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## Nematicidal Activity of some Nanoemulsions of Monoterpenes on Tomato Root-Knot Nematodes (*Meloidogyne javanica*)

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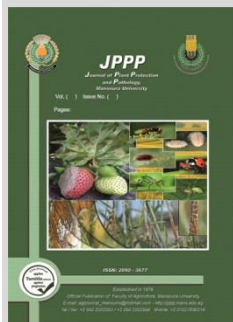


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### ABSTRACT

The present study was initiated to explore at the nematicidal activity of some nanoemulsions of monoterpenes, i.e. (R)-carvone, cinnamaldehyde, citral, geraniol and pulegone against tomato knot-root nematode *M. javanica*, in both laboratory and under greenhouse conditions. The results show that the pure and NE-monoterpenes tested considerably and severely decreased egg hatching at doses ranging from 25 to 1000 µg/ml. NE-Cinnamaldehyde, significantly, showed higher J2 mortality (90.67 %) and egg-hatching inhibition (8.67) than pure cinnamaldehyde. The most successful therapy for reducing root galls and the number of egg masses was NE- Cinnamaldehyde, which showed 90.3% and 92.8% reduction, followed by NE- Pulegone 84.2% reduction, respectively. The population of J2 in soil was considerably decreased after using the tested nanoemulsion monoterpenes. NE- Cinnamaldehyde was the most effective treatment, suppressing the final population of *M. javanica* by 81.8%. Beyond oxamyl, NE-Cinnamaldehyde was the most efficient treatment for increasing the weights and lengths of both fresh shoots and roots in infected tomato plants, consecutively. Meanwhile, pulegone measured the intermediate values of fresh root and shoot weights as well as for lengths. Normal and nanoemulsion cinnamaldehyde ranked the first as it increased polyphenoloxidase (PPO) activity, compared to the other treatments followed by nanoemulsion and normal pulegone. The highest enzyme activity was noticed 7 and 15 days after treatment. Peroxidase activity (POD) also increased by nanoterpene treatments in a trend similar to that of the PPO. Consequently, these effective monoterpene nanoemulsions could be effective potentially and environmentally safe to control tomato knot-root nematode.

**Keywords:** Nanoemulsion(NE), monoterpenes, knot-root nematode, tomato, Induced resistance.



### INTRODUCTION

Plant-parasitic nematodes are harmful to most economical important crops worldwide and are pathogens of hard control (Crow, 2007). The widespread use of insecticides to manage nematodes has a number of negative consequences for the environment and human health, and the formation of nematode resistance. As a result, more attention has been placed on developing alternative pest management techniques that are as effective as synthetic pesticides, remain safe for farmers, consumers, and the environment, and are inexpensive (Fernandez *et al.*, 2001).

Rapid advances in nanoformulations (NFs) of numerous agrochemical agents have raised new expectations in the agriculture industry's industrial and consumer sectors (Kah, 2015; Balaure *et al.*, 2017; Badawy *et al.*, 2017). This is primarily to reduce sensitivities and enhance the stability of compounds with rapid volatilization and breakdown rates, which including plant essential oils (EOs) and their primary components, monoterpenes (Abdelrasoul *et al.*, 2018; Marei *et al.*, 2018; Abdelrasoul *et al.*, 2020). The active ingredient's uptake, absorption, and bioavailability can all be improved using nanoformulations. Companies that manufacture pesticides utilise nanoemulsions, this might be oil- or water-based and include insecticidal or herbicidal nanoparticle inhibitors (200-400nm) (Madhuri *et al.*, 2010; Sekhon 2014). NEs have been produced using a variety of ways, encompassing

both low- and high-energy methods (Ultrasonic emulsification). The high energy technique is characterised by its speed and efficiency in the production of nanoemulsions with tiny droplet diameters and small size dispersion (Ghosh *et al.*, 2013). Monoterpenes, the primary components of plant essential oils, have been shown to be potent antibacterial agents in the form of nanoemulsions (Zhang *et al.*, 2014; Ghosh *et al.*, 2014; Zahi *et al.*, 2015; Ma *et al.*, 2016; Li *et al.*, 2017; Abdelrasoul *et al.*, 2018, Abdelrasoul *et al.*, 2020)

In light of this, the current work was started to look into the nematicidal activity of certain monoterpenes found in normal and nanoemulsions, i.e. (cinnamaldehyde, citral, (R)-carvone, geraniol and pulegone) on tomato knot-root nematode *M. javanica*, in laboratory and under greenhouse conditions.

### MATERIALS AND METHODS

#### Chemicals and reagents

Five monoterpenes (Fig. 1) were used in the present study. These were geraniol (98%), cinnamaldehyde (98%) and pulegone (92%) were obtained from ACROS Organics Company, New Jersey, USA while citral (95%) and R-carvone (98%) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). The nematicide, Oxamyl (Vydate®24%L) (*N,N*-dimethyl-2-methylcarbamoyloxyimino-2-(methylthio) acetamide) was supplied by Dupont company and was used as positive control.

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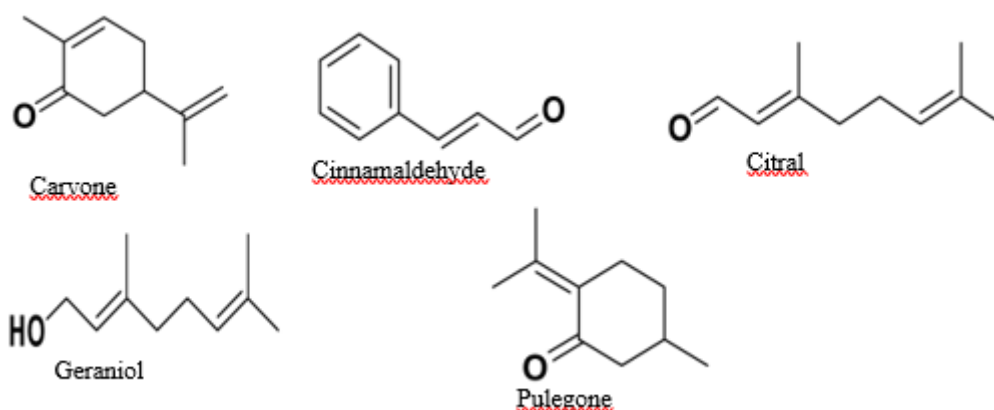


Figure 1. The monoterpenes structure that was employed in the present study.

### Preparation of nanoemulsions

The nanoemulsions of the monoterpenes examined were formed using a high-energy ultrasonication method as described by Abdelrasoul *et al.*, (2020) and illustrated in Fig.

(2). the optimal conditions for ultrasonic emulsification included a sonication power of 75 kHz and pulses or cycles of 9 cycle/sec for 15 minutes (Ultrasonic Homogenizers HD 2070). (Anjali *et al.*, 2012).

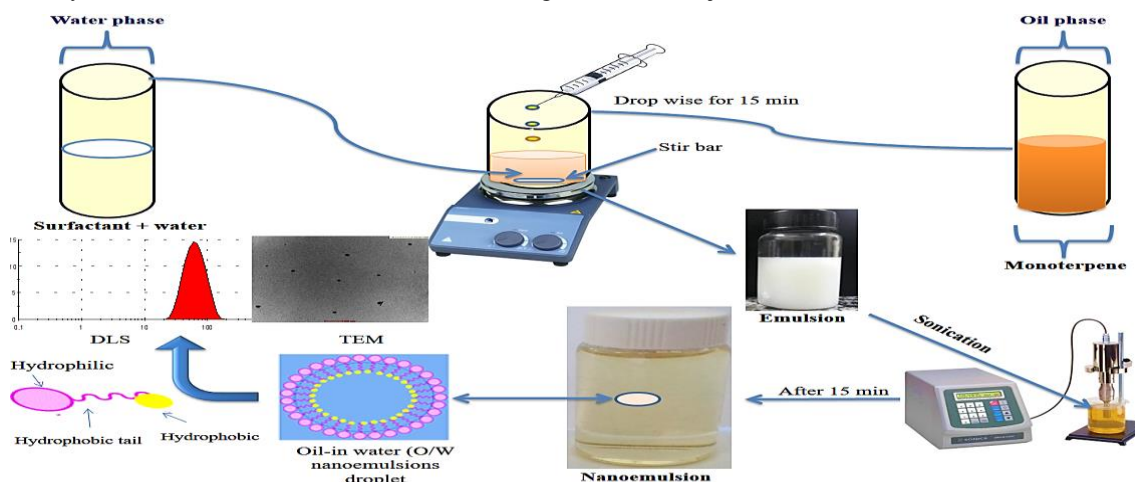


Figure 2. The preparation of various nanoemulsions of the tested monoterpenes is shown schematically.

### Root-knot nematode culture:

Culture of *M. javanica* (Kofoid and White) Chitwood was isolated from tomato plant (*Solanum lycopersicon* cv. Elisa) grown in a greenhouse. Perianal patterns of adult females and the morphology of second stage juveniles were used to identify root-knot nematodes (Hartman and Sasser, 1985; Jepson, 1987). Root knot nematode egg masses were incubated for 48 hours at room temperature ( $25 \pm 2^\circ\text{C}$ ) in sterilized distilled water of sodium hypochlorite (NaOCl) solution (Hussey and Barker, 1973). Newly born second stage juveniles (J2) were collected every day. Toxicity experiments were run on *M. javanica* second-stage juveniles (J2) and eggs.

### Laboratory experiments

#### The in vitro nematicidal activity of certain monoterpenes in normal and nanoemulsion formulations against *M. javanica*

##### 1-Effect on the second-stage juveniles (J2) mortality of *M. javanica*

Nematicidal activities for both normal and NE-monoterpenes, i.e. cinnamaldehyde, citral, (R)-carvone, geraniol and pulegone were assessed on second-stage juveniles (J2) of *M. javanica* under laboratory conditions. The mortality of J2 was calculated by combining 1 ml of water solution containing 50 freshly hatched J2 with 1 ml of

double concentrations, of (100, 500 and 1000  $\mu\text{g}/\text{ml}$  for normal and 25, 50 and 100  $\mu\text{g}/\text{ml}$  for nanoemulsion) of tested monoterpenes in glass vial and incubated at  $25 \pm 2^\circ\text{C}$  for 48 h (Khan *et al.* 2016). Following incubation period, J2s were transferred into distilled water for 24 h, and then active and dead nematodes were counted under the microscope (Olympus CX41RF, Olympus Optical Co., LTD). Abbott's formula (Abbott, 1925) was used to determine the percent mortality:

$$\text{Juvenile mortality (\%)} = \frac{(T-C)}{(100-C)} * 100$$

The percentages of deceased juveniles in the treatment and control groups are T and C, respectively.

As a control, glass vials holding 1 ml of sterile distilled water with new-hatched J2s suspension were used. The positive control was Oxamyl 24% SL (5 ml/l, DuPont Company, USA), a chemically produced nematicide. For each concentration, five repetitions with four vials per replicate were utilised, and the experiment was replicated three times.

##### 2. Effect on egg hatching of *M. javanica*

The in vitro nematicidal activity of the normal and NE- monoterpenes, cinnamaldehyde, citral, (R)-carvone, geraniol and pulegone, at the concentrations of 100, 500 and 1000  $\mu\text{g}/\text{ml}$  (in sterile distilled water with tween 0.5%) for normal and 25, 50 and 100  $\mu\text{g}/\text{ml}$  for nanoemulsion

monoterpenes in sterile distilled water were tested on *M. javanica*. 1 ml of water suspension containing 100 nematode eggs was transferred to a glass vial containing 1 ml of double concentrations of tested monoterpenes solutions and incubated at 25 °C for three days for the egg hatching test. Each treatment was replicated four times. Hatched juveniles were counted under a light microscope (Olympus CX41RF, Olympus Optical Co., LTD) after incubation, and the egg hatching percentage was calculated.

**Greenhouse experiment:**

**1- Efficacy of certain monoterpenes in normal and NE-formulations to control *M. javanica* on tomato.**

The most effective monoterpenes and concentration revealed in the previous in vitro test, i.e. cinnamaldehyde and pulegone at 100 µg/ml, were further tested for their efficacy to control *M. javanica* under greenhouse conditions. Both normal emulsions and nanoemulsions of cinnamaldehyde and pulegone were tested to evaluate their efficacious against *M. javanica* at the concentration of 100 µg/ml compared to oxamyl at the recommended rate (3L per feddan) i.e. 100 ml/pot. 30-day-old uniform tomato seedlings (*Solanum lycopersicon* cv. Elisa) were transplanted singly in a plastic container (20 cm -diameter and 15 cm depth filled with 3 Kg mixture of 3 sand: 1 peat moss v: v). For ten days, transplants were allowed to recuperate from transplanting shock, and agricultural activities were continued as usual. In each container, 5000 eggs of root-knot nematode were injected in holes 5-7 cm deep surrounding the plant within a two-centimeter radius. Each treatment had four duplicates, including non-inoculated and inoculated controls that were not treated. Greenhouse temperature ranged between 25-30°C.

**2. Effect of certain monoterpenes in normal and NE-formulations on activity of the defense-related enzymes of tomato plants inoculated with *M. javanica*.**

Activity of defense-related enzymes for nanoemulsion and normal of cinnamaldehyde and pulegone were determined to reveal effect of the conducted monoterpene treatments in both the normal and nanoemulsion forms on activity of the defense-related enzymes in tomato against *M. javanica*.

The activities of polyphenoloxidase (PPO) and peroxidase (POD) were measured in the leaves at 0, 1, 3, 7, and 15 days after inoculation, with three replicates for each treatment. Mayer *et al.* (1965) measured the activity of polyphenol oxidase (EC 1.14.18.1) and represented it as a change in OD min<sup>-1</sup> mg<sup>-1</sup> protein. The activity of peroxidase (EC 1.11.1.7) was measured as described by Chen *et al.* (2000) and represented as a change in OD min<sup>-1</sup> g<sup>-1</sup> of fresh tissue.

**3. Effect of certain monoterpenes in normal and NE-formulations on plant growth of tomato plants inoculated with *M. javanica*.**

After 60 days, the plants' root systems were carefully removed from the stem. The total numbers of egg masses per root system and galls as well as, the final J2 population were counted. At the end of the experiment, Henderson and Tilton's (1955) equation was used to calculated the percent reduction in galls, egg masses, and nematode population density. Roots were dyed for 15 minutes in an aqueous solution of Phloxine B stain (0.15 g/l water) before being gently rinsed in tap water (Holbrook *et al.*, 1983). Plant growth characteristics were measured and represented as a

percentage increase in shoot and root lengths (in centimetres) and fresh weights (in grammes).

**Analytical statistics**

Using SPSS 20.0 software, data was analysed using one-way analysis of variance (ANOVA) (Statistical Package for Social Sciences, USA).

**RESULTS AND DISCUSSION**

**Toxicity of normal and NE- monoterpenes against J2 juveniles and egg hatching of *M. javanica***

In the bioassay test, the effects of normal and NE-monoterpenes, cinnamaldehyde, citral, (*R*)-carvone, geraniol and pulegone on J2 of *M. javanica* at concentrations of (100, 500 and 1000 µg/ml for normal) and (25, 50 and 100 µg/ml for nanoemulsion) were evaluated as shown in Tables (1 and 2).

**Table 1. The in vitro effect of certain monoterpenes on J2 mortality %, and egg-hatching % of *M. javanica*.**

Treatment	Con. µg/ml	J2 mortality (%)	Hatching (%)	Reduction % hatching 72h
Water		2.67 <sup>k</sup>	46.67 <sup>a</sup>	-
Tween+Water		6.67 <sup>j</sup>	44.00 <sup>ab</sup>	5.71
Pulegone	100	22.67 <sup>g</sup>	21.67 <sup>lg</sup>	53.57
	500	51.67 <sup>cd</sup>	19.33 <sup>g</sup>	58.57
	1000	77.33 <sup>b</sup>	14.67 <sup>h</sup>	68.57
Genaniol	100	13.33 <sup>i</sup>	35.67 <sup>c</sup>	23.57
	500	33.67 <sup>f</sup>	31.67 <sup>d</sup>	32.14
	1000	49.00 <sup>d</sup>	24.00 <sup>f</sup>	48.57
Carvone	100	9.33 <sup>j</sup>	42.00 <sup>b</sup>	10.00
	500	23.33 <sup>g</sup>	36.00 <sup>c</sup>	22.86
	1000	49.00 <sup>d</sup>	27.67 <sup>e</sup>	40.71
Cinnamaldhyde	100	21.33 <sup>g</sup>	23.00 <sup>fg</sup>	50.71
	500	53.00 <sup>c</sup>	19.67 <sup>g</sup>	57.86
	1000	80.33 <sup>b</sup>	14.00 <sup>h</sup>	70.00
Citral	100	8.33 <sup>j</sup>	43.00 <sup>b</sup>	7.86
	500	17.67 <sup>h</sup>	42.33 <sup>bi</sup>	9.29
	1000	45.00 <sup>e</sup>	32.67 <sup>cd</sup>	30.00
Oxamyl		96.67 <sup>a</sup>	5.67	87.86
Significant		0.001	0.003	-

At P=0.05, values in each column with the same letter(s) are not significantly different

**Table 2. The in vitro effect of certain monoterpenes in the nanoemulsion forms on J2 mortality %, and egg-hatching % of *M. javanica*.**

Treatment	Con. µg/ml	J2 mortality (%)	Hatching (%)	Reduction% hatching 72h
Water		3.67 <sup>j</sup>	46.00 <sup>a</sup>	-
Tween+Water		7.33 <sup>ij</sup>	43.00 <sup>ab</sup>	6.52
Pulegon	25	28.67 <sup>g</sup>	19.67 <sup>g</sup>	57.25
	50	59.67 <sup>e</sup>	17.33 <sup>g</sup>	62.32
	100	88.67 <sup>b</sup>	12.33 <sup>h</sup>	73.19
Genaniol	25	19.67 <sup>h</sup>	21.00 <sup>g</sup>	54.35
	50	49.67 <sup>f</sup>	19.33 <sup>g</sup>	57.97
	100	74.33 <sup>c</sup>	17.33 <sup>g</sup>	62.32
Carvon	25	11.67 <sup>i</sup>	28.67 <sup>d</sup>	37.68
	50	34.67 <sup>g</sup>	24.33 <sup>ef</sup>	47.10
	100	72.67 <sup>c</sup>	19.00 <sup>g</sup>	58.70
Cinnamaldhyde	25	30.00 <sup>g</sup>	19.67 <sup>g</sup>	57.25
	50	69.00 <sup>cd</sup>	11.33 <sup>h</sup>	75.36
	100	90.67 <sup>ab</sup>	8.67 <sup>hi</sup>	81.16
Citral	25	11.00 <sup>i</sup>	40.67 <sup>b</sup>	11.59
	50	18.00 <sup>h</sup>	36.67 <sup>c</sup>	20.29
	100	63.67 <sup>de</sup>	27.67 <sup>de</sup>	39.86
Oxamyl		95.33 <sup>a</sup>	7.33 <sup>i</sup>	84.06
Significant		0.001	0.003	-

At P=0.05, values in each column with the same letter(s) are not significantly different

Results clarified that the tested normal and NE-monoterpenes, significantly and drastically, increased J2 mortality % and reduced hatching eggs and the effect increased with increasing compound concentration. However, nanoemulsion forms of all tested compounds consistently showed higher effect even at tenth of the concentration applied by the normal monoterpene forms. Meanwhile, NE- cinnamaldehyde at 100ug/ml showed the highest J2 mortality % and egg-hatching inhibition being 90.67% and 81.16, respectively which were not significantly different from the oxamyl nematicide effect which showed 95.33% and 84.06% for both parameters, respectively. This was followed by NE-Pulegone at 100ug/ml with J2 mortality % and egg-hatching inhibition values being 88.67% and 73.19%, respectively. Several research have found that NEs-essential oils (EOs) improve the physical characteristics and antibacterial properties of conventional emulsions (Buranasuksombat *et al.* 2011; So Pedro *et al.* 2013; Bilia *et al.* 2014; Guerra-Rosas *et al.* 2017; Abdelrasoul *et al.* 2020). Their findings revealed that NEs were more effective than monoterpenes in terms of antibacterial activity. This is likely owing to the fact that fat particle nano-structures may transport primary oil to the cell membrane's surface, but undiluted oil fails or cannot effectively interact with cell membranes. The findings are similarly supported by (Zhang *et al.*, 2014), who found that NEs-essential oils significantly increased antibacterial activity of D-limonene. Lambert *et al.*, 2001, found that EOs containing carvacrol and thymol as monoterpenes, such as

thyme oil, had a high bactericidal effect. Furthermore, the primary components of clove, lemon, and rosewood essential oils, eugenol and citral, were discovered to disrupt a wide range of bacteria (Friedman *et al.*, 2004).

**Greenhouse experiments:**

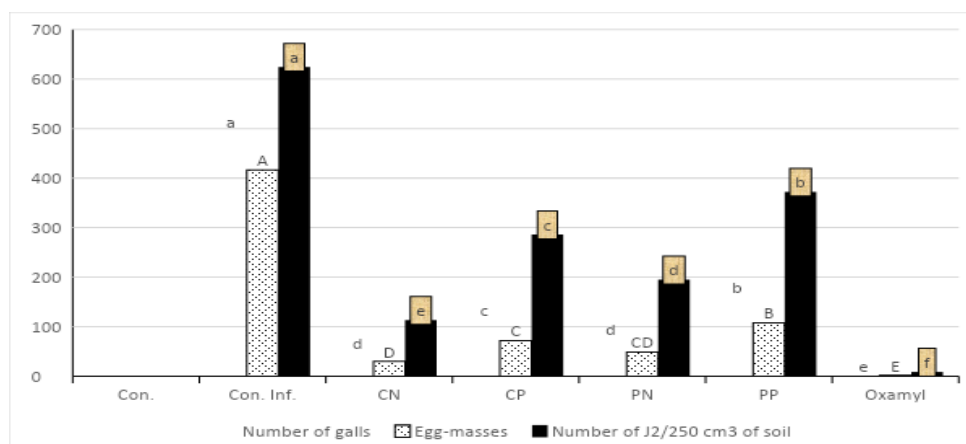
**1-The in vivo nematocidal activity of monoterpenes in normal and NE- formulations to *M. javanica* on tomato plants**

Table (3) and Fig.3 indicate the number of galls and egg masses per root system, as well as the final population, as a function of the monoterpenes examined. The highest numbers of egg masses per root system and galls and the final population, were recorded in the untreated inoculated control (UI control). The nematocidal activity of the two nanoemulsion, dramatically reduced root galls, egg masses and final population of *M. javanica* infecting tomato plants at the application rate of 100 µg/ml. Meanwhile, the NE-Cinnamaldehyde treatment was the most successful in reducing root galls, with the lowest number of galls (48) and a 90.3 percent decrease. Also, the most effective treatment for lowering the number of egg masses was NE-Cinnamaldehyde, which reduced the number of egg masses by 92.8 percent, followed by pulegone, which reduced the number of egg masses by 88.2 percent. On the other hand, application of the tested compound, dramatically decreased the population of J2 in soil where NE- Cinnamaldehyde was the most effective and suppressed the final population of *M. javanica* by 81.8 percent.

**Table 3. Effects of cinnamaldehyde and pulegone monoterpenes in both the normal emulsion and NE- formulations on *M. javanica* disease parameters on tomato under greenhouse conditions.**

Treatment	Number of galls	Reduction %	Egg-masses	Reduction %	Number of J2*	Reduction %
Control(non-inoculated)	0.00 <sup>e</sup>	-	0.00 <sup>e</sup>	-	0.0 <sup>f</sup>	-
Control (infected)	494 <sup>a</sup>	-	416 <sup>a</sup>	-	624 <sup>a</sup>	-
NE-Cinnamaldehyde	48 <sup>d</sup>	90.3	30 <sup>d</sup>	92.8	113 <sup>e</sup>	81.8
P-Cinnamaldehyde	113 <sup>c</sup>	77.1	72 <sup>c</sup>	82.8	286 <sup>c</sup>	54.2
NE-Pulegone	75 <sup>d</sup>	84.8	49 <sup>cd</sup>	88.2	195 <sup>d</sup>	68.8
P-Pulegone	159 <sup>b</sup>	67.8	108 <sup>b</sup>	74.1	372 <sup>b</sup>	40.4
Oxamyl	2.0 <sup>e</sup>	99.6	2.0 <sup>e</sup>	99.5	8.67 <sup>f</sup>	98.6
Significant	0.002	-	0.001	-	0.003	-

At P=0.05, values in each column with the same letter(s) are not significantly different.\* Number of J2/250 cm<sup>3</sup> of soil, NE, nanoemulsion; P, pure (normal monoterpenes)



**Fig. 3. Effects of cinnamaldehyde and pulegone monoterpenes in both the normal emulsion and nanoemulsion formulations on *M. javanica* disease parameters on tomato under greenhouse conditions. Con., control(non-inoculated); Con. inf., control (infected); CN, NE-Cinnamaldehyde; CP, normal cinnamaldehyde; PN, NE-Pulegone; PP, normal pulegone**

Essential oils containing various monoterpenes (Abo-Elyoussr *et al.*, 2009, Onifade, 2007, and Pérez *et al.*, 2003) or organic amendments derived from essential oil-rich plants (Pérez *et al.*, 2003 and Silva *et al.*, 2006) have been used to protect plants against phytonematodes. On nematodes, the mode of action of essential oils and monoterpenes is unknown. However, certain essential oils have been shown to exhibit genotoxic action in *Drosophila melanogaster* by activating octopaminergic receptors and interfering with GABA receptors (Enan, 2001 and Kostyukovsky *et al.*, 2002). (Priestley *et al.*, 2003). As typical lipophiles, essential oils and terpenoids interact with the cytoplasmic membrane of yeasts, distorting the structure of polysaccharides, fatty acids, and phospholipids, as well as causing depolarization of mitochondrial membranes, allowing radicals, cytochrome C, calcium ions, and proteins to leak out (Bakkali, *et al.*, 2008). The presence of phenols, aldehydes, and alcohols in essential oils has been linked to their *in vitro* cytotoxic action (Bruni *et al.*, 2004 and Oka *et al.*, 2000).

**2. Effect of monoterpenes in normal and NE-formulations on Defense-related enzymes and induced resistance in tomato plants inoculated with *M. javanica*.**

**Polyphenol oxidase activity (PPO)**

Data in Table (4) showed that the different treatments with the two tested monoterpenes in both the

normal and NE-formulations significantly increased PPO activity, particularly 7 and 15 days after treatment, in tomato plants grown in soil infested with *M. javanica* compared to the untreated inoculated control and also with the nematicide Oxamyl treatment. However, NE-cinnamaldehyde was the most effective and showed 0.412 and 0.579 (OD min<sup>-1</sup>g<sup>-1</sup> of fresh tissue) PPO activity, 7 and 15 days after treatment compared with 0.248 and 0.320 (OD min<sup>-1</sup>g<sup>-1</sup> of fresh tissue), respectively, for the untreated infected control. This was followed by the N-cinnamaldehyde, NE-Pulegone, and N-Pulegone, respectively.

**Peroxidase activity (POD)**

Concerning POD activity as affected by the monoterpenes treatments, data Results in Table (4) showed a trend similar to that obtained by monoterpenes treatments on PPO activity and this was most evident 7, and 15 days after treatment. NE-Cinnamaldehyde was the most effective followed by the N-cinnamaldehyde, NE-Pulegone, and N-Pulegone, respectively.

These findings are in agreement with Abdelrasoul *et al.* (2020) as revealed that PPO and POD activity significantly increased with NE- Cinnamaldehyde treatment in potato leaves compared with control and the resistance of the potato was associated with high PPO and POD enzyme activities.

**Table 4. Effects of some normal and NE- monoterpenes on activities of peroxidase (POD) and polyphenol oxidase (PPO) enzymes in the roots of tomato plants infected with *M. javanica* under greenhouse conditions.**

Treatment	PPO activity (OD min <sup>-1</sup> g <sup>-1</sup> of fresh tissue)				POD activity (OD min <sup>-1</sup> g <sup>-1</sup> fresh tissue)			
	Time(days)				Time(days)			
	0	3	7	15	0	3	7	15
Control	0.202	0.208	0.173d	0.219e	0.228	0.240	0.262b	0.228d
Control (infected)	0.205	0.212	0.248c	0.320cd	0.233	0.258	0.294b	0.326c
NE-Cinnamaldehyde	0.219	0.244	0.412a	0.579a	0.248	0.264	0.424a	0.552a
N-Cinnamaldehyde	0.214	0.239	0.403a	0.554a	0.257	0.261	0.414a	0.530a
NE-Pulegone	0.215	0.225	0.320b	0.461b	0.243	0.256	0.327b	0.482ab
N-Pulegone	0.209	0.212	0.288bc	0.358c	0.248	0.258	0.310b	0.426b
Oxamyl	0.218	0.227	0.269bc	0.253de	0.268	0.257	0.311b	0.324c
Significant	N.S.	N.S.			N.S.	N.S.		

At p=0.05, values in each column preceded by a distinct letter (s) are significantly different. N.S. = not significant

**3-Effect of the tested monoterpenes in the normal and NE-formulations on tomato growth inoculated with *M. javanica* :**

In pot experiments under greenhouse conditions, the results presented in Table (5), showed that *M. javanica* infection decreased all tested plant growth parameters in the untreated inoculated plants as compared with the non-inoculated tomato plants. However, the different monoterpenes treatments and Oxamyl improved and increased tomato plant growth parameters in the inoculated

treated plants. Oxamyl showed the highest shoot growth values being 51g, 14g, and 50cm for fresh weight, dry weight, and length, respectively, and 19.7g, 4.7g, and 19.7cm for the same growth parameters for the root system, respectively. However, NE-Cinnamaldehyde treatment showed growth values for most parameters not significantly different from the Oxamyl nematicide. Meanwhile, most growth parameters with NE-Pulegone treatment were not significantly different from NE-Cinnamaldehyde treatment (Table 5).

**Table 5. Under greenhouse conditions, the effects of certain monoterpenes on growth parameters of tomato plants infected with *M. javanica*.**

Treatment	Shoot system						Root system					
	weight (g)			length			weight (g)			length		
	Fresh	I	Dry	I	cm	I	Fresh	I	Dry	I	cm	I
Control	50.0 <sup>a</sup>	-	15.7 <sup>a</sup>	-	48.7 <sup>ab</sup>	-	21.0 <sup>a</sup>	-	5.0 <sup>a</sup>	-	18.0 <sup>b</sup>	-
Control (infected)	20.3 <sup>c</sup>	-	6.3 <sup>e</sup>	-	30.0 <sup>d</sup>	-	11.0 <sup>e</sup>	-	2.0 <sup>c</sup>	-	12.7 <sup>c</sup>	-
NE-Cinnamaldehyde	42.7 <sup>b</sup>	110	12.3 <sup>bc</sup>	95	42.7 <sup>bc</sup>	42	17.7 <sup>abc</sup>	61	4.3 <sup>ab</sup>	117	17.7 <sup>b</sup>	39
N-Cinnamaldehyde	36.0 <sup>c</sup>	77	9.5 <sup>cd</sup>	50	34.0 <sup>d</sup>	13	13.3 <sup>cde</sup>	21	3.3 <sup>abc</sup>	67	21.7 <sup>a</sup>	71
NE-Pulegon	39.7 <sup>bc</sup>	95	10.7 <sup>c</sup>	68	40.7 <sup>c</sup>	36	16.3 <sup>bcd</sup>	48	4.0 <sup>abc</sup>	100	17.7 <sup>b</sup>	39
N-Pulegon	27.7 <sup>d</sup>	36	8.2 <sup>d</sup>	30	33.7 <sup>d</sup>	12	12.3 <sup>de</sup>	12	2.7 <sup>bc</sup>	33	18.0 <sup>b</sup>	42
Oxamyl	51.0 <sup>a</sup>	151	14.0 <sup>ab</sup>	121	50.0 <sup>a</sup>	67	19.7 <sup>ab</sup>	79	4.7 <sup>ab</sup>	133	19.7 <sup>ab</sup>	55

At P=0.05, values in each column with the same letter(s) are not significantly different.

As a consequence, these findings revealed that none of the chemicals were phytotoxic at the concentrations evaluated. These findings are consistent with those of (Echeverrigaray *et al.*, 2010 and Oka *et al.*, 2000).

## CONCLUSION

The conversion of monoterpenes to nanoemulsions greatly enhanced its nematocidal activity against the major plant pathogenic nematode *M. javanica*, according to the findings of this study. Because of their potential to promote the application as a nematocidal activity, nanoemulsions may be particularly effective transmission systems for essential oils and their compounds, potentially providing an alternative to high-risk chemical nematocides. Meanwhile, the study found that nanoemulsion was effective on root-knot nematodes *M. javanica* in tomato without causing phytotoxicity to the plant or non-parasitic nematodes.

### Conflict of interest

None.

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## فاعلية بعض المستحلبات النانوية للتربينات الأحادية كمبيدات نيماتودية ضد نيماتودا تعقد الجنور ( ميلويدوجان جافانكا ) في الطماطم

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تم تقييم النشاط النيماتودي لبيعض المستحلبات النانوية لمركبات التربينات الاحاديةPulegone و Geraniol · Citral · Cinnamaldehyde (R) - Carvone ضد نيماتودا تعقد الجنور في الطماطم *M. javanica* معمليا وداخل الصوبة الزراعية. وأشارت النتائج إلى أن التربينات الاحادية لكلا من المركبات النقية والمستحلبات النانوية قد قل بشكل كبير من فقس البيض بتركيزات متفوتة من ( ٢٥ إلى ١٠٠٠ ميكروجرام / مل). وأظهر مستحلب النانو لمركب Cinnamaldehyde تأثيرا معنويا كأعلى نسبة موت للطور البرقي الثاني J2 وتثبيت فقس البيض عن المركب النقي Cinnamaldehyde. ولقد كان لمستحلب النانو لمركب Cinnamaldehyde هو الأكثر فاعلية لتقليل تعقد الجنور وعدد كتل البيض بنسبة ٩٠,٣ و ٩٢,٨٪ على التوالي ، ويليها مركب Pulegone بنسبة انخفاض ٨٤,٨٪. وأيضا ادي إلى انخفاض كبير في تعداد النيماتودا النهائي في التربة J2 . وكان مستحلب النانو لمركب Cinnamaldehyde هو العلاج المتفوق الذي قمع تعداد النيماتودا النهائي في التربة لـ *M. javanica* بقيمة تخفيض ٨١,٨٪. بالإضافة إلى مبيد الأوكساميل ، وكان مستحلب النانو Cinnamaldehyde هو المركب الأكثر فاعلية لزيادة كل من الأوزان وأطوال الفروع الطازجة والجنور على التوالي. بينما سجل مستحلب النانو لمركب Pulegone القيم الوسطية لأوزان الجنور وكذلك للأطوال، وسجل مركب Cinnamaldehyde في صورته النقية ومستحلب النانو المرتبة الأولى حيث زاد من نشاط انزيم (PPO) بنسبة ٧٣,١٣ و ٨٠,٩٪ على التوالي مقارنة بالكنترول. كما لوحظ أن نزيم (PPO) لمستحلب النانو Pulegone أعلى من المركب النقي والكنترول. وقد كان نشاط انزيم البيروكسيداز (POD) أعلى بشكل ملحوظ في النباتات المستحبة مقارنة بكل من الكنترول السلبي (UU) والإيجابي (UI) ، كما لوحظ أعلى نشاط لإنزيم POD بعد ١٥ يوما من الإصابة. وبناء على ما تقدم يمكن أن تكون المستحلبات النانوية للتربينات الاحادية فعالة وآمنة بيئيا للسيطرة على نيماتودا تعقد جنور لنبات الطماطم.