Research Article

Possible Application of *Hyoscyamus albus*, *Mercurialis annua* and *Volutaria tubuliflora* Extracts as Green Nematicides in Managing *Meloidogyne incognita* Infecting Newly Planted Guava Seedlings

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Abstract | Methanolic shoot extracts of *Hyoscyamus albus*, *Mercurialis annua* and *Volutaria tubuliflora* at the concentrations 100, 250, 500, 1000 and 2000 mg L^{-1} significantly exhibited real mortality (12.4 - 100%) of second stage juveniles of the root-knot nematode *Meloidogyne incognita* under *in vitro* conditions. The nematicidal action of *H. albus* extract (LC_{50} = 302 mg L^{-1}) appeared to be the best, followed by *V. tubuliflora* (LC_{50} = 390 mg L^{-1}) and *M. annua* (LC_{50} = 465 mg L^{-1}). GC-MS analysis of the studied plant extracts revealed presence of palmitic acid, linoleic acid methyl ester, linolenic acid methyl ester, phytol, linoleic acid, hyoscyamine, diethylhexyl phthalate, hentriacontane, octacosane, tetratetracontane and β-sitosterol as prevalent bioactive compounds occurred in *H. albus* extract. *M. annua* extract was rich in linolenic acid methyl ester and carbonic acid eicosyl vinyl ester, whereas docosahexaenoic acid methyl ester and linolenic acid methyl ester were the most abundant constituents of *V. tubuliflora*. Considerable levels of *M. incognita* management on guava seedlings (Banaty cv.) were achieved by soil treatment with all the studied plant extracts either individually applied at their LC_{95} values and/or their binary and ternary combinations applied at half and one third of LC_{95} values along two consecutive seasons, recorded relative nematicidal efficacy (81.14 - 102%) of the nematicide Oxamyl 24% that was served as a comparative chemical treatment. Co-toxicity factors (ranged from -8.35 to -12.10) of plant extract combinations along the studied seasons confirmed that the examined extracts had an additive effect between each other. Growth performance of guava seedlings and their leaves content of chlorophylls a, b and the total one were significantly increased in all treatments as compared to those of untreated check and/or blank treatments. These findings indicate that the tested plant extracts could be suggested as hopeful alternatives to Oxamyl 24% SL in the future management of *M. incognita* on newly planted guava.

Received | June 30, 2025; **Accepted** | August 10, 2025; **Published** | November 18, 2025
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Citation | El-Sherbiny, A.A. and S.E. Hammad. 2025. Possible application of *Hyoscyamus albus*, *Mercurialis annua* and *Volutaria tubuliflora* extracts as green nematicides in managing *Meloidogyne incognita* infecting newly planted guava seedlings. *Pakistan Journal of Nematology*, 43(2): 158-173.
DOI | https://dx.doi.org/10.17582/journal.pjn/2025/43.2.158.173
Keywords | .....?

Introduction

Guava, *Psidium guajava* L. (family Myrtaceae) is one of the most economic and popular fruit trees extensively planted in Egypt and many tropical and sub-tropical regions worldwide. Guava fruits are good source of ascorbic acid (vitamin C), other vitamins, minerals, protein and carbohydrates. Great nutritional and pharmacological benefits of guava including antioxidant, antidiabetic, anti-hyperglycemic, antipyretic, anti-hypertensive, analgesic, hypolipidemic, hepatoprotective, antidiarrheal, anti-allergy, laxative, spasmolytic, anti-inflammatory, anticancer, immuno modulatory, antiparasitic, antibacterial, antifungal, anti-malarial, wound healing and cough and cold curing activities were reviewed (Uzzaman *et al*., 2018; Gavhane *et al*., 2022).

Egypt is ranked the 33rd class among the guava producing countries providing 135.1 k tons of the total global guava production for 2022 (https://worldpopulationreview.com/countryrankings/guava-production-by-country).

Phytoparasitic nematodes (PPNs) as well as the root-knot nematodes (RKNs), *Meloidogyne* spp. are of great importance as a real threat of guava and other fruit trees production worldwide. RKNs are considered seriously damaging soil pest of guava trees that may resulting in potential yield loss (Abd-Elgawad, 2014). Thus, it needs intensive attention of the growers and researchers to manage their damage on guava trees. Despite synthetic nematicides providing great levels of nematode management, but fears of their wrong use and possible risks of their accumulated residuals in soil and/or fruit tissues that caused environmental pollution and damaging human health, inspire many researchers all over the world to investigate other non-chemical approaches hope to be highly effective and more safe. Natural nematicides that are developed from plant extracts origin are of the promising alternatives.

Many alkaloids, fatty acids, tannins, saponins, polyphenols, flavonoids, steroids, glycosides, essential oils, monoterpenoids, diterpenoids, triterpenoids, sesquiterpenes, glucosinolates and isothiocyanates that naturally delivered from many plant species as secondary metabolites were found to possess toxic bioactive constituents that effectively suppressed nematodes. These phytochemical compounds may have proposed as renewable and hopeful source of botanical nematicides expected to be relatively safe than the synthetic ones (Chen and Song, 2021; Mwamula *et al*., 2022).

Wild plants naturally grow and widely distribute in deserts and uncultivated lands following rain falls are considered a promising source of phytochemical compounds effectively suppress nematodes (Renčo *et al*., 2014).

White henbane, *Hyoscyamus albus* (family Solanaceae) is an annual or a biennial toxic plant wildly grows in dry uncultivated grounds and field margins. Previous studies on *H. albus* showed that it has a unique chemical composition including alkaloids, terpenoids, flavonoids, tannins (Mobin *et al*., 2015) and fatty acids (Keskin *et al*., 2016). These phytochemical compounds exerted many valuable pharmacological and biological activities included antioxidant, anticancer, antiallergic, antidepressant, antihyperuricemic, anticonvulsant, antiasthmatic, antisecretory, antidiabetic, antidiarrhoeal, Ca^{2+} channel blocking, cardioprotective, hepatoprotective, antihyperuricemic, hypotensive, anti-Parkinsonian, insecticidal, antimicrobial and antifungal activities (Alghazeer *et al*., 2012; Bonde and Pinjari, 2023). Recently, good insecticidal activity of *H. albus* extract against *Aphis gossypii* and *Phenacoccus solenopsis* was achieved by Eldesouky *et al*. (2024).

Dog's mercury *Mercurialis annua* (family Euphorbiaceae) is an annual wild herb grows in desert and semi-arid regions. *M. annua* has been naturally used in the folk medicine for its diuretic, laxative, antiemetic and antioxidant properties, in addition to its benefits in the treatment of warts, eye microbial infections (Doukkali *et al*., 2016). Moreover, promising anticancer, anti-inflammatory, and antimicrobial potentials of different shoot extracts of *M. annua* were reported by Assaf *et al*. (2013) and Al-Douri *et al*. (2022). Other considerable biological activities of *M. annua* extracts included strong insecticidal activity against *Tribolium confusum* (Nasr *et al*., 2021) and high antimicrobial activities towards certain species of human-pathogenic bacteria and fungi (Aboukhalaf *et al*., 2024) were recently reported. Certain flavonoids, coumarins, phenolic acids, alkaloids, terpenes, steroids and other miscellaneous compounds are the main bioactive phytochemicals that naturally found in *M.*

annua and other *Mercurialis* species (Blanco-Salas *et al*., 2019).

Egyptian knapweed, *Volutaria tubuliflora* (Murb.) Sennen (formerly *Amberboa tubuliflora*) (family Asteraceae) is an annual herb naturally grows in grasslands and dunes. Very little informations regarding its biological activities is available in the literature.

However, potential cytotoxic and antibacterial activities towards *Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus subtilis* of *V. tubuliflora* sesquiterpene lactones were earlier reported by Khafagy *et al.* (1979). The pharmaceutical and biological potentials of *Volutaria* species are due to the presence of many constituents such as triterpenoids (cycloartanes), sesquiterpenes, flavonoids, alkaloids, fatty acids, lignans, sterols, tannins and coumarins (Khan *et al*., 2010; Oshkondali *et al*., 2019).

Great nematicidal properties of *H. albus* extract were earlier reported by Haseeb and Butool (1996), where its standard extract effectively inhibited egg hatching of *M. incognita* and provided an excellent (100%) J_2s mortality under laboratory conditions. Otherwise, no available reviewed data found regarding nematicidal activity of *M. annua* and *V. tubuliflora* so far.

Accordingly, the aims of the present study were to investigate the nematicidal activity of the previously mentioned plant species on viability of *M. incognita* J_2s under laboratory conditions and furtherly study their efficacies in managing nematode infecting guava seedlings under orchard conditions.

Materials and Methods

Plants collection and extraction

Fresh shoot parts (leaves, flowers and/or fruits) of the wildly grown plants namely *Hyoscyamus albus*, *Mercurialis annua* and *Volutaria tubuliflora* (Figure 1) were collected from different suburbs of Alexandria city, Egypt during March-May, 2021. Plant species were scientifically identified based on the morphological descriptions given by Täckholm (1974) and the updated online literature reviews. The gathered shoots were completely air dried in clean dark place for two weeks, coarsely ground in a home mill and a weight (300 g, each) was extracted three times in total 2 L methanol/plant for one week under laboratory conditions (air temperature 25±2˚C), then filtrated through Whatman filter paper No. 1. Following filtration, the solvent (methanol) was evaporated from each extract under reduced pressure using the rotary evaporator (RE-111 Buchi Rotavapor supplemented by B-461 Water Bath, Artisan Technology Group®) to yield crude sticky pastes with aromatic scent weighted 71.3, 63.5 and 55.9 g for *H. albus, M. annua* and *V. tubuliflora*, respectively. All the above plant extracts were strictly collected and kept in dark glass bottles (ca. 100 ml) in the refrigerator at 4-5˚C till conducting the bioassays and orchard trials.

Gas chromatography-mass spectrometry (GC-MS)

Crude plant extractive pastes (≈ 0.2g, each) were sent to the Institute of Graduate Studies and Research, Alexandria University, Shatby, Alexandria for the chemical analysis and identification of their bioactive constituents using a Trace GC Ultra/ Mass Spectrophotometer ISQ (Thermo Scientific® Germany) instrument equipped with the column type (TG-1 MS) under the following optimized conditions: the used solvent was dichloromethane, the carrier gas was helium with average velocity (39 cm s^{-1}), flow rate (1 ml/min) and run time (47.86 min) and the concentration of injected sample was 10 µg/ml. The oven temperature program was held at 80 ˚C to 280 ˚C and the temperature of right injector was 250 ˚C. All mass spectra were recorded in the electron impact ionization (EI) 70 electron volts and identification of the phytochemical constituents was performed on the basis of mainlib search.

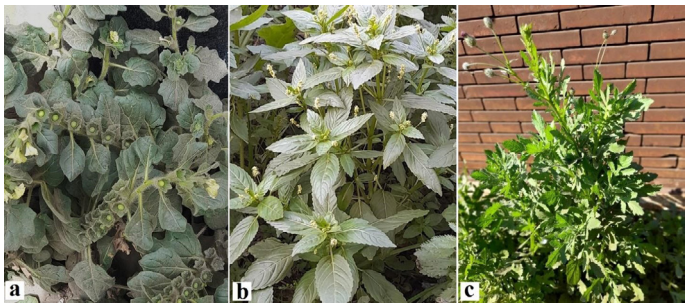

Figure 1: *An overview of the studied wild plants Hyoscyamus albus (a), Mercurialis annua (b) and Volutaria tubuliflora (c) naturally wild grown in Alexandria suburbs.*

Inoculum source of nematode juveniles

As a sustainable source of nematode inoculum, pure culture of the root-knot nematode *Meloidogyne incognita* (Cofoid and White) (Chitwood) was maintained on eggplants cv. Black Beauty grown in 23

cm diameter pots containing 2:1 sandy clay solarized soil mixture for three months under greenhouse conditions. Whenever needed to inoculum, heavily galled eggplant roots were carefully discarded from potted soil, washed with tap water, cut into small pieces and the nematode eggs were extracted in 0.5% sodium hypochlorite (NaOCl) solution for 2 min, followed by completely removing NaOCl residuals by a gentle stream of tap water (Hussey and Barker, 1973). Baermann tray technique was applied to the extracted eggs suspension for 72 hr under laboratory conditions in order to induce eggs hatching (Hooper *et al*., 2005) and the freshly emerged J_2s were collected in 50 ml distilled water for the bioassay.

Bioassay

A net weight (0.5 g) of each plant extractive paste was separately subjected to the formulation via dissolving in 1 ml of DAT mixture (1:3:2 DMSO: Acetone: Tween-80) according to Wiranto *et al.* (2009). The formulated stock extractives were processed to investigate their nematicidal activities against J_2s of *M. incognita* at the concentrations (100, 250, 500, 1000 and 2000 mg L^{-1}). Double folds of the desired concentrations of each extract were prepared by dissolving appropriate volumes of the above formulated mixtures up to 10 ml distilled water to fix stock solutions. One ml of the active nematode J_2s suspension (including approximately 250-300 active J_2s) were decanted into clean test glass vials (ca. 10 ml, each) over 1 ml of double fold of each desired concentration in order to adjust the studied ones into vials. J_2s in distilled water and those in DAT mixture were served as check and blank treatments, properly. The bioassay was assigned as factorial experiment in complete randomized design, where plant extracts and their concentrations were the main factors. All treated vials were replicated three times and kept under laboratory temperature (25°C±2) for 72 hr. Eventually, active and dead J_2s of all treatments were microscopically checked and counted. Mortality of nematode J_2s was featured as dead juveniles that had erected body shape and easily distinguished from the alive ones that had curved bodies. Moreover, in order to definitely differentiate dead J_2s from the motile ones, an aqueous solution (0.062-0.50%) of potassium permanganate was used, where the dead J_2s will stained light to dark brown, whereas the motile ones stay clear (Jatala, 1975).

Orchard trials

A private orchard naturally infested by *M. incognita* located at Rosetta province, Behera governorate was selected for performing the field trials during the interval (April to July) of the consecutive growing seasons (2022 - 2023). Five experimental plots were maintained for carrying out the present trials. Each plot comprised ten labeled 30-cm diameter plastic pots randomly distributed into holes (0.5m × 0.5m), filled with 10 kg *M. incognita* - infested soil that collected from an infested field at the trial location. The experimental soil consisted of 93% sand, 6% clay and 1% silt, EC 0.918 ds m^{-1} and pH 8.26. Initial population (Pi) of nematode J_2s was determined via nematode extraction from representative 400 g soil samples collected from each pot after thoroughly mixing and processed to method of Cobb's wet-sieving and centrifugal sucrose floatation techniques. Stainless sieves that used in the nematode extraction were under U.S. standardization of pore opening diameters 850, 150 and 38µ (Ayoub, 1980).

The field experiments were assigned to evaluate relative nematicidal efficacy of crude methanolic extracts of *H. albus, M. annua* and *V. tubuliflora* applied as soil drench individually at their LC_{95} values and in their binary and ternary combinations at half and one third of LC_{95} values, as compared to the synthetic nematicide Oxamyl 24% SL that was applied at the recommended dose (3 L/feddan) in managing *M. incognita* on newly planted guava seedlings.

Individual plant extracts and their combinations were processed for soil treatment by dissolving the proper weights of crude extractive pastes in appropriate volumes of DAT mixture as previously mentioned (Wiranto *et al*., 2009), then irrigation water (pH = 7.07 and EC = 0.429 ds m^{-1}) was added up to 3 L into clean plastic bottles (ca. 5 L), well manually shacked for few minutes to allow pastes to properly mix before soil drenching (500 ml/seedling).

One year aged uniform healthy guava (Banaty cv.) seedlings with 24-26 true leaves were obtained from a certified fruit nursery at Rosetta province, Behera governorate and transplanted (one/pot). One-week after establishment, all treatments were applied as the following:

- Check (untreated seedlings)
- Blank (treated seedlings with the volume of DAT mixture that served in dissolving plant extractive pastes.

- The synthetic liquid nematicide Oxamyl 24% SL applied at the recommended dose (3 L/feddan).
- *Hyoscyamus albus* extract applied at 1512 mg L^{-1}.
- *Mercurialis annua* extract applied at 2382 mg L^{-1}.
- *Volutaria tubuliflora* extract applied at 2074 mg L^{-1}.
- A binary combination of *H. albus* (756 mg L^{-1}) + *M. annua* (1191 mg L^{-1}).
- A binary combination of *H. albus* (756 mg L^{-1}) + *V. tubuliflora* (1037 mg L^{-1}).
- A binary combination of *M. annua* (1191 mg L^{-1}) + *V. tubuliflora* (1037 mg L^{-1}).
- A ternary combination of *H. albus* (504 mg L^{-1}) + *M. annua* (794 mg L^{-1}) + *V. tubuliflora* (691 mg L^{-1}).

Oxamyl 24% SL was applied once (one week after transplanting), whereas all plant extracts and blank treatments were applied twice (the 1st soil drench was one week after transplanting and the 2nd one was one week later). Treatment-free seedlings were served as untreated check. In order to maintain nematicidal action of plant extracts and to avert probable breakdown of their bioactive constituents, all treatments were applied in the early morning following regular irrigation time by 2-3 days to avoid possible leaching of extracts or Oxamyl. All the treated pots were replicated five times and organized in a randomized complete block design (RCBD) according to Snedecor and Cochran (1990).

Seedlings received their requirements of water and nutritional fertilizers along all the experimental times. Three months after treatments, the trials were terminated and nematode infection parameters were recorded. Number of root galls and nematode egg masses were visually counted after root staining in an aqueous solution (0.15 g/L water) of Phloxine B for 1520 min to indicate egg masses in red color (Daykin and Hussey, 1985). Final population (Pf) of nematode including number of J_2s in kg soil extracted from soil samples based on the above mentioned method of Ayoub (1980) plus the total count of eggs extracted from roots using NaOCl technique previously described by Hussey and Barker (1973) was estimated. In addition, growth criteria of guava seedlings including plant heights, fresh weights of shoot and root systems were measured. Moreover, chlorophyll content of guava leaves was extracted by acetone according to method of Ašimović *et al.* (2016) and the chlorophylls a and b contents were determined spectrophotometrically via readings of absorbance (A) at the wave lengths 647 and 663, respectively using Milton Roy Spectronic 601 (Artisan® TG) and these readings were involved into the following formulas proposed by Lichenthaler (1987) to calculate leaves content of chlorophyll a, b and the total one:

$$Chlorophyll\ a = 12.25\ A_{663} - 2.79\ A_{647}$$
$$Chlorophyll\ b = 21.50\ A_{647} - 5.10\ A_{663}$$
$$Total\ chlorophyll\ (a+b) = 7.15\ A_{663} - 18.71\ A_{647}$$

Reduction percentages (R%) of nematode Pf were calculated using Mulla's equation (Mulla *et al.*, 1971) where:

$$R\ (\%) = 100 - \left[\left(\frac{\text{Pi check}}{\text{Pi treatment}}\right) x \left(\frac{\text{Pf treatment}}{\text{Pf check}}\right)\right] x\ 100$$

Furtherly, R (%) of nematode Pf was corrected to the blank based on the adapted Abbott's 316 equation as follows:

$$\text{Corrected R (\%)to blank} = \frac{\text{R\% treatment} - \text{R\% blank}}{100 - \text{R\% blank}} \times 100$$

Eventually, relative nematicidal efficacy (RNE %) of all plant extracts individually and their combinations to Oxamyl 24% SL were calculated as follows:

$$\text{RNE (\%)} = \left[1 - \left(\frac{\text{R\% Oxamyl} - \text{R\% plant extract(s)}}{\text{R\% Oxamyl}}\right)\right] \times 100$$

Furthermore, co-toxicity factor of binary and ternary plant extract combinations was determined based on the following equation:

$$\text{Co - toxicity factor} = \left(\frac{\text{Observed effect (\%)} - \text{Expected effect (\%)}}{\text{Expected effect (\%)}}\right) \times 100$$

According to Mansour *et al.* (1966), the co-toxicity factor was served to categorize results into three different classes, where the positive factor (+20 or more) indicated to synergism, the negative factor (-20 or less) meant antagonism, while the intermediate values 333 between –20 and +20 considered that the combination had an additive effect.

Expected effect (%) of the binary combinations was calculated using Limpel's equation (Richer, 1987) as follows:

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

Where; E= the expected effect of the binary combination of plant extracts; X = the observed effect provided by the extract 1 individually; Y= the observed effect provided by the extract 2 individually.

Whereas, expected effect (%) of the ternary combination was performed according to Colby (1967), as the following:

$$E\,(\%) = (X + Y + Z) - \left(\frac{XY + XZ + YZ}{100}\right) + \left(\frac{XYZ}{10000}\right)$$

Where; E = the expected effect of the ternary combination of all plant extracts. X = the observed effect provided by the extract 1 individually. Y = the observed effect provided by the extract 2 individually. Z = the observed effect provided by the extract 3 individually.

Data collection and statistical analysis

Mortality percentages of nematode J_2s (M%) in the bioassay were calculated for each replicate according to the formula: M (%) = (No. dead J_2s ÷ Total J_2s) × 100 and then corrected for all treatments to the blank using Abbott' equation (Abbott, 1925) as follows:

$$\text{Corrected M (\%)} = \left(\frac{\text{M (\%) treatment} - \text{M (\%) blank}}{100 - \text{M (\%) blank}}\right) \times 100$$

Finally, the corrected mortality percentages of each plant extract were analyzed via the Probit software to estimate their LC_{50} and LC_{95} values based on Finney (1971). The resulted data of bioassay and orchard trials were statistically analyzed and the differences among treatments were determined based on least significant difference (LSD) values at the level of probability 363 (0.05%) using SAS software (SAS, 2004).

Results and Discussion

The bioassay results showed that all tested concentrations of the examined plant extracts significantly (P = 0.05) reduced viability of *M. incognita* J_2s causing mortality percentages ranged from 12.4 to 100% as compared to control and blank treatments (Table 1). The mortality percentages were strongly (P=0.05) influenced by all concentrations of each extract and the 371 highest concentration (2000 mg L^{-1}) exhibited 100% J_2s mortality (Figure 2). The nematicidal action of *H. albus* extract (LC_{50} = 302 mg L^{-1}) appeared to be the best in suppressing *M. incognita* J_2s viability, followed by *V. tubuliflora* (LC_{50} = 390 mg L^{-1}) and *M. annua* (LC_{50} = 465 mg L^{-1}) (Table 1). Generally, J_2s mortality (%) increased linearly (P = 0.0001) with increasing concentrations of the studied plant extracts (Figure 2). Potent nematicidal activity of *H. albus* against *M. incognita* was earlier documented by Haseeb and Butool (1996) and the present results highly supported this result. Otherwise, no reports are available in the literature regarding the nematicidal properties of *M. annua* and *V. tubuliflora* as yet. Thus, our findings offered these plants as new sources of promising nematicidal constituents.

Table 1: *Mortality (%)* of the second stage juveniles (J_2s) exposed for 72 h to different concentrations of methanolic shoot 420 extracts of Hyoscyamus albus, Mercurialis annua and Volutaria tubuliflora under laboratory conditions (25 ± 2°C).*

Plant extract (E)	Check (distilled water)	Blank (DAT mixture)	Concentration (C) at mg L^{-1}					Overall mean (E)	LC_{50} (mg L^{-1})	Fiducial limits (mg L^{-1})	LC_{95} (mg L^{-1})	Fiducial limits (mg L^{-1})	Slope	Chi square
			100	250	500	1000	2000							
Hyoscyamus albus	1.66^{m}	9.71^{l}	20.04^{j} ±2.10	35.97^{h} ±3.35	59.32^{e} ±2.31	92.98^{b} ±1.83	100^{a} ±0	45.67^{A}	302	263 - 346	1512	1179 - 1943	2.35 ± 3.31	15.60
Mercurialis annua			12.40^{kl} ±1.02	22.30^{j} ±2.99	43.35^{g} ±2.59	74.81^{d} ±2.82	100^{a} ±0	37.75^{C}	465	408 - 530	2382	1820 - 3121	2.32 ± 3.11	19.81
Volutaria tubuliflora			14.55^{k} ±2.27	29.32^{i} ±2.50	51.52^{f} ±2.17	80.33^{c} ±2.68	100^{a} ±0	41.01^{B}	390	341 - 447	2074	1587 - 2713	2.27 ± 0.03	13.09
Overall mean (C)	1.66^{G}	9.71^{F}	15.66^{E}	29.2^{D}	51.40^{C}	82.71^{B}	100^{A}							

Data are means of three replicates.

*Mortality (%) = (No. dead J_2s ÷ total No. J_2s) x 100 * J_2s mortality (%) was corrected using Abbott's formula (Abbott, 1925), where distilled water and blank act as checks. Percentages of J_2s mortality within a column/row superscripted by the same small or capital letter(s) are not significantly different according to Fisher's least significant difference at P= 0.05. LC_{50} and LC_{95} values were obtained from the Probit analysis according to (Finney, 1971).*

Table 2: *Phytochemical compounds of the methanolic dried shoots extract of Hyoscyamus albus as delivered from GC-MS analysis.*

Retention time (min)	Compound name	Area (%)	Molecular formula	Molecular weight
31.61	Lauric acid, methyl ester (syn. Methyl laurate)	3.17	C13H26O2	214
44.52	Hexadecanoic acid, methyl ester (syn. Methyl palmitate)	4.06	C17H34O2	270
45.20	n-Hexadecanoic acid (syn. Palmitic acid)	6.91	C16H32O2	256
45.39	Eicosane	1.74	C20H42	282
46.43	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (syn. linoleic acid, methyl ester)	9.81	C19H34O2	294
46.49	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (syn. Linolenic acid, methyl ester)	8.28	C19H32O2	292
46.60	Phytol	11.26	C20H40O	296
46.69	Methyl stearate	1.63	C19H38O2	298
46.95	9,12-Octadecadienoic acid (Z,Z)- (syn. Linoleic acid)	7.43	C18H32O2	280
47.80	Hyoscyamine	4.99	C17H23NO3	289
48.64	Scopolamine	1.95	C17H21NO4	303
49.54	Diethylhexyl phthalate	7.51	C24H38O4	390
50.27	Pentacosane	3.30	C25H52	352
50.38	11,14-Eicosadienoic acid, methyl ester	1.50	C21H38O2	322
51.32	Hentriacontane (syn. 3,11-Dimethylnonacosane)	5.06	C31H64	436
51.60	Octacosane	4.51	C28H58	394
53.02	Tetratetracontane	6.94	C44H90	618
54.19	Cholesterol	5.47	C27H46O	386
57.03	β-Sitosterol	4.48	C29H50O	414
Total identified		100		

Table 3: *Phytochemical compounds of the methanolic dried shoot extract of Mercurialis annua as revealed from GC-MS analysis.*

Retention time (min)	Compound name	Area (%)	Molecular formula	Molecular weight
44.53	Hexadecanoic acid, methyl ester (syn. Methyl palmitate)	3.58	C17H34O2	270
45.17	n-Hexadecanoic acid (syn. Palmitic acid)	2.76	C16H32O2	256
46.43	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (syn. linoleic acid, methyl ester)	3.45	C19H34O2	294
46.50	9,12,15-Octadecatrienoic acid methyl ester, (Z,Z,Z)- (syn. Linolenic acid, methyl ester)	21.32	C19H32O2	292
46.60	Phytol	7.26	C20H40O	296
46.70	Stearic acid methyl ester (syn. Methyl stearate)	2.24	C19H38O2	298
46.95	9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (syn. Linolenic acid)	6.42	C18H30O2	278
47.43	Neophytadiene	1.28	C20H38	278
47.94	Octacosane	2.72	C28H58	394
49.16	Carbonic acid, eicosyl vinyl ester (syn. Isoamyl ricinoleate)	12.76	C23H44O3	368
49.36	Docosanoic acid, methyl ester	2.43	C23H46O2	354
49.54	Diisooctyl phthalate	2.82	C24H38O4	390
49.71	17-Pentatriacontene	2.79	C35H70	490
50.28	Octacosane	5.86	C28H58	394
50.44	Linolenic acid, 2-hydroxy-1-(hydroxymethyl), ethyl ester (Z,Z,Z)- (syn. Linolenoylglycerol)	5.60	C21H36O4	352
51.60	Tetratetracontane	1.53	C44H90	618
51.67	Octatriacontyl pentafluoropropionate	3.69	C41H77F5O2	696
55.61	Campesterol	2.31	C28H48O	400
56.09	Stigmasterol	3.22	C29H48O	412
57.03	β-Sitosterol	5.96	C29H50O	414
Total identified		100		

Results of the orchard trials showed great levels of *M. incognita* management along the studied growing seasons. All treatments by plant extracts provided reduction percentages (73.37 - 88.10%) of root galling, (73.65 - 89.86%) of nematode egg masses and (75.37 - 91.27%) of nematode Pf, while Oxamyl 24% SL achieved 86.11, 88.52 and 90.25% reductions of root galling, no. egg masses and nematode Pf, respectively in the 1st season (Table 5). Correspondingly, appreciable levels of nematode reduction (72.03–87.01%) of root galls, (70.05–87.17%) of egg masses and (70.56–88.66%) of nematode Pf were achieved by all plant extracts versus those given by Oxamyl (83.70, 84.49 and 86.96%, respectively) in the 2nd season (Table 6).

Luckily, all treatments with plant extracts gave a considerable RNE to Oxamyl reached to (83.51-101.1%) and (81.14-102%) in the 1st and 2nd seasons, consequently. Therefore, they may have selected as new source of plants possess promising nematicidal compounds and could be considered alternatives of Oxamyl.

Results of GC-MS analysis confirmed that *H. albus* extract (Figure 3a) is rich in many bioactive chemical constituents, where phytol (11.26%), 9,12-octadecadienoic acid (Z,Z)- methyl ester (9.81%), 9,12,15-octadecatrienoic acid methyl ester (Z,Z,Z)- (8.28%), diethylhexyl phthalate (7.51%), 9,12-octadecadienoic acid (Z,Z)- (7.43%), palmitic acid (6.91%), hentriacontane (5.06%), hyoscyamine (4.99%), octacosane (4.51%) and β-sitosterol (4.48%) were the most prevalent compounds representing >65% of the total extract content (Table 2). However, other bioactive compounds were associated in low area percentages such as hexadecanoic acid methyl ester (4.06%), lauric acid methyl ester (3.17%), scopolamine (1.95%) and methyl stearate (1.63%). These promising findings are in agreement with those provided by other authors (Mahmood *et al.*, 1985; Keskin *et al.*, 2016; Ashraf *et al.*, 2019).

The major phytochemical constituents identified in *M. annua* extract (Figure 3b) were linolenic acid methyl ester (21.32%), followed by carbonic acid eicosyl vinyl ester (12.76%), phytol (7.26%), linolenic acid (6.42%), β-sitosterol (5.96%) and octacosane (5.86%) that representing approximately 60% of the total chemical composition (Table 3). These results are similar to those found by Al-Douri and Shakya (2019), Blanco-Salas *et al.* (2019), Nasr *et al.* (2021) and Al-Douri *et al.* (2022). Other ingredients such as hexadecanoic acid methyl ester, stigmasterol, diisooctyl phthalate, n-hexadecanoic acid and methyl stearate that found in relatively low area percentages (2.24-3.58%) are of nematicidal consideration too.

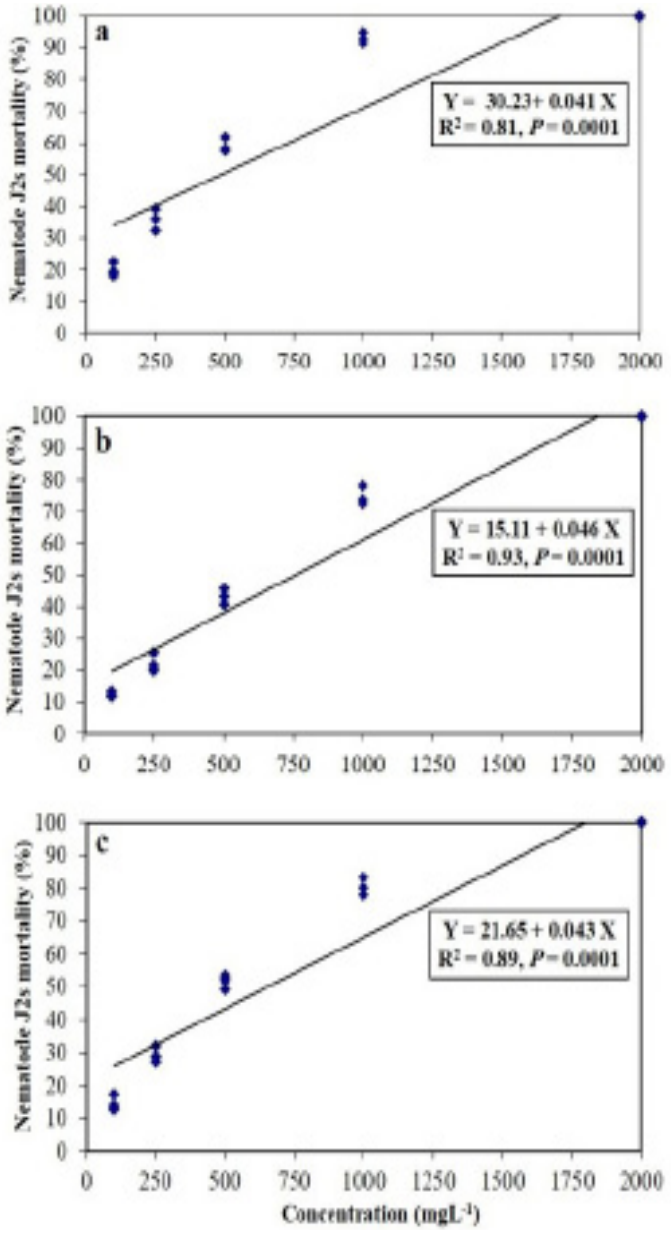

Figure 2: *Linear regression models of Meloidogyne incognita J_2s mortality (%) caused by different concentrations of the methanolic shoot extracts of Hyoscyamus albus (a), Mercurialis annua (b) and Volutaria tubuliflora (c) after 72 hr exposure time under laboratory conditions.*

Octacosane was identified in 4.51% of *H. albus* (Table 2) and 5.86% of *M. annua* extracts (Table 3). This observation is identical to that was given by Soliman *et al.* (2017) who detected that constituent in 5.19% among the major compounds of *Artemisia judaica* shoot extract. Similarly, tetratetracontane

was detected in 6.94% and 1.53% of *H. albus* and *M. annua* extracts, respectively (Tables 2-3). This observation is in agreement with Elsayed *et al.* (2025) who identified that constituent within the bioactive ingredients of *Dysphania ambrosioides* (formerly, *Chenopodium ambrosioides*). Surprisingly, nematicidal properties of the phytochemical constituents of *A. judaica* (Soliman *et al.*, 2017) and *C. ambrosioides* (Bai *et al.*, 2011) are well documented in the literature. Carbonic acid eicosyl vinyl ester (syn. Isoamyl ricinoleate) was detected in a considerable occurrence (12.76%) in *M. annua* extract (Table 3). Unfortunately, no article discussed its biological activity against PPN so far. However, a potential bactericidal effect of this compound towards certain human-pathogenic bacteria was reported by Narasimhan *et al.* (2007). So, it may suggest that the nematicidal activity of *M. annua* extract in the current investigation is probably attributed to carbonic acid eicosyl vinyl ester beside linolenic acid methyl ester the well-known nematicidal phytochemical constituent (Gu *et al.*, 2005).

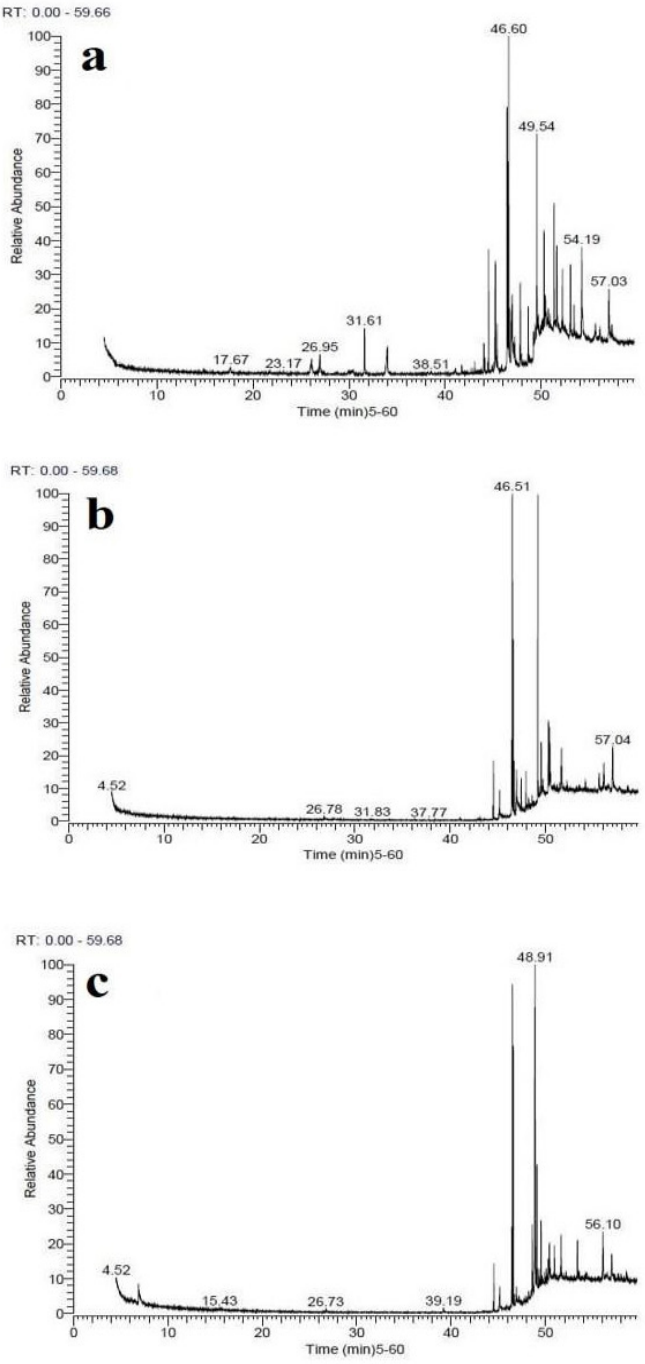

Figure 3: *GC-MS chromatograms of the identified phytochemical constituents in methanolic extracts of Hyoscyamus albus (a), Mercurialis annua (b) and Volutaria tubuliflora (c).*

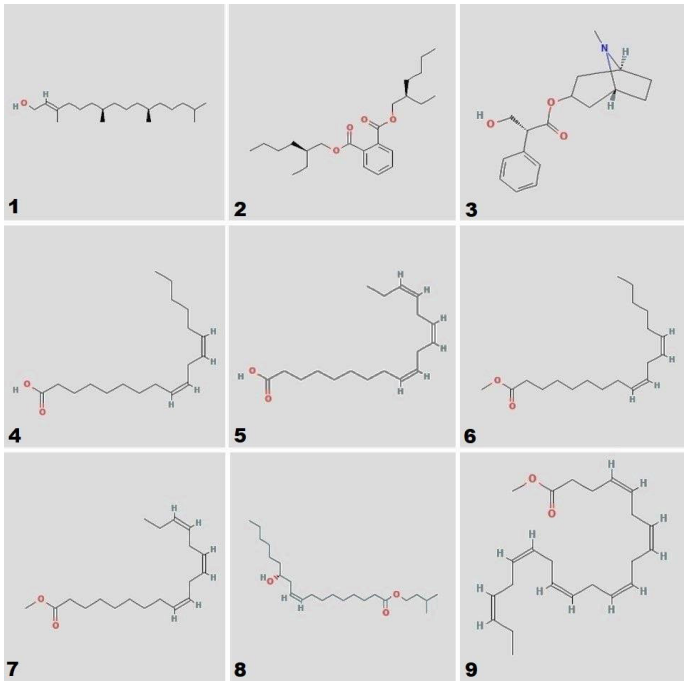

Figure 4: *Chemical structures of the most prevalent bioactive phytochemical constituents delivered from GC-MS analysis of the studied plants: phytol (1), diethylhexyl phthalate (2), hyoscyamine (3), linoleic acid (4), linolenic acid (5), linoleic acid methyl ester (6), linolenic acid methyl ester (7), carbonic acid, eicosyl vinyl ester (8) and docosahexaenoic acid methyl ester (9).*

Twenty phytochemical constituents were detected in the *V. tubuliflora* extract (Figure 3c). The cardenolide (docosahexaenoic acid methyl ester), linolenic acid methyl ester, phytol, pregnenolone and docosahexaenoic acid were the major bioactive compounds identified in 31.99, 12.88, 9.59, 5.39 and 5.06% of the total extract content, respectively (Table 4). This result is in agreement with Khan *et al.* (2004) who confirmed the presence of docosahexaenoic acid methyl ester in the extract of *Volutaria ramosa*. Fortunately, nematicidal properties of the cardenolides found in *Nerium indicum* leaves extract were previously reported by Wang *et al.* (2009). Therefore, it has suggested that docosahexaenoic acid

methyl ester may exhibit a nematicidal performance against nematode in the present study. On the other hand, doconexent (syn. docosahexaenoic acid) occurred in *V. tubuliflora* extract was previously extracted from certain seaweed species and found to possess a considerable nematicidal activity towards gastrointestinal-parasitic nematodes infecting human (Bonde *et al.*, 2021).

It worth mentioning that the most commonly identified phytochemical compounds (Figure 4) and even those associated with low area percentages (Tables 2, 3, 4) were possessing nematicidal properties and should keep interest.

Nematicidal properties of lauric acid methyl ester, linoleic acid methyl ester and nhexadecanoic acid (Gu *et al.*, 2005), linolenic acid methyl ester (Mahawer *et al.*, 2025), hexadecanoic acid methyl ester (Lu *et al.*, 2020; Mahawer *et al.*, 2025), stearic acid methyl ester (Lu *et al.*, 2020), linoleic acid (Panda *et al.*, 2020), linolenic acid (Páez-León *et al.*, 2022), phytol (Fujimoto *et al.*, 2021), diethylhexyl phthalate (Sharma *et al.*, 2018), hyoscyamine and scopolamine (Babaali *et al.*, 2021), β-sitosterol (Xia *et al.*, 2025), stigmasterol (Velasco-Azorsa *et al.*, 2021) and campesterol (Rocha *et al.*, 2020) that identified in the current investigation were fortunately reported in the literature. Thus, nematicidal potentials of the studied plant extracts are referred to the above mentioned constituents either acted individually or in their combinations between each other.

On the other hand, growth criteria of guava seedlings and their leaves content of chlorophylls a, b and total one of all treatments along both the studied seasons significantly improved as compared to those recorded for check and/or blank (Tables 5-6). In general, the binary and ternary combinations of all plant extracts exhibited good levels of nematode management and improvement of growth criteria better than those given by individual plant extracts. Enhancement of the guava growth criteria and the improvement of their leaves chlorophyll contents are due to suppressing nematode population effectively occurred as a result of the applied treatments. Recent results of Mnyambo *et al.* (2024) greatly supported our findings.

Table 4: *Phytochemical compounds of the methanolic dried shoots extract of Volutaria tubuliflora as resulted from GC-MS analysis.*

Retention time (min)	Compound name	Area (%)	Molecular formula	Molecular weight
6.90	2-Butenoic acid, 4-hydroxy-, methyl ester	1.96	C5H8O3	116
44.53	Hexadecanoic acid, methyl ester (syn. Methyl palmitate)	2.49	C17H34O2	270
45.16	n-Hexadecanoic acid (syn. Palmitic acid)	1.92	C16H32O2	256
46.42	9,12-Octadecadienoic acid, methyl ester (syn. linoleic acid, methyl ester)	3.00	C19H34O2	294
46.50	9,12,15-Octadecatrienoic aci, methyl ester, (Z,Z,Z)- (syn. Linolenic acid, methyl ester)	12.88	C19H32O2	292
46.60	Phytol	9.59	C20H40O	296
46.92	9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (syn. Linolenic acid)	1.87	C18H30O2	278
48.60	Doconexent (syn. Docosahexaenoic acid)	5.06	C22H32O2	328
48.90	4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, (all-Z)- (syn. Cardenolide = Docosahexaenoic acid methyl ester)	31.99	C23H34O2	342
49.09	5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)- (syn. Pregnenolone)	5.39	C21H32O2	316
49.16	1-Hexadecanol, 2-methyl-	0.73	C17H36O	256
49.36	10,12-Pentacosadiynoic acid	1.43	C25H42O2	374
49.54	Diisooctyl phthalate	2.07	C24H38O4	390
50.30	1-Eicosanol	1.50	C20H42O	298
50.43	Linolenic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester(Z,Z,Z)-	2.59	C21H36O4	352
50.94	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	1.71	C16H28O3	268
51.67	Octatriacontyl pentafluoropropionate	2.58	C41H77F5O2	696
53.39	Octadecanedioic acid	3.29	C18H34O4	314
56.10	Stigmasterol	4.80	C29H48O	412
57.02	β-Sitosterol	3.15	C29H50O	414
Total identified		100		

Table 5: *Effect of soil application of crude methanolic shoot extracts of the wild plants Hyoscyamus albus (Ha), Mercurialis annua (Ma) and Volutaria tubuliflora (Vt) individually and in their combinations, comparing to the synthetic nematicide Oxamyl 24%SL for managing Meloidogyne incognita (Mi) on guava seedlings (Banaty cv.) under Rosetta orchard conditions, Behera governorate during the 1st season (April - July, 2022).*

Treatment	Pi + SD	No. galls/ root	R* (%)	No. egg masses /root	R* (%)	Pf	R* (%)	RNE (%) to Oxam-yl	Co-tox-icity factor	Plant height (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Leaves content (mg/g fresh weight)		
													Chloro-phyll a	Chlo-rophyll b	Total chloro-phyll
Untreated check (*Mi* only)	1677±287	362a	-	152a	-	26868[a]	-	-	-	43.2[c]	14.85[c]	8.97[d]	4.61[f]	5.77[e]	10.38[e]
Blank (DAT + *Mi*)	1825±316	353a	2.49	148a	2.63	26295[a]	10.07	-	-	44.8[c]	16.03[c]	10.02[d]	4.75[f]	5.99[e]	10.74[e]
Oxamyl 24% SL + *Mi*	2028±219	49c	86.11	17c	88.52	2851[c]	90.25	-	-	58.2ab	26.35ab	17.95bc	7.51ab	9.67[b]	17.18[b]
Hyoscyamus albus + *Mi*	1971±424	58c	83.57	21c	85.81	3493[c]	87.70	97.17	-	55.8ab	24.17ab	16.47[c]	7.08cde	8.66[c]	15.74[c]
Mercurialis annua + *Mi*	1794±275	94b	73.37	39b	73.65	6367[b]	75.37	83.51	-	53.4[b]	22.23[b]	15.89[c]	6.72[e]	7.96[d]	14.68[d]
Volutaria tubu-liflora + *Mi*	1849±287	61bc	82.72	22c	85.14	3681[c]	86.18	95.49	-	57.4ab	25.05ab	18.14bc	6.94[de]	9.45[b]	16.39bc
(*Ha* + *Ma*) + *Mi*	2089±309	65bc	81.58	24c	83.78	4018bc	86.65	96.01	-10.64	54.6ab	23.34ab	17.03[c]	7.39bc	10.68[a]	18.07[a]
(*Ha* + *Vt*) + *Mi*	1753±349	46c	86.97	18c	87.84	2940[c]	88.36	97.91	-10.11	58.4ab	26.22ab	20.35ab	7.80[a]	10.47[a]	18.27[a]
(*Ma* + *Vt*) + *Mi*	1594±373	55c	84.42	20c	86.48	3322[c]	85.53	94.77	-11.46	56.2ab	24.57ab	18.72abc	7.30bcd	10.89[a]	18.19[a]
(*Ha* + *Ma* + *Vt*) + *Mi*	2005±473	42c	88.10	15c	89.86	2522[c]	91.27	101.1	-8.35	58.8[a]	27.28[a]	21.13[a]	7.86[a]	10.94[a]	18.80[a]

*Data are means of five replicates. Values within a column superscripted by the same letter(s) are not significantly different according to Fisher's least significant difference at P= 0.05. Pi = initial population of second stage juveniles (J_2s) in kg soil. Pf = final population of second stage juveniles (J_2s) in kg soil + no. eggs extracted from roots. * Reduction percentages of nematode populations were corrected to the check treatment using Mulla's equation (Mulla et al., 1971), followed by correction to the blank treatment using adapted Abbott's formula (Abbott, 1925). RNE (%) = Relative Nematicidal Efficacy (%) of the plant extract(s) to the chemical nematicide Oxamyl 24% SL.*

Values resulted from calculating co-toxicity factor of the binary and ternary combinations of plant extracts were ranged between -8.35 and -11.46 in the 1st season (Table 5) and from -9.87 to -12.10 in the 2nd one (Table 6). These findings confirmed that the examined plant extracts had an additive effect between each other (Mansour *et al.*, 1966).

The nematicidal mechanisms of plant extracts and their bioactive constituents were formerly discussed by some authors. Like most chemical nematicides which provide their nematicidal performance via inhibition of nematode acetylcholinesterase "AChE" (Ebone *et al.*, 2019), many of plant alkaloids, essential oils and other phytochemical compounds were also act in *in vitro* AChE tests (Ebadollahi *et al.*, 2021; Coqueiro *et al.*, 2023). Moreover, Eldesouky *et al.* (2024) confirmed that mode of the insecticidal action of *H. albus* extract against *A. gossypii* and *P. solenopsis* is due to inhibition of AChE of the tested insects. This observation indicates that *H. albus* extract probably act its nematicidal action in the current study through the same way.

Furthermore, Ibrahim *et al.* (2013) confirmed that sesquiterpene lactones extracted from *Volutaria ramosa* showed potential inhibitory activity against AChE. On the other hand, it was noticed that cardenolides of *Nerium oleander* has another physiological action similar to that of cardiac glycosides. They act via inhibition of membrane Na^+/K^+ ATPase pump which resulting in deficit in conduction of electrical potential (Cheeke, 1998). Fortunately, *V. tubuliflora* extract is rich in the cardenolide (docosahexaenoic acid methyl ester), then its mode of nematicidal action in the present study is rather to be attributed to the above mentioned mechanisms.

Table 6: *Effect of soil application of crude methanolic shoot extracts of the wild plants Hyoscyamus albus (Ha), Mercurialis annua (Ma) and Volutaria tubuliflora (Vt) individually and in their combinations, comparing to the synthetic nematicide Oxamyl 24%SL for managing Meloidogyne incognita (Mi) on guava seedlings (Banaty cv.) under Rosetta orchard conditions, Behera governorate during the 2nd season (April – July, 2023).*

Treatment	Pi + SD	No. galls / root	R* (%)	No. egg masses /root	R* (%)	Pf	R* (%)	RNE (%) to Ox-amyl	Co toxicity factor	Plant height (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Leaves content (mg/g fresh weight)		
													Chloro-phyll a	Chlo-rophyll b	Total chloro-phyll
Untreated check (*Mi* only)	2192±327	473a	-	194a	-	34602^{a}	-	-	-	50.8^{c}	17.54^{d}	11.89^{e}	4.04^{f}	5.12^{f}	9.16^{e}
Blank (DAT + *Mi*)	1979±284	454a	4.02	187a	3.61	33213^{a}	6.32	-	-	52.2^{c}	18.73^{d}	12.45^{e}	4.23^{f}	5.46^{f}	9.69^{e}
Oxamyl 24% SL + *Mi*	2505±447	74c	83.70	29c	84.49	4831^{c}	86.96	-	-	69.8^{a}	31.65ab	22.75abc	6.39^{d}	8.91^{b}	15.30^{b}
Hyoscyamus albus + *Mi*	2299±354	85c	81.28	35c	81.28	5752^{c}	83.08	95.54	-	65.6ab	28.71abc	19.94cd	6.35^{d}	7.60^{d}	13.95^{c}
Mercurialis annua + *Mi*	1998±455	127b	72.03	56b	70.05	8698^{b}	70.56	81.14	-	63.8^{b}	26.33^{c}	17.98^{d}	5.96^{e}	6.71^{e}	12.67^{d}
Volutaria tubuli-flora + *Mi*	2175±348	90c	80.17	37c	80.22	6077^{c}	81.11	93.27	-	67.4ab	29.86abc	22.19abc	6.26de	8.16^{c}	14.42^{c}
(*Ha* + *Ma*) + *Mi*	2401±411	82c	81.93	33c	82.35	5408^{c}	84.77	97.48	-10.79	64.8ab	28.57abc	21.84bc	7.03ab	9.32ab	16.35^{a}
(*Ha* + *Vt*) + *Mi*	2254±261	67c	85.25	26c	86.10	4249^{c}	87.25	100.33	-9.87	68.6ab	30.35abc	24.06ab	6.61cd	9.77^{a}	16.38^{a}
(*Ma* + *Vt*) + *Mi*	2012±369	78c	82.82	31c	83.42	5056^{c}	83.01	95.46	-12.10	66.2ab	27.84bc	22.37abc	6.81bc	9.32ab	16.13ab
(*Ha* + *Ma* + *Vt*) + *Mi*	2370±267	59c	87.01	24c	87.17	3973^{c}	88.66	102.0	-10.50	69.4ab	33.16^{a}	25.28^{a}	7.21^{a}	9.43^{a}	16.64^{a}

*Data are means of five replicates. Values within a column superscripted by the same letter(s) are not significantly different according to Fisher's least significant difference at P= 0.05. Pi = initial population of second stage juveniles (J_2s) in kg soil. Pf = final population of second stage juveniles (J_2s) in kg soil + no. eggs extracted from roots. * Reduction percentages of nematode populations were corrected to the check treatment using Mulla's equation (*Mulla *et al.*, 1971*), followed by correction to the blank treatment using adapted Abbott's formula (*Abbott, 1925*). RNE (%) = Relative Nematicidal Efficacy (%) of the plant extract(s) to the chemical nematicide Oxamyl 24% SL.*

Lastly, good nematicidal performance of *H. albus*, *M. annua* and *V. tubuliflora* and their combinations in managing *M. incognita* on guava seedlings under orchard conditions in the present investigation may encourage us to consider these plant extracts as new promising alternatives to Oxamyl and could involve them in nematode management strategies.

Acknowledgment

Thanks are introduced to Aida Abdelhameed (Institute of Graduate Studies and Research, Alexandria University) for her sincere efforts in the GC-MS analysis of studied botanical extracts. Appreciation is extended to our dear colleague Dr. Gaber M. Abdelgalil (Plant Protection Research Institute (PPRI), for his kind favor in determination chlorophyll contents of guava leaves.

Novelty Statement

Searching for natural sources of nematicidal phytochemicals for use as relatively safe alternatives to synthetic nematicides and studying their possible application in nematode management under field conditions.

Author's Contribution

Amr Ali El-Sherbiny: Proposed the research work idea. Participated in methodology, laboratory tests, field application, collecting data, statistical analysis and writing of the research work.
Sandy El-Sayed Hammad: Participated in methodology, laboratory tests, field application, collecting data, statistical analysis and reviewing the manuscript.

Generative AI and AI-assisted technology statement
The authors have declared that no generative AI or AI-assisted technologies were used to create this manuscript.

Conflict of interest
The authors have declared no conflict of interest.

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