing the pot soil and watering. Then, the plants were treated with the tested six preparations of extracts (ethanol, ethyl acetate, and hexane extracts of *M. azedarach* (green and ripe fruits) at a concentration equal to approximately 10 times the LC50 value, which was determined in the laboratory experiments in the current study Table(7). Also, oxamyl treatment was applied based on its field recommended rate as a soil drench and the non-treated control received water only. The pots were arranged in a randomized block design, consisting of eight treatments and four replications. After 45 days from nematode inoculation, plants were gently removed from pots. All nematode parameters (number of galls and egg masses per root system, the number of 2nd juveniles per pot, and the average number of eggs/egg mass were counted (Hussey, 1973). Plant growth criteria were also estimated.

The nematode reproduction factor (RF) was calculated according to the formula:

$$\mathbf{RF = (Pf/Pi)}$$

Pi = nematode initial inoculum, Pf = nematode final population.

Statistical analysis: Data were subjected to the analysis of variance test (ANOVA) and a significant difference among the treatments was portioned by Duncan's multiple range test by using a software computer program (Costat, 1998) at probability levels of P = 0.05.

RESULTS AND DISCUSSION

Extraction yield and qualitative phytochemical analysis

Table (2) shows the extraction yield of green and ripe *M. azedarach* fruits using three different solvents. Accordingly ethanol extract has the highest extraction yield of both ripe (REth) and green (GEth) fruits. The lowest yield, on the other hand, was observed for n-hexane extract (green fruit, GH), whereas the other extracts were in the order of RH, GE, and RE. Also, this table shows the phytochemical screening of *M. azedarach* green and ripe fruit solvent extracts. With the exception of ethanolic ripe extract (REth), all extracts were negative for flavonoids, and tannins. All fruit solvent extracts, on the other hand, showed positive terpenoids and steroids, with the exception of ethanolic green extract (GEth), which tested negative terpenoids. Finally, all of the extracts tested showed the presence of alkaloids among the extract components, with the exception of ethyl acetate green fruit extract GE, which showed a negative alkaloid test.

Table 2. Extraction yield and qualitative analysis of phytochemicals contained in different solvent extracts of *M. azedarach* ripe and green fruits.

Phytochemical compounds	Ripe fruit extract			Green fruit extract		
	n-Hexane (RH)	Ethylacetate (RE)	Ethanol (REth)	n-Hexane (GH)	Ethyl acetate (GE)	Ethanol (GEth)
Terpenoids	+	+	+	+	+	-
Alkaloids	+	+	+	+	-	+
Flavonoids	-	-	-	-	-	-
Tannins	-	-	-	-	-	-
Steroids	+	+	+	+	+	+
Saponins	-	-	+	-	-	+
% yield of extract	6.37	2.82	18.4	1.6	5.28	14.52

+ = present − = absent RH = ripe fruit hexane extract, RE = ripe fruit ethyl acetate extract, REth = ripe fruit ethanol extract, GH = green fruit hexane extract, GE = green fruit ethyl acetate extract, and GEth = green fruit ethanol extract.

Chemical compositions of *M. azedarach* ripe and green fruits extracts:

Chemical ingredients using GC-MS spectrum analysis of REth crude extract of *M. azedarach* ripe fruit is shown in Table (3). The main components of REth were fatty acid esters such as hexanoic and hexadecanoic acid ethyl esters, 6-methyl-2-heptanol, 1,3-butadiene-1-carboxylic acid, also known as vinyl acrylic acid, 5-hydroxymethyl furfural, and 2(3H) furanone, according to the chromatograms and qualitative phytochemical analysis. The molecular formula, retention time and % area are shown in Table (3).

Table 3. The main chemical components of ethanol ripe fruits extract of *Melia azedarach*

Compound name	Mol. Formula /Mol. Weight	Retention time (RT). Min.	% area
Hexanoic acid ethyl esters	$C_8H_{16}O_2$ / 144.214	7.457	8.26
Hexadecanoic acid ethyl esters	$C_{18}H_{36}O_2$ / 300.5	18.963	4.07
6-methyl-2-heptanol	$C_8H_{18}O$ / 130.228	13.351	20.18
Vinyl acrylic acid	$C_5H_6O_2$ / 72.06	13.740 – 14.221	6.35
5-hydroxymethyl furfural	$C_6H_6O_3$ / 126.11	10.387 – 10.639	13.59
2(3H) furanone	C_4H4O_2 / 84.07	13.574 – 13.683	6.44

Physicochemical properties of prepared green fruit crude extracts of *M.azedarach* (10% oil in water emulsion (EW) formulation)

The physicochemical parameters of green fruit crude extract. 10% emulsion in water (EW) after different storage conditions are shown in Table (4). According to the results presented, the hot storage sample of the GE 10% EW formulation had the maximum viscosity value (84.25 cP), whereas the cold storage sample of the GEth 10% EW formulation had the lowest (26.95 cP). The surface tension of the investigated formulations revealed that hot storage samples had the maximum surface tension as compared to the initial sample, exhibiting values of 35.278, 29.474 dyne/cm and 35.339, 30.950 dyne/cm for GE and GEth 10% EW, respectively.

Data also demonstrated that formulation density has not changed much since it was recorded 0.9960-1.0030 g/cm3; 1.0064-1.0119 g/cm3, and 1.0015-1.0057 g/cm3 for GH, GE, and GEth 10% EW formulations. The pH results revealed that all generated formulations are acidic in nature, and the recorded pH values were slightly elevated by storage condition, ranging from 5.19 – 4.66, 4.14–3.99, and 5.18–4.96 for GH, GE, and GEth 10% EW samples, respectively. Finally, in cold and hot storage, acidity increases in GH and GEth by-0.485-0.490 and 0.488-0.451, respectively. pH data is additionally corroborated by acidity records, which show how strong the hydrogen ion concentration is in comparison to concentrated H_2SO_4.

Table 4. Physicochemical properties of green fruit crude extracts of *Melia azedarach* 10% EW formulations.

Physical properties	GH 10% EW			GE 10% EW			GEth 10% EW		
	Initial	Cold	Hot	Initial	Cold	Hot	Initial	Cold	Hot
Viscosity (cP)	47.49	36.54	52.54	28.03	44.85	84.25	26.99	26.95	53.74
Surface tension (dyne/cm)	29.65	29.82	30.79	29.47	31.96	35.27	30.95	30.28	35.33
Density (g/cm3)	0.9960	1.0000	1.0030	1.0064	1.0089	1.0119	1.0015	1.0056	1.0057
pH	5.19	5.01	4.66	4.14	4.12	3.99	5.18	5.14	4.96
Acidity	0.108	0.485	0.490	1.201	0.961	1.390	0.194	0.488	0.451

Initial = pre-storage samples at zero time, cold = storage sample at 0◦C, and hot = storage sample at 54 ◦C GH= n-hexane extract of green fruit of *M. azerdarach*, GE= ethyl acetate extract of green fruit of *M. azerdarach* and GEth= ethanol extract of green fruit of *M. azerdarach*.

Physicochemical properties of prepared ripe fruit crude extracts of *M. azedarach* (10% oil in water emulsion (EW) formulation):

Table (5) shows the physicochemical parameters of the ripe fruit crude extract, 10% emulsion oil in water (EW) formulation. The viscosity parameter revealed a minor difference between recorded viscosity at starting and storage temperatures for all ripe extracted formulations. Surface tension was measured to be 29.617, 31.946, and 36.540 dyne/cm for hot storage samples, while it was 29.286, 30.808, and 31.171 dyne/cm for RH, RE, and REth 10% EW, respectively. It is also obvious from this table that there is dispersion in the surface tension of cold storage samples when compared to initial sample recording 28.854, 30.397, and 29.129 dyne/cm for the prior formulation sequence.

The density of the produced oil in water emulsion was similarly altered by storage temperature, but only in a limited range. The table clearly shows that the hot storage sample has the maximum density 0.9875, 1.0049, and 1.0081 g/cm3. Cold storage samples also showed an increase in the density of stored formulation amounted to 0.9855, 1.0048, and 1.0073 g/cm3, respectively, for RH, RE, and REth 10% EW. The pH of the developed formulation revealed that all formulations had acidic properties. The RE 10% EW formulation produced strongly acidic samples, with pH values of 4.1, 4.02, and 4.07 for initial, cold, and hot storage samples, respectively. In addition, cold storage samples had the greatest acidic percentage compared to conc. H_2SO_4 1.491, 1.518, and 1.807% for the preceding sample order. The lowest acidic pH values for RH10% EW formulation were 6.52, 6.53, and 6.38 and their acidity percentage were 0.012, 0.139, and 0.292 as H_2SO_4, whereas the pH values for REth 10% EW were 4.46, 4.28, and 4.40 and their acidity percentage were 0.240, 0.527, and 0.532 for initial, cold, and hot storage samples, respectively.

Table 5. Physicochemical properties of ripe fruit crude extracts of *Melia azedarach* 10% EW formulations.

Physical properties	RH 10% EW			RE 10% EW			REth 10% EW		
	Initial	Cold	Hot	Initial	Cold	Hot	Initial	Cold	Hot
Viscosity (cP)	3.07	3.76	3.96	18.75	18.52	19.29	43.51	47.74	48.2
Surface tension (dyne/cm)	29.28	28.854	29.617	30.80	30.39	31.94	31.17	29.13	36.54
Density (g/cm3)	0.9789	0.9855	0.9875	0.9939	1.0048	1.0049	0.9957	1.0073	1.0081
pH	6.52	6.53	6.38	4.10	4.02	4.07	4.46	4.28	4.40
Acidity	0.012	0.139	0.292	1.491	1.518	1.807	0.240	0.527	0.532

Initial = pre-storage samples at zero time, cold = storage sample at 0◦C, and hot = storage sample at 54 ◦C RH= n-hexane extract of ripe fruit of *M. azerdarach*, RE= ethyl acetate extract of ripe fruit of *M. azerdarach* and REth= ethanol extract of ripe fruit of *M. azerdarach*.

Physicochemical properties of spray solution prepared from 10% oil in water emulsion (EW) formulation of fruit crude extracts

The physicochemical properties of soft and hard water spray solutions for the manufactured fruit crude extract, 10% EW formulation utilising a 5% rate of dilution are shown in Table (6).

Table 6. Physicochemical properties of hard and soft water spray solutions for crude extracts of *Melia azedarach* 10% EW formulation.

EW formulation	Emulsion stability (ml)						Viscosity (cP)						Surface tension (dyne/cm)						Conductivity (µs)						pH					
	Initial		Cold		Hot		Initial		Cold		Hot		Initial		Cold		Hot		Initial		Cold		Hot		Initial		Cold		Hot	
	SW[a]	HW[b]	SW	HW	SW	HW	SW	HW	SW	HW	SW	HW	SW	HW	SW	HW	SW	HW	SW	HW	SW	HW	SW	HW	SW	HW	SW	HW	SW	HW
RH	0.5	0.6	0.3	0.3	0.7	0.8	1.61	1.29	1.38	1.20	1.34	1.26	30.37	30.08	30.32	30.15	30.29	30.18	128.1	544	115.3	539	121.8	550	6.71	6.23	6.22	5.88	5.15	4.90
RE	----	---	----	---	---	----	1.48	1.25	1.49	1.27	1.44	1.37	31.64	31.29	31.92	31.87	32.56	31.58	276	654	269	648	275	630	3.85	3.907	3.933	3.91	4.033	4.063
REth	----	---	----	---	---	----	1.17	0.81	1.47	1.30	2.09	2.07	31.84	31.32	31.66	31.05	31.06	31.03	215	601	247	615	228	586	4.267	4.20	4.217	4.223	4.21	4.19
GH	----	---	----	---	---	----	1.57	1.56	1.88	1.84	1.49	1.46	31.14	30.98	31.18	30.91	31.43	31.26	141.1	544	138.5	544	150.9	560	4.76	4.65	4.67	4.67	4.42	4.50
GE	----	---	----	---	---	----	1.33	1.30	1.57	1.45	1.67	1.60	30.77	30.68	31.20	30.84	31.79	31.16	250	618	249	569	220	541	3.91	3.87	3.96	3.96	4.15	4.19
GEth	----	---	----	---	---	----	1.37	1.35	1.68	1.67	1.72	1.66	31.91	30.47	31.62	31.59	31.74	31.32	373	680	390	715	319	649	5.28	5.17	5.21	5.23	5.22	5.20

[a]SW: soft water [b] HW: hard water RH = ripe fruit hexane extract RE = ripe fruit ethyl acetate extract REth = ripe fruit ethanol extract GH = green fruit hexane extract GE = green fruit ethyl acetate extract, and GEth = green fruit ethanol extract.

The physical and chemical properties of all evaluated formulations were satisfactorily tested for emulsion stability. The volume of the cream layer did not surpass 2 mL, and no cream or oil separation or sedimentation was detected after 30 minutes. For all tested 5% spray solutions, the tabulated data demonstrated that the viscosity of soft water spray solution is greater than that of hard water spray solution. Despite the foregoing, the maximum and lowest viscosity values reported for soft and hard water spray solutions of REth 10% EW for hot and initial storage samples were 2.09 and 0.81cP respectively. Similarly, surface tension declared that for all prepared formulations along the same lines, soft water spray solutions have higher surface tension values than hard water spray solutions. The soft water spray solution of a hot storage sample of RE 10% EW formulation had the maximum surface tension 32.567 dyne/cm, whereas the hard water spray solution of the RH 10% EW starting sample had the lowest surface tension 30.08 dyne/cm. The electrical conductivity of hard water spray solution was always higher than that of soft water, as hard water includes more Ca^{2+} and Mg^{2+} ions. The electrical conductivity of hard water spray solution was measured to be 539.0–715.0 s, while the soft water solution was measured to be 115.3–390.0s.On the other hand, all of the pH values reported for the prepared spray solution were acidic. Additionally, the greatest pH value recorded for the soft water spray solution of the initial RH 10% EW sample was 6.71 and the lowest value recorded for RE 10% EW was 3.85. Furthermore, storage conditions clearly had a moderate effect on the pH of the formulation hard and soft spray solutions.

Nematicidal activity of *M.azedarach* ripe and green fruit crude extracts against J2 of *M. incognita*

Laboratory experiment.

The laboratory experiment was carried out to assess the LC_{50} values of the studied extracts (hexane, ethyl acetate, and ethanol) of *M. azedarach* fruits (green and ripe) against J2s of *M. incognita* after 48 hours. The results shown in Table (7) and Fig. (1) revealed that ethanol and hexane extracts of ripe fruits had the highest nematicidal activity with LC_{50} values of 206.43 and 254.75 ppm, respectively, followed by GH, GE, and GEth with LC_{50} values of 341.196, 469.226, and 483.009, respectively, while RE had the least nematicidal activity LC_{50} = 805.811 ppm.

Table 7. LC_{50}, and slope values of *Melia azedarach* extracts against J2s of root-knot nematode, *Meloidogyne incognita* after 48 hours under laboratory conditions.

Extracts	LC_{50}	Slope	Index
RH	254.755	1.769	81.032
RE	805.811	2.027	25.618
REth	206.432	2.806	100.000
GH	341.196	3.353	60.502
GE	469.226	2.637	43.994
GEth	483.009	2.449	42.739

RH = ripe fruit hexane extract, RE = ripe fruit ethyl acetate extract, REth = ripe fruit ethanol extract, GH = green fruit hexane extract, GE = green fruit ethyl acetate extract, and GEth = green fruit ethanol extract.

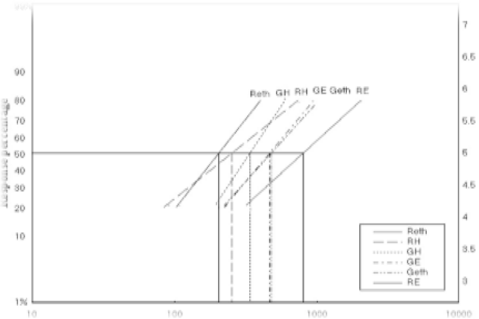


Fig. 1. Toxicity regression lines of *Melia azedarach* green and ripe fruits extracts on J2s of root-knot nematode, *Meloidogyne incognita* under laboratory conditions.

RH = ripe fruit hexane extract, RE = ripe fruit ethyl acetate extract, REth = ripe fruit ethanol extract, GH = green fruit hexane extract, GE = green fruit ethyl acetate extract, and GEth = green fruit ethanol extract.

Ripe fruit extracts demonstrated higher nematicidal activity than green fruit ones extracted by the same solvent, with the exception of ethyl acetate extracts.

Greenhouse experiment

Table (8) and Fig. (2) illustrate the nematicidal activity of *M. azedarach* ripe and green fruit extracts in comparison to Oxamyl 24% SL as a reference registered nematicide against *M. incognita* infecting tomato roots under greenhouse conditions.

Table 8. Effect of *Melia azedarach* formulations and oxamyl on the development and reproduction of root-knot nematode *Meloidogyne incognita* infecting tomato plants under greenhouse conditions.

Treatments	No. of galls	No. of egg masses	No. of juveniles in soil (J2s)	No. of developmental stages	No. of eggs/ egg masses	RF
GEth	5.00^{b}	9.33^{bc}	101.67^{d}	6.00^{bc}	79.33^{cd}	0.86
GE	6.33^{b}	9.33^{bc}	122.67^{b}	6.00^{bc}	80.00^{cd}	0.88
GH	7.33^{b}	13.00^{b}	112.00^{c}	9.33^{b}	101.00^{bc}	1.45
REth	5.67^{b}	9.00^{bc}	100.67^{d}	5.33^{bc}	92.67^{bcd}	0.95
RE	6.33^{b}	11.00^{bc}	106.67^{cd}	9.67^{b}	105.00^{b}	1.28
RH	4.00^{b}	6.33^{c}	69.67^{f}	4.00^{c}	69.33^{d}	0.52
Oxamyl	3.67^{b}	7.33^{bc}	84.33^{e}	2.67^{c}	83.33^{bcd}	0.71
Control	18.33^{a}	41.33^{a}	155.67^{a}	23.00^{a}	163.33^{a}	6.97
LSD at 5%	3.51	5.22	9.16	4.79	21.95	

*** Differences between means in each column followed by the same small letter (s) are not significant at P=0.05 according to Duncan's multiple range tests.RH = ripe fruit hexane extract, RE = ripe fruit ethyl acetate extract, REth = ripe fruit ethanol extract, GH = green fruit hexane extract, GE = green fruit ethyl acetate extract, and GEth = green fruit ethanol extract.**

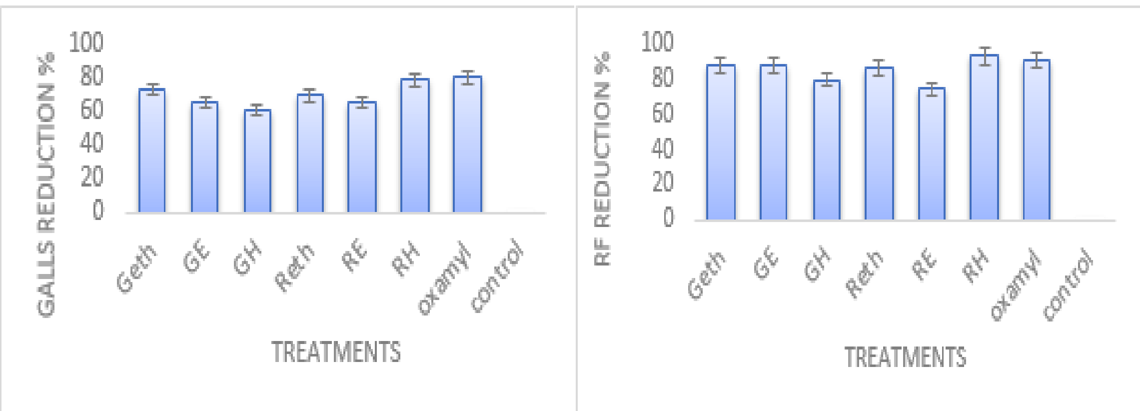


Fig. 2. The reduction percentage of root galling and reproduction factor of root-knot nematode, *Meloidogyne incognita* as affected by *Melia azedarach* formulations and oxamyl.

RH = ripe fruit hexane extract, RE = ripe fruit ethyl acetate extract, REth = ripe fruit ethanol extract, GH = green fruit hexane extract, GE = green fruit ethyl acetate extract, and GEth = green fruit ethanol extract.

All treatments considerably reduced the enumerated variable parameters, namely the number of galls, J2s in soil, developmental stages within the root, egg masses, eggs/single egg mass, and lastly, the nematode reproduction rate, compared to the untreated control. In general, no significant difference in the number of root galls was detected between the treatments with *M. azedarach* extract (oil in water formulation) and the oxamyl treatment. Compared to the untreated control, oxamyl and RH treatments reduced the number of galls/root by 80 and 78.18%, respectively and the nematode reproduction factor by 89.88 and 92.55%, respectively (Fig 2).

Table (9) shows the effect of treatments on the growth of tomato plants infected with *M. incognita.* The findings indicate that all treatments greatly increased tomato growth criteria as evaluated by the fresh weight of shoots and roots as well as the length of both systems compared to untreated control plants. Surprisingly, the REth and GH treatments increased the shoot weight131.73 and 105.15%, respectively and root weight 206.21 and 237.71%, respectively of tomato plants. Furthermore, the other treatments significantly improved plant growth parameters.

Table 9. Effect of tested formulations and oxamyl on growth of tomato plants infected with root-knot nematode,*Meloidogyne incognita.*

Treatments	Shoot length	% Increase	Root length	% Increase	Shoot weight	% Increase	Root weight	% Increase
GEth	34.67^{cd}	15.56	18.00^{bc}	25.58	6.15^{b}	53.24	2.35^{b}	68.26
GE	34.67^{cd}	15.56	23.67^{a}	65.12	5.79^{bc}	44.19	2.65^{b}	89.74
GH	45.00^{ab}	50.00	21.00^{ab}	46.51	8.23^{a}	105.15	4.72^{a}	237.71
REth	51.67^{a}	72.22	21.00^{ab}	46.51	9.30^{a}	131.73	4.28^{a}	206.21
RE	36.67^{bcd}	22.22	19.00^{abc}	32.56	5.87^{bc}	46.26	2.58^{b}	84.49
RH	41.67^{bc}	38.89	20.67^{ab}	44.19	7.44ab	85.47	3.02^{b}	116.23
Oxamyl	35.67^{bcd}	18.89	22.67^{ab}	58.14	6.11^{b}	52.33	2.62^{b}	87.59
Control	30.00^{d}	0.00	14.33^{c}	0.00	4.01^{c}	0.00	1.40^{c}	0.00
LSD at 5%	8.7		4.95		1.84		0.84	

*** Differences between means in each column followed by the same small letter (s) are not significant at P<0.05 according to Duncan's multiple range tests.RH = ripe fruit hexane extract, RE = ripe fruit ethyl acetate extract, REth = ripe fruit ethanol extract, GH = green fruit hexane extract, GE = green fruit ethyl acetate extract, and GEth = green fruit ethanol extract.**

The current study demonstrated a solvent extraction series for *M. azedarach* plant (green and ripe fruits). It also showed nematicidal activity of these crude extracts and their oil in water emulsion formulations against the root-knot nematode, *M. incognita*, infecting tomato plants in the laboratory and greenhouse. At the extract formulation levels, *M. azedarach* plant solvent extracts of green and ripe fruit revealed good and similar nematicidal potency. According to CIPAC MT 36.3 (2003) the developed oil in water emulsion (EW) formulation demonstrated good emulsion stability tests, with creamy separation not exceeding 1 mL. This means that the tested formulations could be used in the field without any separation or foam hazards. The acquired results are also consistent with the findings of researchers El-Sisi *et al.*, (2009); Elkhiat *et al.,* (2016), (2022). They claimed that if the physicochemical qualities, such as emulsion stability, do not exceed 5 ml, the tested formulation is regarded to have passed satisfactorily.

The viscosity records revealed an equivocal behaviour of the studied formulations, with hot storage samples recording higher values than the initial ones. This can be explained in terms of surfactant autoxidation and crystallisation caused by temperature effects (Kerwin, 2008; Kishore *et al.,* 2011). The fluctuation in viscosity results may be caused by autoxidation or crystallisation of polysorbate surfactant under different storage conditions (Kerwin, 2008; Kishore *et al.,*2011), or by surfactant self-assembly into aggregates of various shapes and sizes above their critical micelle concentration (CMC), which leads to changes in the intermolecular interaction between surfactant and water molecules. As a result, spherical micelles become rod like or even flexible, affecting both density and viscosity of the formulation (Gautam *et al.,* 2014; Sikorska *et al.,* 2016; Kroll *et al.,* 2022).

The presence of Ca^{2+} and Mg^{2+} ions, on the other hand, changes the composition of the electric double layer formed at the solution surface, resulting in a decrease in electrostatic force between particles and curling of molecular chains, resulting in a reduction in molecular size and a decrease in both the surface tension and viscosity of hard water spray solution. The degree of compressing increases as the electrolyte content increases and hence the viscosity decreases (Giribabu *et al.,* 2007; Fainerman *et al.,* 2012;; Zhenhua *et al.,* 2020).Furthermore, storage temperature has been proven to have a significant impact on surfactant molecule orientation, which in turn influences agrochemical surface tension (Zdziennicka *et al.,* 2017).

Phytochemical analysis indicated that *M. azedarach* fruits are rich in secondary metabolism compounds such as alkaloids, nicotine, saponins, terpenoids, and flavonoids (Mahdhi *et al.,* 2020). Alkaloids, which kill 90 to 100% of *M. incognita*, are one of the most promising and well-known classes of plant metabolites with pest control capabilities and a high rate of nematicidal activity (Asif *et al.,* 2017; Almadiy *et al.,* 2018).

Early research revealed that numerous plants, including *M. azedarach* and its derived phytochemicals, had nematicidal potential against a wide variety of phytoparasitic nematodes, including root-knot nematode, *M. incognita.*The results demonstrated that *M. azedarach* fruit extracts had good potential nematicidal potency in both *in vivo* and *in vitro* bioassays. *M. azedarach* ethanol extract exhibits highly effective nematicidal action against root-knot nematode, *M.incognita* which may be attributed to its ovicidal and larvicidal activities, according to Kepenekci *et al.,* (2016). Similarly, Ntalli, *et al.,* (2010a,2018) demonstrated the nematicidal activity of powder and ripe fruit methanol, and water extracts in comparison to furfural and oxamyl as reference nematicide.

According to our findings, using *M. azedarach* extracts as a soil drench against *M. incognita* on tomato plants improved plant growth metrics. This study largely confirmed previous observations that *M. azedarach* ripe fruit water extract increased plant growth in the *M. incognita* pot experiment, possibly by activating plant defence systems (Cavoski *et al.,* 2012; Ntalli *et al.,* 2018)

The inclusion of carboxylic acids and aldehydes, p-coumaric acid and p-hydroxybenzoic acid derivatives, furfural and 5-hydroxymethylfurfural, may explain the nematicidal action displayed by the extracts and their formulation (Ntalli, *et al.,* 2010 b; Aoudia *et al.,* 2012; Ntalli *et al.,* 2020)

Plant extract emulsion formulation, particularly oil in water emulsion (EW), may be crucial in ensuring either active component release and increased product stability or enhanced biological properties (Pavoni *et al.,* 2019). Numerous studies have demonstrated that formulations improve the efficacy, stability, and even bioavailability of botanical extracts(Tadros *et al.,* 2004 ; Liang *et al.,* 2012; Ali *et al.,* 2017; Tadros *et al.,* 2018 , Pavoni *et al.,* 2019).

CONCLUSION

Future studies should focus on the findings from the current investigation regarding the effectiveness and usefulness of fruit extracts of *M. azedarach* on root-knot nematode, identifying the chemical components and formulation development to be easy and applicable. In order to find new, less toxic and eco-friendly pest control agents, natural products are considered alternative options to potentially active compounds. Otherwise, preparing these botanicals as a formulation is an important step toward the development of potential plant-based materials into commercial products, and this approach may increase the number of eco-friendly options for root-knot nematode management.

We provide the recommendations based on the results obtained from the potential of ripe and green fruits extracts of *Melia azedarach* plant to control the root-knot nematode *M. incognita*. Finally, we can advise that future research should focus on the leaf extract of *M. azedarach* as a cost-effective and environmentally sustainable alternative to synthetic pesticides.

REFERENCES

Abbasi, M. W., Ahmed, N., Zaki, M. J., and Shaukat, S. S. (2008). Effect of *Barleria acanthoides* Vahl. on root-knot nematode infection and growth of infected okra and brinjal plants. Pakistan Journal of Botany, 40(5),2193–2198.

Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. J. Econ. Entomol, 18(2),265–267.

Abegunde, S. M., and Ayodele-Oduola, R.(2015). Comparison of efficiency of different solvents used for the extraction of phytochemicals from the leaf, seed and stem bark of *Calotropis Procera*. International Journal of Science and Research, 4(7), 835–838.

Akhtar, Y., Yeoung, Y.-R., and Isman, M. B. (2008). Comparative bioactivity of selected extracts from Meliaceae and some commercial botanical insecticides against two noctuid caterpillars, *Trichoplusia ni* and *Pseudaletia unipuncta*. Phytochemistry Reviews, *7*(1), 77–88.

Alfy, H., Ghareeb, R. Y., Soltan, E., and Farag, D. A. (2020). Impact of chitosan nanoparticles as insecticide and nematicide against *Spodoptera littoralis*, *Locusta migratoria*, and *Meloidogyne incognita*. Plant Cell Biotechnology Molecular Biology, 21, 126–140.

Ali, E. O. M., Shakil, N. A., Rana, V. S., Sarkar, D. J., Majumder, S., Kaushik, P., Singh, B. B., and Kumar, J. (2017). Antifungal activity of nano emulsions of neem and citronella oils against phytopathogenic fungi, *Rhizoctonia solani and Sclerotium rolfsii.* Industrial Crops and Products, 108, 379–387.

Almadiy, A. A., Nenaah, G. E., and Shawer, D. M. (2018). Facile synthesis of silver nanoparticles using harmala alkaloids and their insecticidal and growth inhibitory activities against the khapra beetle. Journal of Pest Science, 91(2), 727–737.

Aoudia, H., Ntalli, N., Aissani, N., Yahiaoui-Zaidi, R., and Caboni, P. (2012). Nematotoxic phenolic compounds from *Melia azedarach* against *Meloidogyne incognita*. Journal of Agricultural and Food Chemistry, *60*(47), 11675–11680.

Ascher, K. R. S., Schmutterer, H., Mazor, M., Zebitz, C. P. W., and Naqvi, S. N. H. (2002). The Persian lilac or Chinaberry tree: L. In The neem tree A. Juss. and other meliaceous plants: sources of unique natural products for integrated pest management, medicine, industry and other purpose*s* . Neem foundation, 770–820.

Asif, M., Tariq, M., Khan, A., and Siddiqui, M. A. (2017). Biocidal and antinemic properties of aqueous extracts of *Ageratum* and *Coccinia* against root-knot nematode, *Meloidogyne incognita in vitro*. The Journal of Agricultural Sciences ,12(2),108-122.

ASTM (American Society of Testing and Materials (2016). Standard Test Methods for Density and Specific Gravity by Rodulph Densitometer, D–4052, 1-9.

ASTM (American Society of Testing and Materials) (2014). Standard Test Methods for Surface and Interfacial Tension of Solution of Surface–Active Agents, D–1331–14, 1-5.

ASTM (American Society of Testing and Materials) (2015). Standard Test Methods for Rheological Properties of Non–Newtonian Materials by Rotational (Brookfield Viscometer DV+II pro.), D 2196–15, 1-5.

Campos, E. V. R., Proença, P. L. F., Oliveira, J. L., Bakshi, M., Abhilash, P. C., and Fraceto, L. F. (2019). Use of botanical insecticides for sustainable agriculture: Future perspectives. Ecological Indicators, 105, 483–495.

Carpinella, M. C., Defago, M. T., Valladares, G., and Palacios, S. M. (2003). Antifeedant and insecticide properties of a limonoid from *Melia azedarach* (Meliaceae) with potential use for pest management. Journal of Agricultural and Food Chemistry, 51(2), 369–374.

Cavoski, I., Chami, Z. Al, Bouzebboudja, F., Sasanelli, N., Simeone, V., Mondelli, D., Miano, T., Sarais, G., Ntalli, N. G., and Caboni, P. (2012). *Melia azedarach* controls *Meloidogyne incognita* and triggers plant defense mechanisms on cucumber. Crop Protection, *35*, 85–90.

Chitwood, D. J. (2002). Phytochemical based strategies for nematode control. Annual Review of Phytopathology, 40 (1), 221–249.

CIPAC Handbook (Collaborative International Pesticides Analytical Council Limited) (2005) Volume L, MT 191,143-144.

CIPAC Handbook (Collaborative International Pesticides Analytical Council Limited) (1995). Volume F, MT 32, 103-104.

CIPAC Handbook (Collaborative International Pesticides Analytical Council Limited), (2003) Volume K, MT 36.3, 137-139.

CIPAC Handbook (Collaborative International Pesticides Analytical Council Limited) (1999). Volume J, MT 39.3,46.3 , 75.3 , 126-127, 128-130, 152 -153.

Costat. (1998). Microcomputer program analysis, CoHort software, Berkely, CA, USA.

Dawar, S., Sattar, A., and Zaki, M. J. (2008). Seed dressing with biocontrol agents and nematicides for the control of root knot nematode on sunflower and okra. Pak J Bot, 40(6), 2683–2691.

El-Sisi, A. G., Ahmed, M. H. M., Ahmed, I. S., and El-Akhal, R. M. (2009). Correlation between constituents, physicochemical properties of locally formulated chlorpyrifos as 48% EC and its insecticidal efficiency against cotton leaf worm. Egyptian Journal of Chemistry, 52(6), 759–777.

Elkhiat, Z. K., Aly, A. F., Shaban, S., and Ibrahim, N. (2022). Formulations of chlorpyrifos-ethyl in non-conventional water-based oil in water emulsion (EW) and micro emulsion (ME), and their insecticidal evaluation against pink bollWorm *(P. gossypiella)*, and migratory locust *(L. migratoria)*. Egyptian Journal of Chemistry [Preprint].

Elkhiat, Z. K., El-Sayed, W., Abdel-Megeed, M. I., and Farg, E.S. M. (2016). The effectiveness of adjuvants on different formulations of lambda-cyhalothrin against cotton leaf worm , middle east journal of applied sciences issn 2077-4613 , (6) 3 ,541-552.

Fainerman, V. B., Lylyk, S. V, Aksenenko, E. V, Kovalchuk, N. M., Kovalchuk, V. I., Petkov, J. T., and Miller, R. (2012). Effect of water hardness on surface tension and dilational visco-elasticity of sodium dodecyl sulphate solutions. Journal of Colloid and Interface Science, 377(1), 1–6.

Finney, D. J. (1952). Probit analysis: a statistical treatment of the sigmoid response curve. Cambridge university press, Cambridge.

Gautam, P. K., Gautam, R. K., Rai, R., and Pandey, J. D. (2014). Thermodynamic and transport properties of sodium dodecylbenzenesulphonate (SDBS) in aqueous medium over the temperature range 298.15 K to 333.15 K. Journal of Molecular Liquids, 191, 107–110.

Giribabu, K., Reddy, M. L. N., and Ghosh, P. (2007). Coalescence of air bubbles in surfactant solutions: Role of salts containing mono-, di-, and trivalent ions. Chemical Engineering Communications, 195(3), 336–351.

Guan, W., Zhao, X., Dickson, D. W., Mendes, M. L., and Thies, J. (2014). Root-knot nematode resistance, yield, and fruit quality of specialty melons grafted onto Cucumis metulifer. HortScience, 49(8), 1046–1051.

Harborne. (1998). Phytochemical methods a guide to modern techniques of plant analysis. springer science \& business media.

Harborne, J. B., Mabry, T. J., and Mabry, H. (1975). The flavonoids, chapman and hall, London. Phytochemistry, 12, 3963–3968.

Hartman, K. M., and Sasser, J. N. (1985). Identification of *Meloidogyne* species on the basis of differential host test and perineal-pattern morphology, 69-77.

Hussein, H. J., and Al-Marzoqi, A. H. (2020). The antibacterial efficacy of the secondary metabolites extracted from *(Melia azedarach* L.*)* leaves against pathogenic microorganisms isolated from burns and gingivitis infections. EurAsian Journal of Biosciences, 14(1), 561–565.

Hussey, R. S. (1973). A comparison of methods of collecting inocula of *Meloidogyne spp*., including a new technique. Plant Dis. Rep., 57, 1025–1028.

Ibrahim, I. K. A. (2011). Nematode pests parasitic on agricultural field crops. Manshaat El. Maaref, Alexandria, 2010.

Ismail, A. R., Jamaludin, S., Chan, Y. P., Rosnah, I., and Hazimah, A. H. (2010). Performance of palm-based emulsions in water (EW)-insecticide formulations against insect pests on chilli and brinjal. Journal of oil palm research, 22, 774–780.

Isman, M. B. (2006). Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annu. Rev. Entomol., 51, 45–66.

Jordan, S. (2018). Yield to the resistance: The impact of nematode resistant varieties on alfalfa yield. Natural Resource Modeling, 31(2), e12150.

Karssen, G., Wesemael, W., And, and Moens, M. (2013). *Root-knot nematode* InR. N. Perry, & M. Moens (Ed.),Plant nematology,73–108.

Kerwin, B. A. (2008). Polysorbates 20 and 80 used in the formulation of protein biotherapeutics: structure and degradation pathways. Journal of Pharmaceutical Sciences, 97(8), 2924–2935.

Kishore, R. S. K., Pappenberger, A., Dauphin, I. B., Ross, A., Buergi, B., Staempfli, A., and Mahler, H.-C. (2011). Degradation of polysorbates 20 and 80: studies on thermal autoxidation and hydrolysis. Journal of Pharmaceutical Sciences, 100(2), 721–731.

Kroll, P., Benke, J., Enders, S., Brandenbusch, C., and Sadowski, G. (2022). influence of temperature and concentration on the self-assembly of nonionic ciej surfactants: a light scattering study. acs omega, 7(8), 7057–7065.

Kumar, A., Li, S., Cheng, C.-M., and Lee, D. (2015). Recent developments in phase inversion emulsification. Industrial \& Engineering Chemistry Research, 54(34), 8375–8396.

Li, Y., Feng, Y., Yu, G., Li, J., Zhou, Y., and Liu, Y. (2020). Preparation and characterization of oil-in-water emulsion based on eco-friendly emulsifiers. Colloids and Surfaces A: Physicochemical and Engineering *Aspects*, 602, 125024.

Liang, R., Xu, S., Shoemaker, C. F., Li, Y., Zhong, F., and Huang, Q. (2012). Physical and antimicrobial properties of peppermint oil nanoemulsions. Journal of Agricultural and Food Chemistry, 60(30), 7548–7555.

Mahdhi, M., Tounekti, T., Abada, E., Al-Faifi, Z., and Khemira, H. (2020). Diversity of arbuscular mycorrhizal fungi associated with acacia trees in southwestern Saudi Arabia. Journal of Basic Microbiology, 60(4), 322–330.

Malar, T. R. J. J., Antonyswamy, J., Vijayaraghavan, P., Kim, Y. O., Al-Ghamdi, A. A., Elshikh, M. S., Hatamleh, A. A., Al-Dosary, M. A., Na, S. W., and Kim, H.-J. (2020). In-vitro phytochemical and pharmacological bio-efficacy studies on *Azadirachta indica* A. Juss and *Melia azedarach* Linn for anticancer activity. Saudi Journal of Biological Sciences, 27(2), 682–688.

Mulqueen, P. (2003). Recent advances in agrochemical formulation. Advances in Colloid and Interface Science, 106(1–3), 83–107.

Navarrete, X., Ron, L., Viteri, P., and Viera, W. (2018). Parasitism of the root knot nematode *Meloidogyne incognita* (Kophoid and White) chitwood in five wild Solanaceae species. Journal National Faculty of Agronomy Medellín, 71(1), 8367–8373.

Nicolopoulou-Stamati, P., Maipas, S., Kotampasi, C., Stamatis, P., and Hens, L. (2016). Chemical pesticides and human health: the urgent need for a new concept in agriculture. Frontiers in Public Health, 4, 148.

Ntalli, N. G., Menkissoglu, S. U., Doitsinis, K., Kalomoiris, M., Papadakis, E.-N., Boutsis, G., Dimou, M., and Monokrousos, N. (2020). Mode of action and ecotoxicity of hexanoic and acetic acids on *Meloidogyne javanica*. Journal of Pest Science, 93(2), 867–877.

Ntalli, N. G., Monokrousos, N., Rumbos, C., Kontea, D., Zioga, D., Argyropoulou, M. D., Menkissoglu-Spiroudi, U., and Tsiropoulos, N. G. (2018). Greenhouse biofumigation with *Melia azedarach* controls *Meloidogyne* spp. and enhances soil biological activity.Journal of Pest Science,91(1),29–40.

Ntalli, N G, Menkissoglu, S. U., and Giannakou, I. (2010 a). Nematicidal activity of powder and extracts of *Melia azedarach* fruits against *Meloidogyne incognita*. Annals of Applied Biology, 156(2), 309–317.

Ntalli, N G, Vargiu, S., Menkissoglu-Spiroudi, U, and Caboni, P. (2010 b). Nematicidal carboxylic acids and aldehydes from *Melia azedarach* fruits. Journal of Agricultural and Food Chemistry, 58(21), 11390–11394.

Padilla-Hurtado, B., Morillo-Coronado, Y., Tarapues, S., Burbano, S., Soto-Suárez, M., Urrea, R., and Ceballos-Aguirre, N. (2022). Evaluation of root-knot nematode (*Meloidogyne* spp.) population density for disease resistance screening of tomato germplasm carrying the gene Mi-1. Chilean Journal of Agricultural Research, 82(1), 157–166.

Pavoni, L., Pavela, R., Cespi, M., Bonacucina, G., Maggi, F., Zeni, V., Canale, A., Lucchi, A., Bruschi, F., and Benelli, G. (2019). Green micro- and nanoemulsions for managing parasites, vectors and pests. Nanomaterials, 9(9), 1285.

Rachokarn, S., Piyasaengthong, N., and Bullangpoti, V. (2008). Impact of botanical extracts derived from leaf extracts *Melia azedarach* L.(Meliaceae) and *Amaranthus viridis* L.(Amaranthaceae) on populations of *Spodoptera exigua* (Hübner)(Lepidoptera: Noctuidae) and detoxification enzyme activities. Communications in Agricultural and Applied Biological Sciences, 73(3), 451–457.

Raddy M., H., F., A.-L. M., Ali;, F. A. F., and A., M. S. (2018). Nematicidal Potential Impact of Phosphodiesterase (PDE) Inhibitors against Root-Knot Nematode, *Meloidogyne incognita. E*gyptian Academic Journal of Biological Sciences, F. Toxicology and Pest Control, 10(1), 85–90.

Ralmi, N., Khandaker, M. M., and Mat, N. (2016). Occurrence and control of root knot nematode in crops: a review. Aust J Crop Sci, 11(12), 1649–1654.

Sikorska, E., Wyrzykowski, D., Szutkowski, K., Greber, K., Lubecka, E. A., and Zhukov, I. (2016). Thermodynamics, size, and dynamics of zwitterionic dodecylphosphocholine and anionic sodium dodecyl sulfate mixed micelles. Journal of Thermal Analysis and Calorimetry, 123(1), 511–523.

Tadros, T. F. (1995). Surface and colloid chemistry in advanced ceramics processing: edited by Robert J. Pugh and Lennart Bergsrom, Surfactant Science Series, 51(8247-9098-7), 376.

Tadros, T. F. (2018). Surfactants in agrochemicals. routledge, 280.

Tadros, T. F., Izquierdo, P., Esquena, J., and Solans, C. (2004). Formation and stability of nano-emulsions. Advances in Colloid and Interface Science, 108, 303–318.

Wagner, H., and Ulrich-Merzenich, G. (2009). Synergy research: approaching a new generation of phytopharmaceuticals. Phytomedicine, 16(2–3), 97–110.

Whitehead, A. G., and Hemming, J. R. (1965). A comparison of some quantitative methods of extracting small vermiform nematodes from soil. Annals of Applied Biology, 55(1), 25–38.

Win, P. P., Kyi, P. P., Maung, Z. T. Z., Myint, Y. Y., Cabasan, M. T. N., and De Waele, D. (2020). Crop rotation sequencing to minimize yield losses of summer-irrigated lowland rice in Myanmar caused by the rice root-knot nematode *Meloidogyne graminicola*. International Journal of Pest Management, 66(4), 319–331.

Xiao, Z., Liu, M., Jiang, L., Chen, X., Griffiths, B. S., Li, H., and Hu, F. (2016). Vermicompost increases defense against root-knot nematode (*Meloidogyne incognita*) in tomato plants. Applied Soil Ecology, 105, 177–186.

Xie, G., Cui, H., Dong, Y., Wang, X., Li, X., Deng, R., Wang, Y., and Xie, Y. (2016). Crop rotation and intercropping with marigold are effective for root-knot nematode (*Meloidogyne* sp.) control in angelica (*Angelica sinensis*) cultivation. Canadian Journal of Plant Science, 97(1), 26–31.

Zdziennicka, A., Szymczyk, K., Krawczyk, J., and Jańczuk, B. (2017). Components and parameters of solid/surfactant layer surface tension. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 522, 461–469.

Zhang, B. (2014). Development of 5% Abamectin EW formulation. Journal of Chemical and Pharmaceutical Research, 6(6), 28–32.

Zhenhua, Y., Zhan, J., Wang, H., Zheng, H., Xie, J., and Wang, X. (2020). Analysis of influencing factors on viscosity of agar solution for capsules. Journal of Physics: Conference Series, 1653(1), 12059.

تجهيز مستحضرات صديقة للبيئة في صورة مستحلبات زيت في الماء من مستخلصات ثمار *Melia azedarach* ودراسة التأثير الأبادي لها ضد نيماتودا تعقد الجذور (*Meloidogyne incognita*) علي نبات الطماطم تحت ظروف الصوبة

هبة حجاج فهمي [1] ، حسني محمد راضي [2] ، علي فهمي علي[1] ، ذكية كمال لطفي الخياط [1]

[1]قسم بحوث مستحضرات المبيدات ، المعمل المركزي للمبيدات ، مركز البحوث الزراعية ، الدقي ، جيزة ، مصر .
[2]قسم وقاية النبات ، كلية الزراعة ، جامعة الأزهر ، مصر .

الملخص

تهدف هذه الدراسة إلى تجهيز مستحضرات صديقة للبيئة و تقدير النشاط الأبادي النيماتودي لمستخلصات الهكسان وخلات الإيثيل والإيثانول للثمار الخضراء و الناضجة لنبات *Melia Azedarach* والمعروف في مصر بأسم (zanzalacht) ضد الطور الثاني من نيماتودا تعقد الجذور *Meloidogyne incognita* تحت ظروف المعمل والصوبة علي نبات الطماطم. أظهرت النتائج أن مستخلص الأيثانول للثمار الناضجة حقق أكبر نسبة نشاط أبادي نيماتودي وكانت قيمة التركيز النصفي المميت له بعد 48 ساعة يساوي 206.43 جزء في المليون .تم تحديد المكونات الكيميائية لمستخلص إيثانول الفاكهة الناضجة بواسطة طيف، GC-MS وكانت كالأتي : إستر إيثيل الأحماض الدهنية (28.44٪) ، 5-هيدروكسي فورفورال (13.59٪) ، 2 (3H) فورانون (6.44٪) ، وحمض أكريليك الفينيل (6.35٪).) كمكونات رئيسية. تم عمل مستحضرات في صورة مستحلبات زيت في الماء صديقة للبيئة للمستخاصات محل الدراسة ، وتم تقييم خواصها الفيزيائية والكيميائية تحت ظروف التخزين المختلفة ودراسة النشاط الأبادي للمستحضرات المجهزة ضد النيماتودا ومقارنتها مع مبيد تجاري Vydate 24 % SL تحت ظروف الصوبة .علاوة على ذلك ، أظهرت نتائج تجارب الصوبة أن جميع المعاملات قللت بشكل ملحوظ من مرض نيماتودا تعقد الجذور وتثبيط نموها مقارنةً بنبات الطماطم غير المعامل . كما أظهرت النتائج المتحصل عليها عدم وجود فروق ذات دلالة إحصائية بين المعاملة بمستخلصات *M. Azedarach* وVydate 24 % SL حيث كانت نسبة الخفض في عدد العقد الجذرية (80 و 78.18٪ لمبيد النيماتودا التجاري والمستحضر المجهز RH 10%EW على التوالي). أدت جميع المعاملات إلى زيادة أوزان وأطوال الساق والجذور مقارنةً بالنباتات غيرالمعاملة . وبناءاً عليه يمكن أستخدام مستخلصات *M. azedarach* كبديل آمن وجوهري لمبيدات النيماتودا التقليدية في أطار الإدارة المتكاملة لنيماتودا تعقد الجذور .

الكلمات الدالة : ثمار *Melia azedarach* ، *التحليل* الكروماتوجرافي الغازي/التحليل بالمطياف الكلي ، مستحضر مستحلب زيت في ماء ، نيماتودا تعقد الجذور (*Meloidogyne incognita*) ، نبات الطماطم .