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Nematicidal Performance of *Ipomoea carnea* and *Pluchea dioscoridis* Extracts towards *Tylenchulus semipenetrans* and their Possible Application in Nematode Management on Washington Navel Oranges

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ABSTRACT

Crude methanolic shoot extracts of the perennial weeds, *Ipomoea carnea* and *Pluchea dioscoridis* were tested for their nematicidal activity towards second stage juveniles (J_2s) of the citrus nematode, *Tylenchulus semipenetrans* under laboratory conditions. All extracts at the concentrations 100, 250, 500, 1000 and 2000 mg/L significantly suppressed J_2s viability causing mortality ranged from 13.82 to 100% as compared to check and blank treatments. Mortality (%) of J_2s was linearly increased with increasing concentrations of plant extracts. Extract of *P. dioscoridis* (LC_{50} = 249 mg/L) appeared to be stronger than *I. carnea* (LC_{50} = 345 mg/L). Results of GC-MS analysis of *I. carnea* and *P. dioscoridis* extracts revealed the presence of 33 and 36 different chemical compounds, respectively. The most prevalent compounds present in *I. carnea* extract were Cyclopentane, 1-acetyl-1,2 epoxy, 2-Octenal, (E) and Thymol, whereas Ethanone, 1-(2-methylcyclopropyl)-, Cholestan-3-ol, 2-methylene-, (3á,5à)-, cis-5,8,11,14,17-Eicosapentaenoic acid and Undecanoic acid were the most occurred in *P. dioscoridis* extract. Soil application with *I. carnea* and *P. dioscoridis* extracts alone at their LC_{95} values (1637, 1340 mg/L, respectively) and in their combination at half LC_{95} values of each (819 and 670 mg/L for *I. carnea* and *P. dioscoridis*, respectively) and with the chemical nematicide Oxamyl 24% SL at the recommended dose (3 L/feddan) significantly reduced final population densities (Pf) and reproduction factor (Rf) of *T. semipenetrans* during two subsequent growing seasons (March-September, 2023 and 2024) on Washington Navel orange trees growing in an orchard at Rosetta province, Behera governorate. Oxamyl 24% SL achieved a valuable reduction of Pf reached to 84.07% and 81.08% for the 1st and 2nd seasons, respectively, whereas extracts of *I. carnea, P. dioscoridis* and their combination provided considerable reduction levels of nematode Pf ranged between 79.03-86.48% and 75.64-84.01% for the 1st and 2nd seasons, respectively. It is worthy to note that Co-toxicity factor of the combination of the two extracts recorded the values (-10.2 and -11.87) for the 1st and 2nd seasons, respectively that means there is an additive effect between each other. All extracts alone and their combination appeared to be effective as Oxamyl 24% SL recording a considerable relative nematicidal efficacy (%) ranged from 93.3 to 103.6% of the chemical nematicide. This finding introduced these extracts as promising natural alternatives to Oxamyl that hope to be environmentally safe in the future.

***Keywords*:** citrus nematode, orange, plant extracts, perennial weeds, *Ipomoea carnea*, *Pluchea dioscoridis*, GC-MS, phytochemical composition, nematode management.

INTRODUCTION

Orange, *Citrus sinensis* (L.) Osbeck (family Rutaceae) is one of the most important popular fruit in the horticultural industry to the farmers. Orange fruits are rich source of vitamin C, essential minerals, flavonoids, organic acids and volatile oils. Their nutritional and health

benefits including anti-carcinogenic, cardiovascular & hyperglycemia properties, antioxidant, anti-inflammatory, antiallergic, analgesic, anti-anxiety, antidepressant, antiallergic and anti-microbial activities were reviewed by Abobatta (2019). Egypt is ranked the 5th category among the 10 top oranges producing countries providing 8% (3.7 Million tons in 2023/2024) of the total global oranges production (USDA, 2024).

The citrus nematode (*Tylenchulus semipenetrans* Cobb) is considered one of the most damaging soil pests causing the slow decline disease in orange orchards in Egypt and worldwide resulting in potential fruit yield losses and should be subjected to management (Abd-Elgawad et al., 2016). However, many chemical nematicides achieved good levels of nematode control, but concerns of their wrong application and accumulated residuals in soil and fruit tissues encouraged many nematologists to study other non-chemical methods hope to be more safe. Therefore, numerous research works focused on the isolation and identification of new active compounds derived from plants toxic to nematodes (Desaeger et al., 2020). Plant extracts offer many biochemical substances that are classified as secondary metabolites (including alkaloids, flavonoids, tannins, saponins, glycosides, essential oils, polyphenols, monoterpenoids, diterpenoids, pentacyclic triterpenoids, sesquiterpenes, steroids, glucosinolates, isothiocyanates and fatty acids) that found to be toxic to nematodes. These phytochemical constituents occurred in tissues of many plant species showed considerable and promising nematicidal activities that may have proposed as relatively safe alternatives to the use of synthetic nematicides (Chitwood, 2002; Zhang et al., 2012; Renčo et al., 2014; Chen & Song, 2021 and Mwamula et al., 2022).

Pink morning glory, *Ipomoea carnea* Jacq. (family: Convolvulaceae) and marsh fleabane, *Pluchea dioscoridis* (L.) DC. (family: Asteraceae) are common perennial shrubby weeds naturally grown along river beds, banks and field canals in Egypt (Täckholm, 1974). *I. carnea* is rich in fatty acids, essential oils, phenolics, flavonoids, tannins, glycosides and alkaloids (Shaltout et al., 2006; Adsul et al., 2009 and Sadek, 2015). Many valuable pharmacological and medicinal properties of *I. carnea* and its extract were reviewed by Nusrat et al. (2014) and Kunal et al. (2021). These extracts provide antioxidant, immuno-modulatory effect, anti-diabetic, anti-cancer, hepatoprotective, cardiovascular, sedative, anxiolytic, glycosidase inhibitory, anticonvulsant, peroxide production and wound healing potentials. Moreover, extracts of *I. carnea* exhibit diverse biological effects including antibacterial (Filho et al., 2022), antifungal (Kamal et al., 2017), antiviral (Vats et al., 2020), insecticidal (Nassar et al., 2018), anthelmintic (Dhembare and Kakad, 2015), and mosquitocidal (Adsul et al., 2022) activities.

Similarly, *Pluchea dioscoridis* (earlier *Conyza dioscoridis*) is traditionally used in the folk medicine for treatment of certain human diseases (Awaad et al., 2011, Zain et al., 2012 and Zalabani et al., 2013). Previous phytochemical studies had shown that *Conyza* plants are source of many bioactive constituents such as essential oils, terpenoids, phenolic acids, flavonoids, tannins, saponins, glycosides, steroids and alkaloids that provide a wide range of therapeutic and biological activities including antioxidant, anti-inflammatory, antitumor, analgesic, wound healing, allopathic, antidiabetic, anticonvulsant, anti-amnesic, antiplasmodial, antimicrobial, antiviral and insecticidal effects and these activities are attributed to these bioactive constituents (Opiyo, 2023). The volatile constituents of *P. dioscoridis* had promising antimicrobial activity towards certain species of bacteria and fungi (El-Hamouly & Ibrahim, 2003 and Karray et al., 2020). Great control level of the root-knot nematode, *Meloidogyne incognita* was achieved by *P. dioscoridis* when applied as soil amendment of its shoot powder on sugar beet (El-Nagdi and Abd El Fattah, 2011) and on tomato plants (El-Sherbiny and El-Saedy, 2017). Also, soil amendment with shoot powder of *I. carnea* significantly managed *M. incognita* infection on tomato plants (El-Sherbiny and El-Saedy, 2017). Recently, phytochemical constituents of *I. carnea* leaf extract revealed a potential nematicidal activity

towards *M. incognita* infecting carrot under laboratory conditions and in pot experiments (Abdullah et al., 2023).

Based on the considerable biological activities of the above mentioned weed species, the present study is focused on investigation of their nematicidal performance against *T. semipenetrans* under laboratory conditions and conducting further trials to study their relative nematicidal efficacies as compared to the chemical nematicide Oxamyl 24% SL on Washington Navel orange trees under orchard conditions.

MATERIALS AND METHODS

The current investigation was carried out to study the nematicidal efficacy of different concentrations of crude methanolic extracts of *Ipomoea carnea* and *Pluchea dioscoridis* towards the second stage juveniles (J_2s) of citrus nematode (*Tylenchulus semipenetrans*) under laboratory conditions and furtherly evaluate their nematicidal efficacy, comparing to the recommended chemical nematicide Oxamyl 24% SL in the nematode management on Washington Navel orange trees during two consecutive seasons (March - September, 2023 and 2024).

Plants collection and extraction

Ariel parts of *I. carnea* and *P. dioscoridis* (Fig. 1) including leaves, flowers and/or fruits were freshly collected in the early morning from Abis village, Alexandria during July-August, 2022 and traditionally identified according to Täckholm (1974) and their recent scientific genus, species and family names were provided from updated literature reviews. Weeds were left to air drying in a shade place for 10-15 days and following the full dryness, shoots were coarsely ground in a home mill and a weight (1 kg) of each plant powder was extracted three times in 10 litters of methanol in 2 L Erlenmeyer flasks under laboratory temperature (25 ± 2°C) and dark conditions, then mixed in a home blender, filtered through a muslin layer followed by filtration by Whatman™ grade 1 qualitative filter papers. The filtrate was exposed to the rotary evaporator (RE-111 Buchi Rotavapor and B-461 Water Bath, Artisan Technology Group®, USA) to remove the solvent below 40°C. The yielded dark green plant extracted pastes that had aromatic odor and sticky texture (203.5 and 215.7 g for *I. carnea* and *P. dioscoridis*, respectively) were carefully collected and placed in amber glass bottles and kept in the refrigerator (4-5°C) till carrying out laboratory tests and soil application.

Figure 1: An overview of the studied perennial weeds *Ipomoea carnea* (left) and *Pluchea dioscoridis* (right) naturally growing in Abis village, Alexandria.

Mass Spectrum Gas Chromatography (GC-MS)

Crude extracted plant pastes (approximately 0.2g, each) were sent to the High Institute of Public Health, Alexandria University for analysis their chemical compositions using Gas Chromatography Mass Spectroscopy (GC-MS). Their phytochemical constituents were identified using a Trace 1300 GC Ultra/Mass Spectrophotometer ISQ QD (Thermo Scientific) instrument, X-calibur 2.2 software (Thermo X-calibur) under the optimized conditions illustrated in Table (1).

Table 1: Optimized conditions of GC-MS analysis of studied plant extracts.

Solvent type	Methanol
Carrier gas	Helium (average velocity 39 cm/s)
Flow rate	1 mL/min
Run time	51.41 min
Column type	TG-5MS Zebron capillary column (length 30 cm × 0.25 mm ID, 0.25 µm film thickness; Thermo.
The temperature program of the oven	held at 70°C for 1 min then increased from 70°C to 100°C for 1 min (5°C/min), 100-220°C for 2 min (7°C/min), 220-280°C for 2 min (20°C/min) and 280-300°C (20°C/min) for 3 min.
Injector temperature	275°C
Sample volume	1 µL
Injection type	Split
Split ratio	10:1
Electron impact ionization (EI)	70 electron volts
Scanning range	50-600 m/zat five scans per second
MS libraries	NIST database

Bioassay

A weight (0.5 g) of each plant paste was separately dissolved in 1 ml of a mixture of Dimethyl sulfoxide, Acetone and Tween-80 (1:3:2) in glass vials ca. 10 ml (Wiranto et al., 2009). DAT is an abbreviated name of the above mixture. The tested plant extracts were subjected to study their nematicidal potential towards the second stage juveniles (J_2s) of citrus nematode at the desired concentrations 100, 250, 500, 1000 and 2000 mg/L. Double folds of the desired concentrations (200, 500, 1000, 2000 and 4000 mg/L) of each plant extract were processed by dissolving appropriate volumes of the above mixtures up to 10 ml distilled water to prepare the stock solutions

Second stage juveniles (J_2s) of *T. semipenetrans* were extracted from soil samples that were collected from a nematode-infested orange orchard located at Rosetta province, Behera governorate using a combination of Cobb's sieving and centrifugal sugar floatation techniques (Ayoub, 1980). Extracted nematode J_2s were carefully washed by a gentle stream of tap water to remove sugar residuals, followed by Baermann tray technique (Hooper et al., 2005) for 24 hr in order to maintain the motile J_2s only, and then were collected in 50 ml distilled water. One ml of the active nematode J_2s suspension (containing approximately 600 J_2 in distilled water) were poured into the test glass vials (ca. 10 ml) over 1 ml of double fold of each desired concentration to optimize the studied ones. Juveniles in distilled water and those in DAT mixture were served as check and blank treatments, respectively. All test vials were replicated four times and kept in a clean dark place under laboratory temperature (25°C ±2). Three days later, alive and dead J_2s were microscopically counted and viability of J_2s was examined in all test vials. An aqueous solution (0.062-0.50%) of potassium permanganate was served to stain dead J_2s according to Jatala (1975). Mortality percentages were calculated and corrected using Abbott' formula (Abbott, 1925). The corrected mortality percentages of each plant extract were

subjected to the Probit analysis to obtain their LC_{50} and LC_{95} values according to Finney (1971).

Orchard experiments

Mature (17-18 years old) and nearly uniform Washington Navel orange trees (*Citrus sinensis* (L.) Osbeck) growing in a private orchard located at Rosetta province, Behera governorate were randomly selected and labeled for carrying out this study. Physical and chemical properties of the experimental soil and irrigation water (flood system) of the orchard were illustrated in Table 2. Trees were spaced at 4 x 4 m apart (250 trees/feddan) and they received their cultural practices according to recommendations of the Egyptian ministry of agriculture. Orchard experiments were carried out during the subsequent growing seasons (March - September, 2023) and (March - September, 2024) to evaluate the relative nematicidal potential of crude methanolic shoot extracts of *I. carnea* and *P. dioscoridis* at their LC_{95} values and their combination at half LC_{95} values of each, comparing to the chemical nematicide Oxamyl 24% SL applied at the recommended dose (3 L/feddan) in the management of *T. semipenetrans*.

Weed extracts were prepared for soil application by dissolving the appropriate amounts of crude extracted pastes (adjusted to the studied LC_{95} values) in 4 ml of DAT mixture poured in glass vials (ca. 20 ml), then added up to 4 L water poured in clean plastic bottles (ca. 6 L) and well manually shacked for about 2 min in order to allow pastes to mix properly prior to soil drenching with a proposed dose (4 L/tree).

Table 2: Some physical and chemical properties of the experimental soil and irrigation water.

Soil properties	**Water properties**
Particle size distribution: Sand 93%, Silt 1 % and Clay 6 % (**Textural class**: Sandy soil) pH = 8.26 Electrical conductivity (EC) = 0.918 ds m^{-1} Field capacity (FC) = 16.67% Available water (AW) = 12.50% **Exchangeable cations (meq L^{-1}):** Ca^{2+} (1.925), Mg^{2+} (4.125), Na^{+} (3.135), K^{+} (0.495) **Exchangeable anions (meq L^{-1}):** CO_3 (0), HCO_3 (0.495), Cl (2.75), SO_4 (5.955). Permanent Wilting Point (PWP) = 0.17 Sodium Adsorption Ratio (SAR) = 1.80 Exchangeable Sodium Percentage (ESP) = 1.38 $CaCO_3$ = 0.17	pH = 7.07 EC = 0.429 ds m^{-1}

In general, trees were subjected to the following treatments:

- Check (untreated trees).
- Blank (trees treated with the volume of DAT mixture that was used in dissolving crude pastes and formulating plant extracts (4 ml added up to 4 L water/tree).
- The chemical nematicide, Oxamyl 24% SL was applied at 12 ml added up to 4 L water/tree (equivalent to the recommended dose 3 L/feddan).
- *Ipomoea carnea* extract was applied at 1637 mg/L.
- *Pluchea dioscoridis* extract was applied at 1340 mg/L.
- A combination of *I. carnea* at 819 mg/L + *P. dioscoridis* at 670 mg/L.

Soil drenching with Oxamyl 24% SL was applied once, while weed extracts and blank were applied twice (the 1st drench was at the beginning of the season and the 2nd one was three

months later). All treatments were applied in the early morning in order to maintain nematicidal efficacy of Oxamyl 24% SL and to avoid breakdown of the active phytochemical constituents of weed extracts. Soil application with all treatments was done following regular irrigation time by 2 days to prevent possible leaching of extracts or the chemical nematicide. According to Snedecor and Cochran (1990), the experimental treatments were organized in a randomized complete block design (RCBD) and each treatment was replicated five times (30 trees were selected for each season). At the beginnings and the endings of each season, soil and root samples of all trees were collected according to Barker (1985). Five sub-samples were collected from 10-30 cm depth of each tree to form composite sample (approximately 2 kg), which were thoroughly mixed and a representative weight (400 g) was subjected to nematode extraction according to Ayoub (1980).

Roots associated with each soil sample were gently washed with tap water to discard soil particles and about 10 g per tree were cut into 1 cm long segments and then gently macerated in 200 ml tap water using a home blender for 1-2 minutes to extract attached adult females and other developmental stages of nematode from root surface (Southey, 1970). Initial and final population densities of nematode (Pi and Pf, respectively) including population of active J_2s / kg soil + number of the attached adult females and other developmental stages of nematode per 10 g fresh root, were recorded and the nematode reproduction factor (Rf) was calculated according to the formula: Rf = Pf ÷ Pi (Oostenbrink, 1966).

Reduction percentages (R%) of nematode Pf were determined and calculated using the formula proposed by Mulla et al. (1971) as the following:

$$\mathrm{R}\ (\%) = 100 - \left[\left(\frac{\mathrm{C1}}{\mathrm{T1}}\right) \mathrm{x}\ \left(\frac{\mathrm{T2}}{\mathrm{C2}}\right)\right] \mathrm{x}\ 100$$

where: C_1 = nematode density in control before application.
C_2 = nematode density in control after application.
T_1 = nematode density in treatment before application.
T_2 = nematode density in treatment after application.

Finally, reduction percentages of all treatments were corrected to the blank treatment using adapted Abbott's formula (Abbott, 1925), relative nematicide efficacy (RNE %) of all plant extracts and their combination to the chemical nematicide Oxamyl 24% SL, and Co-Toxicity factor of the combination of the two extracts were calculated according to the following formulas:

$$\text{Corrected reduction (\%) to blank} = \left(\frac{\text{R\% of treatment} - \text{R\% of blank}}{100 - \text{R\% of blank}}\right) \mathrm{X}\ 100$$

$$\mathrm{RNE}\ (\%) = \left[1 - \left(\frac{\text{R\% of nematicide} - \text{R\% of plant extract}}{\text{R\% of nematicide}}\right)\right] \mathrm{x}\ 100$$

$$\mathrm{Co - Toxicity\ factor} = \left(\frac{\text{Observed effect (\%)} - \text{Expected effect (\%)}}{\text{Expected effect (\%)}}\right) \mathrm{X}\ 100$$

Expected effect (%) is calculated using Limpel´s formula according to Richer, 1987 as follows:

$$\mathrm{E} = (\mathrm{X} + \mathrm{Y}) - \left(\frac{\mathrm{XY}}{100}\right)$$

where:
E = the expected additive effect of the combination of the two extracts.
X = the observed effect due to the extract 1 alone.
Y = the observed effect due to the extract 2 alone.

Co-Toxicity factor was served to classify results into three different categories. A positive factor (+20 or more) means synergism, a negative factor (-20 or less) is considered antagonism, and the intermediate values between (–20 and +20) indicated that the combination had an additive effect between each other (Mansour et al., 1966).

Data collection and statistical analysis
Analysis of variance (ANOVA) of the obtained results from the bioassay and those given from orchard experiments were statistically analyzed and differences between treatments were performed according to LSD values at the 0.05% level of probability using SAS software (SAS, 1997).

RESULTS AND DISCUSSION

Results of the bioassays revealed that all the studied concentrations of all weed extracts were significantly (*P*=0.05) suppressed J_{2s} viability causing mortality percentages ranged between 13.82 and 100% as compared to check and blank treatments. Generally, extract of *P. dioscoridis* (LC_{50} = 249 mg/L) appeared to be stronger than *I. carnea* (LC_{50} = 345 mg/L) in killing nematode (Table 3). Mortality (%) of J_2s was characterized as dead juveniles that had erected body shape as compared to the alive ones that had curved body shape (Fig. 2) and it linearly (P = 0.0001) increased with increasing the concentrations of the weed extracts (Fig. 3). Nematicidal properties of *I. carnea* and *P. dioscoridis* were previously reported on the root-knot nematode, *M. incognita* by some authors (El-Nagdi and Abd El Fattah, 2011; El-Sherbiny & El-Saedy, 2017; Abdullah et al., 2023). Thus, nematicidal activity of these weed extracts was confirmed on the citrus nematode in the present study. It was noticed that blank treatment that contained a mixture of Dimethyl sulfoxide, Acetone and Tween-80 (1:3:2) at low concentration (4 ml/ 4 L water) gave low and not significant reduction of nematode Pf comparing to the untreated check trees reached to 5.16% (Table 4) and 6.02% (Table 5) for the 1st and 2nd seasons, respectively. This observation confirmed that the nematicidal performance of these weed extracts is definitely attributed to their chemical constituents.
Data of soil application with weed extracts alone and in their combination on the orange trees under orchard conditions showed that all treatments significantly (P=0.05) reduced final populations (Pf) and reproduction factor (Rf) of nematode as compared to those recorded in check and blank treatments along the two experimental seasons (Tables 4&5). The chemical nematicide Oxamyl 24% SL achieved a valuable reduction of Pf reached to 84.07% and 81.08% for the 1st and 2nd seasons, respectively, whereas extract of *I. carnea*, *P. dioscoridis* and their combination provided considerable reduction levels ranged between 79.03-86.48% and 75.64-84.01% for the 1st (Table 4) and the 2nd seasons (Table 5), respectively. It was clearly that all extracts and their combination appeared to be effective as Oxamyl 24% SL recording promising RNE % ranged from 93.3 to 103.6% of the chemical nematicide. Fortunately, these findings are very similar to those reported by other authors during their studies on phytochemical management of root-knot nematodes on some plant hosts using certain plant species and seaweeds that applied as soil amendments (Ibrahim & Ibrahim, 2000; El-Sherbiny & El-Saedy, 2017). Co-toxicity factor of the two extracts combination recorded the values -10.2 (Table 4) and -11.87 (Table 5) for the 1st and 2nd seasons, respectively and that means there is an additive effect between each other.

Table 3: Corrected mortality (%)* of second stage juveniles (J_2s) of *Tylenchulus semipenetrans* exposed for 72 hr to different concentrations of crude methanolic shoot extracts of the perennial weeds *Ipomoea carnea* and *Pluchea dioscoridis* under laboratory conditions (25 ± 2°C).

Plant extract (E)	Control (distilled water)	Blank (DAT mixture)	Concentration (C) at mg/L					Overall mean (E)	LC_{50} (mg/L)	Fiducial limits (mg/L)	LC_{95} (mg/L)	Fiducial limits (mg/L)	Slope	Chi square
			100	250	500	1000	2000							
Ipomoea carnea	2.58^{k}	10.96^{j}	13.82^{i}	35.04^{g}	55.81^{e}	88.14^{c}	100^{a}	43.76^{B}	345	303 - 393	1637	1283 - 2091	2.433 ± 3.38-02	9.56
Pluchea dioscoridis			21.89^{h}	49.87^{f}	67.69^{d}	92.54^{b}	100^{a}	49.36^{A}	249	215 - 288	1340	1036 - 1737	2.249 ± 3.30-02	6.07
Overall mean (C)	2.58^{G}	10.96^{F}	17.86^{E}	42.45^{D}	61.75^{C}	90.34^{B}	100^{A}							

Data are means of four replicates.
J_2s mortality (%) = (No. dead juveniles ÷ total No. juveniles) x 100.
* J_2s mortality (%) was corrected using Abbott's formula (Abbott, 1925), where distilled water and blank were served as checks.
Percentages of J_2s mortality within a column/row superscripted by the same small or capital letter are not significantly different at P= 0.05.
LC_{50} and LC_{95} values were obtained from the Probit analysis (Finney, 1971).
LSD values of E, C and EC were 1.84, 3.44 and 2.31, respectively.

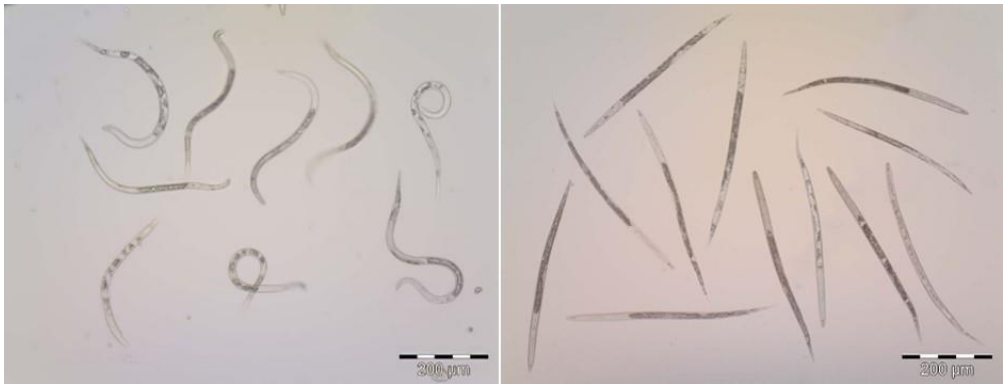

Figure 2: Dead second stage juveniles (right) of *Tylenchulus semipenetrans* exposed to crude methanolic extracts of *Ipomoea carnea* or *Pluchea dioscoridis* at 2000 mg/L for 72 hr versus check alive ones (left).

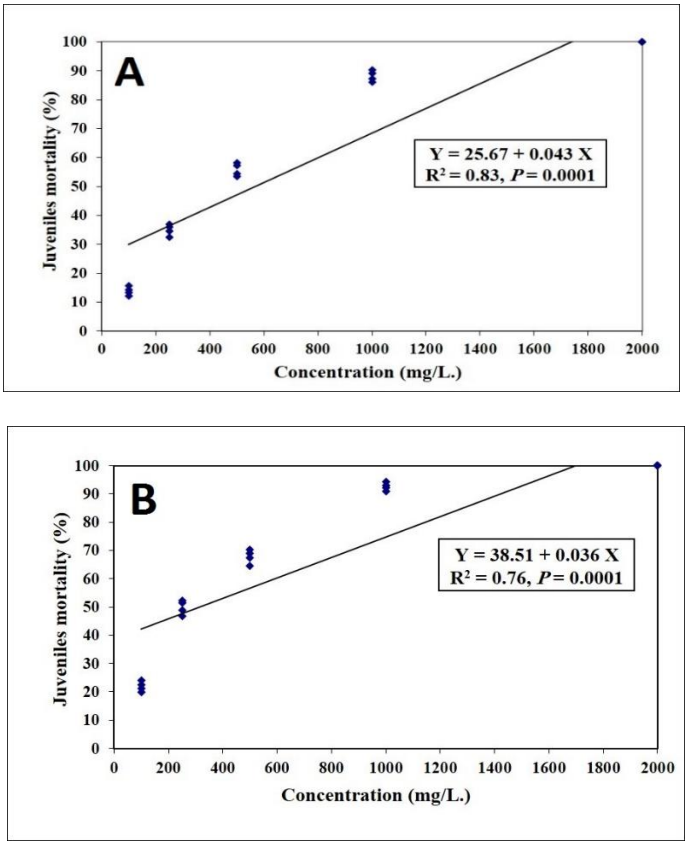

Figure 3: Linear regression models of juveniles mortality (%) of *Tylenchulus semipenetrans* caused by different concentrations of crude methanolic shoot extracts of *Ipomoea carnea* (A) and *Pluchea dioscoridis* (B) after 72 hr exposure time.

Table 4: Effect of soil application of *Ipomoea carnea* and *Pluchea dioscoridis* extracts at their LC_{95} values and their combination at half LC_{95} values of each, as compared to Oxamyl 24% SL for managing *Tylenchulus semipenetrans* on Washington Navel orange trees under orchard conditions during the 1st season (March - September, 2023).

Treatment	Pi ± SD	Pf	Reduction (%)*	Rf	RNE (%)	Co-Toxicity Factor
Untreated check (control)	14154 ± 1683	45686[a]	-	3.24[a]	-	
Blank	13937 ± 1482	43330[a]	5.16	3.12[a]	-	
Oxamyl 24% SL (3 L/feddan)	13258 ± 2124	6468[bc]	84.07	0.49[c]	-	
Ipomoea carnea at 1637 mg/L	14569 ± 2065	9353[b]	79.03	0.64[b]	94.0	
Pluchea dioscoridis at 1340 mg/L	12748 ± 1785	6885[bc]	82.36	0.54[bc]	98.0	
I. carnea at 819 mg/L + *P. dioscoridis* at 670 mg/L	14435 ± 2122	5972[c]	86.48	0.42[c]	102.9	-10.2 (additive effect)
LSD at $P = 0.5$		3204		0.1449		

Data are means of five replicates. Values within a column superscripted by the same letter(S) are not significantly different. Pi (initial population) and Pf (final population) of nematode J_2s in kg soil + No. attached adult females and other developmental stages of nematode per 10 g fresh root. Rf (Reproduction factor) = Pf ÷ Pi.

* Reduction (%) of nematode Pf was corrected to the check treatment using Mulla's formula, followed by correction to the blank treatment using adapted Abbott's formula.

RNE (%) = Relative Nematicidal Efficacy (%) of the plant extract to Oxamyl 24% SL.

Table 5: Effect of soil application of *Ipomoea carnea* and *Pluchea dioscoridis* extracts at their LC_{95} values and their combination at half LC_{95} values of each, as compared to Oxamyl 24% SL for managing *Tylenchulus semipenetrans* on Washington Navel orange trees under orchard conditions during the 2nd season (March - September, 2024).

Treatment	Pi ± SD	Pf	Reduction (%)*	Rf	RNE (%)	Co-Toxicity factor
Untreated check (control)	12055 ± 1690	42422[a]	-	3.53[a]	-	
Blank	11610 ± 1607	39867[a]	6.02	3.46[a]	-	
Oxamyl 24% SL (3 L/feddan)	10948 ± 1823	6851[b]	81.08	0.63[c]	-	
Ipomoea carnea at 1637 mg/L	11782 ± 2385	9492[b]	75.64	0.82[b]	93.3	
Pluchea dioscoridis at 1340 mg/L	10558 ± 1890	6711[b]	80.78	0.64[c]	99.6	
I. carnea at 819 mg/L + *P. dioscoridis* at 670 mg/L	11671 ± 1974	6171[b]	84.01	0.53[c]	103.6	-11.87 (additive effect)
LSD at $P = 0.5$		3785		0.1765		

Data are means of five replicates. Values within a column superscripted by the same letter(S) are not significantly different. Pi (initial population) and Pf (final population) of nematode J_2s in kg soil + No. attached adult females and other developmental stages of nematode per 10 g fresh root. Rf (Reproduction factor) = Pf ÷ Pi.

* Reduction (%) of nematode Pf was corrected to the check treatment using Mulla's formula, followed by correction to the blank treatment using adapted Abbott's formula.

RNE (%) = Relative Nematicidal Efficacy (%) of the plant extract to Oxamyl 24% SL.

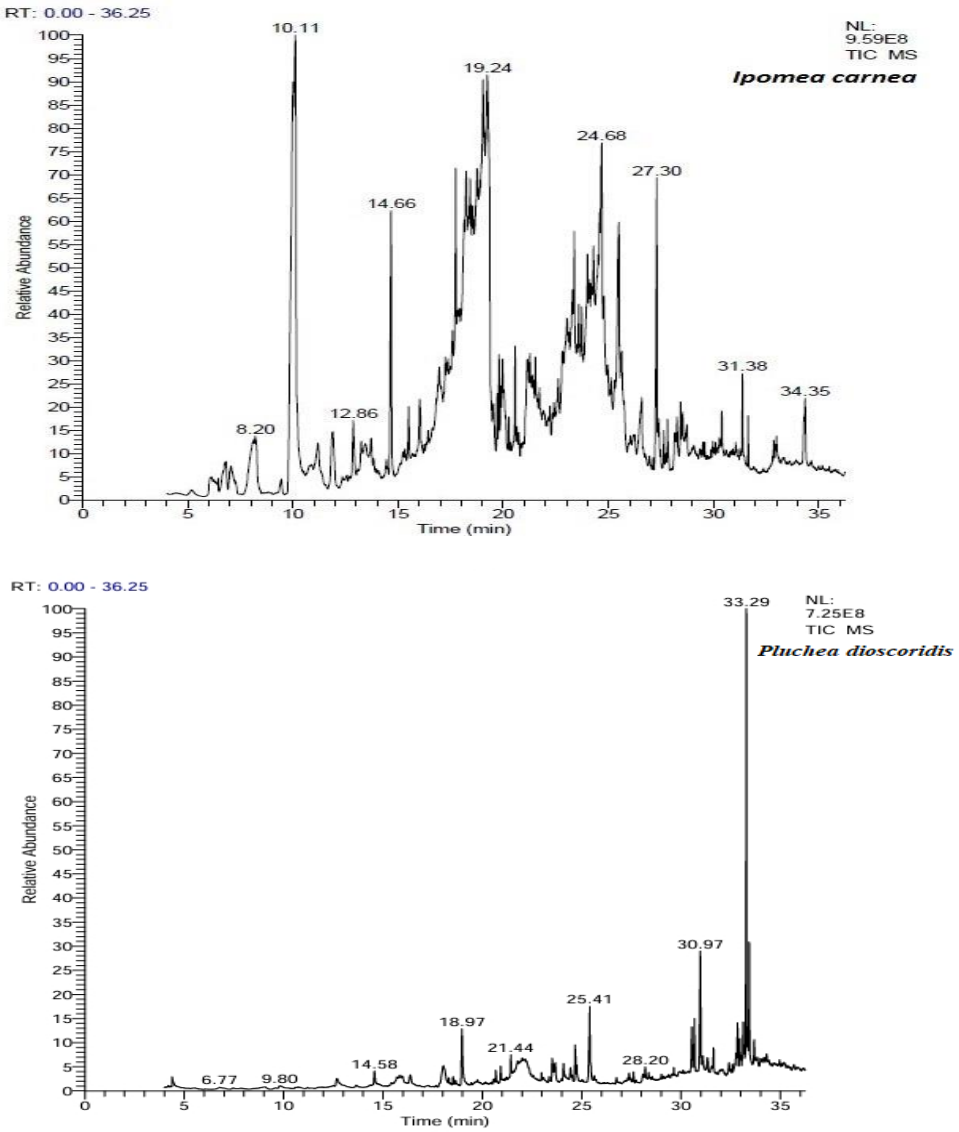

Figure 4: GC-MS chromatograms of crude methanolic shoot extracts of *Ipomoea carnea* and *Pluchea dioscoridis*.

Results of GC-MS analysis of *I. carnea* extract (Fig. 4) revealed the presence of 33 chemical compounds (Table 6). It was appeared that they were similar to those given by several authors *(*Kar et al., 2018*;* Abd-ElGawad et al., 2022; Elhefni et al., 2023; Javaid et al., 2023). Cyclopentane, 1-acetyl-1,2 epoxy; 2-Octenal, (E); and Thymol were the most abundant chemical compounds occurred in the extract of *I. carnea* recording the area percentages 13.65, 12.21 and 5.79%, respectively to represent >30% of the total constituents (Table 6). Cyclopentane, 1-acetyl-1,2-epoxy- (syn. Heptadienoic acid) was previously identified in the extract of other related species of *Ipomoea* and possess considerable antiviral (Padmashree et al., 2018a) and antibacterial (Padmashree et al., 2018b) activities. Unfortunately, no available information regarding its nematicidal properties so far. However, such related compounds namely Trans-1,2-Diethyl cyclopentane and Cyclopentane, 1-methyl-2-propyh- that identified in the extract of *Azadirachta indica* found to possess nematicidal against the root-knot nematodes, *Meloidogyne* spp. (Haroon et al., 2018).

Table 6: GC-MS analysis of crude methanolic shoot extract of *Ipomoea carnea.*

Retention time (min.)	Compound name	Area (%)	Molecular formula	Molecular Weight
8.14	DL-2-Aminoadipic acid	2.85	$C_6H_{11}NO_4$	161
8.20	Valeric acid	1.53	$C_5H_{10}O_2$	102
9.99	Cyclopentane, 1-acetyl-1,2 epoxy	13.65	$C_7H_{10}O_2$	126
10.11	2-Octenal, (E)	12.21	$C_8H_{14}O$	126
11.89	5-Hydroxypipecolic acid	2.11	$C_6H_{11}NO_3$	145
12.87	Phenylacetaldehyde	1.43	C_8H_8O	120
14.66	Thymol	5.79	$C_{10}H_{14}O$	150
16.02	Methyl Cinnamate	1.25	$C_{10}H_{10}O_2$	162
16.95	Ethanamine, 2 (2,6-dimethylphenoxy)-N-methyl. (syn. Methylephedrine)	2.32	$C_{11}H_{17}NO$	179
17.73	9-Hexadecenoic acid, methyl ester, (Z)-	2.48	$C_{17}H_{32}O_2$	268
18.13	Cyclohexyl methanesulfonate	1.39	$C_7H_{14}O_3S$	178
18.23	Lauric acid	1.62	$C_{12}H_{24}O_2$	200
18.43	Octanoic acid, 2-chlorophenyl ester	0.90	$C_{14}H_{19}ClO_2$	254
19.03	1-Methylpyrrolidine 2-carboxylic acid	2.04	$C_6H_{11}NO_2$	129
19.27	7-Hydroxyheptanoic acid	5.18	$C_7H_{14}O_3$	146
19.32	Allyl acetate	3.62	$C_5H_8O_2$	100
19.71	cis-5,8,11,14,17-Eicosapentaenoic acid	0.60	$C_{20}H_{30}O_2$	302
19.89	2,4-Dodecadienal, (E,E)-	0.57	$C_{12}H_{20}O$	180
19.97	9-Hexadecenoic acid	2.85	$C_{16}H_{30}O_2$	254
20.56	10,12-Octadecadiynoic acid	1.51	$C_{18}H_{28}O_2$	276
21.14	Dodecanal	2.82	$C_{12}H_{24}O$	240
21.27	Linolenic acid ethyl ester	1.55	$C_{20}H_{34}O_2$	306
24.00	Linoleic acid	3.80	$C_{18}H_{32}O_2$	280
24.29	Caryophyllene oxide	2.43	$C_{15}H_{24}O$	220
24.68	Hexadecanoic acid, methyl ester (syn. Methyl palmitate)	4.12	$C_{17}H_{34}O_2$	270
25.49	Undecanoic acid	4.06	$C_{11}H_{22}O_2$	186
26.58	Tetradecanoic acid	1.48	$C_{14}H_{28}O_2$	228
27.29	cis-11,14-Eicosadienoic acid methyl ester	5.58	$C_{21}H_{38}O_2$	322
27.81	Methyl 10,11-tetradecadienoate	0.82	$C_{15}H_{26}O_2$	238
30.39	Oleic Acid	1.31	$C_{18}H_{34}O_2$	282
31.38	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	1.17	$C_{19}H_{38}O_4$	330
31.65	Diisooctyl phthalate	0.45	$C_{24}H_{38}O_4$	390
34.35	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan4yl) methyl ester, cis	1.75	$C_{28}H_{44}O_4$	444
Total identified :		97.24		

On the other hand, methanolic shoot extract of *P. dioscoridis* was rich in different bioactive compounds. Totally, 36 phytochemical constituents (Fig. 4) were identified (Table 7). Ethanone, 1-(2-methylcyclopropyl)- (syn.2-Hexenal, (E)-), Cholestan-3-ol, 2-methylene-, (3á,5à)- (syn. Campesterol), cis-5,8,11,14,17-Eicosapentaenoic acid (syn. abietic acid), and Undecanoic acid were the most abundant chemical constituents recording the area percentages 21.00, 13.79, 12.34 and 5.61%, respectively to represent >50% of the total identified compounds. Results of the GC-MS analysis of *P. dioscoridis* extract in the present study agree with those provided by other authors (Ibrahim et al., 2017; Diab et al., 2021; Elgamal et al., 2021; Madboly et al., 2023).

Fortunately, nematicidal properties of the phytochemical constituents 2-Octenal, (E)- (Aissani, 2013), 2-Hexenal, (E)- (Lu et al., 2017), Thymol (Tsao and Yu, 2000; Lei et al., 2010; Nasiou & Giannakou, 2023), Lauric and Oleic acids (Zhang et al., 2012), Undecanoic acid (Cruz-Estrada et al., 2019), Dodecanal (Kim et al., 2008), Linoleic acid (Stadler et al., 1993),

Table 7: GCMS analysis of crude methanolic shoot extract of *Pluchea dioscoridis*.

Retention time (min.)	Compound name	Area (%)	Molecular Formula	Molecular Weight
12.68	Phenol, 4-ethenyl -acetate	0.77	$C_{10}H_{10}O_2$	162
14.58	Phenylacetic acid	1.00	$C_8H_8O_2$	136
15.71	2,3,4,5,6-Pentahydroxy-hexanoic acid propylamide	0.64	$C_9H_{19}NO_6$	237
15.85	Caryophyllene	1.20	$C_{15}H_{24}$	204
16.37	Cyanoacetamide	0.89	$C_3H_4N_2O$	84
18.04	3-Nitrobenzyl alcohol	2.74	$C_7H_7NO_3$	153
18.98	3-hydroxy-4-[(2-methyl-5-nitrophenyl)diazenyl]-*N*-phenylnaphthalene-2-carboxamide	4.46	$C_{24}H_{18}N_4O_4$	426
20.92	Geraniol	1.01	$C_{15}H_{26}O$	222
21.44	1-Octadecyne	1.03	$C_{18}H_{34}$	250
22.01	9-Hexadecenoic acid (syn. Palmitoleic Acid)	3.16	$C_{16}H_{30}O_2$	254
22.07	Cyclohexanone, 4-methyl-	1.02	$C_7H_{12}O$	112
22.15	Pregnanolone	3.64	$C_{21}H_{34}O_2$	318
23.61	Heptanophenone	1.18	$C_{13}H_{18}O$	190
23.67	Caryophyllene oxide	2.24	$C_{15}H_{24}O$	220
24.08	2,5-Octadecadiynoic acid, methyl ester	1.83	$C_{19}H_{30}O_2$	290
24.44	2-Ethyl-5-oxohexyl phthalate	0.90	$C_{16}H_{20}O_5$	292
24.67	9-pentadecenoic acid	2.05	$C_{15}H_{28}O_2$	240
24.75	Dibutyl phthalate	0.91	$C_{16}H_{22}O_4$	278
25.40	Undecanoic Acid	5.61	$C_{11}H_{22}O_2$	186
27.39	9-Octadecenoic acid (Z), methyl ester	0.47	$C_{19}H_{36}O_2$	296
27.60	9-Hexadecenoic acid, methyl ester, (Z)-	0.54	$C_{17}H_{32}O_2$	268
28.08	Linoleic acid	0.71	$C_{18}H_{32}O_2$	280
28.37	Raffinose	0.38	$C_{18}H_{32}O_{16}$	504
29.64	Cyclopropanedodecanoic acid, 2-octyl-, methyl ester	0.58	$C_{24}H_{46}O_2$	366
30.68	4-Butoxybiphenyl	1.97	$C_{16}H_{18}O$	226
31.09	Linolenic acid ethyl ester	2.17	$C_{20}H_{34}O_2$	306
31.33	Silane, dimethyl (2-naphthoxy) hexadecyloxy-	1.14	$C_{28}H_{46}O_2Si$	442
31.63	Diisooctyl phthalate	1.38	$C_{24}H_{38}O_4$	390
32.57	2-Hexadecanol	0.25	$C_{16}H_{34}O$	242
32.84	Farnesoic acid	2.61	$C_{15}H_{24}O_2$	236
33.28	Ethanone, 1-(2-methylcyclopropyl)- (syn. 2-Hexenal, (E)-)	21.0	$C_6H_{10}O$	98
33.43	cis-5,8,11,14,17-Eicosapentaenoic acid (syn. Abietic acid)	12.34	$C_{20}H_{30}O_2$	302
33.68	Cholestan-3-ol, 2-methylene-, (3á,5à)- (syn. Campesterol)	13.79	$C_{28}H_{48}O$	400
33.83	4,9-Decadienoic acid, 2-nitro-, ethyl ester	0.91	$C_{12}H_{19}NO_4$	241
34.17	Involucrin	1.79	$C_{27}H_{38}O_8$	490
34.30	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester,cis-	1.00	$C_{28}H_{44}O_4$	444
Total identified		99.31		

Allyl acetate (Munakata et al., 1959), *Phenylacetaldehyde* (Aissani, 2013 and Wang et al., 2021), Methyl palmitate (Lu et al., 2020) and Caryophyllene oxide (Devrani et al., 2024) occurred in extracts of *I. carnea* and *P. dioscoridis* were reported in the literature.

Therefore, the nematicidal activity of *I. carnea* or *P. dioscoridis* extracts in this study is attributed to the above mentioned constituents either individually or in their combinations. However, Abietic acid found in *P. dioscoridis* extract has been reported as a nematode attractant (Ohri and Kaur, 2009).

In general, mode of nematicidal action of many plant extracts and their constituents was previously discussed by some authors. As several chemical nematicides act their nematicidal performance through inhibition of nematode acetylcholinesterase "AChE" (Ebone et al.,

2019), the alkaloids, essential oils and other phytochemical constituents were also employed in *in vitro* AChE bioassays (Korayem et al., 1993; Ebadollahi et al., 2021; Coqueiro et al., 2023).

The current study introduced these weed extracts as promising natural alternatives to Oxamyl 24% SL hope to be potentially effective in *T. semipenetrans* management and environmentally safe in the future.

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الملخص العربي

الفعالية الإبادية للنيماتودا لمستخلصات حشائش الآيبوميا والبرنوف وإمكانية استخدامهما في مكافحة نيماتودا التدهور البطيء في الموالح على أشجار البرتقال أبو سُرّة

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تمت دراسة الفعالية الإبادية للنيماتودا للمستخلصات الميثانولية الخام للمجاميع الخضرية للحشائش المُعَمِّرَة (الآيبوميا والبرنوف) عند تركيزات ١٠٠، ٢٥٠، ٥٠٠، ١٠٠٠، ٢٠٠٠ ملليجرام / لتر ضِد يرقات الطور الثاني لنيماتودا التدهور البطيء في الموالح تحت ظروف المعمل. أظهرت النتائج أن جميع التركيزات المدروسة قد تسببت في موت اليرقات بنسب معنوية تراوحت من ١٣,٨٢ إلى ١٠٠% ، مقارنة بمعاملتي الشاهد والبلانك، وقد زادت نسبة موت اليرقات معنوياً بزيادة التركيزات المختبرة . وقد تبين أن مستخلص البرنوف كان أقوى معنوياً من مستخلص الآيبوميا ، حيث أن التركيز القاتل لـ ٥٠% من اليرقات لكلا المستخلصين قد بلغ ٢٤٩ ، ٣٤٥ ميلليجرام/لتر ، على الترتيب. أظهرت نتائج تحليل المكونات الكيميائية للمستخلصات تحت الدراسة بتقنية كروماتوغرافيا الغاز - مطياف الكتلة وجود عدد من المكونات الكيميائية بلغ ٣٣ مركب، و ٣٦ مركب لمستخلص الآيبوميا و البرنوف على الترتيب. وقد كانت كل من المركبات الكيميائية Thymol ، 2-Octenal, (E) ، Cyclopentane, 1-acetyl-1,2 epoxy أعلى المركبات تواجداً في مستخلص الآيبوميا، بينما كانت كل من المركبات Ethanone, 1-(2-methylcyclopropyl)- ، Cholestan-3-ol, 2- cis-5,8,11,14,17-Eicosapentaenoic acid ، methylene-, (3á,5à)- و Undecanoic acid هي الأعلى نسبة في مستخلص البرنوف.

أُجريت تجربتين حقليتين في إحدى بساتين البرتقال أبو سُرّة في مركز رشيد – محافظة البحيرة خلال موسمين زراعيين متتاليين (مارس – سبتمبر لعامي ٢٠٢٣ ، ٢٠٢٤م)، لدراسة كفاءة التطبيق الحقلي لتلك المستخلصات عند التركيز القاتل لـ ٩٥% من اليرقات (١٦٣٧ ، ١٣٤٠ ميلليجرام/لتر لكل من الآيبوميا والبرنوف على الترتيب) وكذا مخلوطهما معاً عند أنصاف قيم التركيز القاتل لـ ٩٥% من اليرقات (٨١٩ ، ٦٧٠ ميلليجرام/لتر لكل منهما على التوالي). وقد أظهرت النتائج أن جميع المعاملات قد حققت خفض معنوي كبير في كل من الكثافة العددية النهائية للنيماتودا تراوحت بين ٧٩,٠٣ – ٨٦,٤٨% للموسم الأول و ٧٥,٦٤ – ٨٤,٠١% للموسم الثاني ، مقارنة مع معاملتي الشاهد والبلانك. ومن ناحية أخرى أدت المعاملة بمبيد النيماتودا الكيميائي أوكساميل ٢٤% عند الجرعة الموصى بها ٣ لتر/الفدان (معاملة للمُقارنة) إلى نسبة خفض معنوي في الكثافة العددية النهائية للنيماتودا بلغت ٨٤,٠٧ ، ٨١,٠٨% خلال موسمي الدراسة على الترتيب.

جدير بالذكر أن مخلوط كلا المستخلصين معاً قد حقق قيمة عامل السُمية (Co-toxicity factor) بلغت -١٠,٢ ، -١١,٨٧ في كل من الموسم الأول والثاني على الترتيب ، الأمر الذي يشير إلى أن للمستخلصين تأثير إضافي لبعضهما البعض، كما أن المعاملة الحقلية بكل من المستخلصين بمفردهما وبمخلوطهما معاً قد حققوا كفاءة إبادية للنيماتودا تراوحت بين ٩٣,٣- ١٠٣,٦% من كفاءة مبيد الأوكساميل ٢٤% ، وهذه تعتبر نتائج واعدة لإعتبار تلك المستخلصات بدائل طبيعية لمبيد الأوكساميل ٢٤% يُرجَى أن تكون آمنة على البيئة في المستقبل.