Research Article

Combined use of Aqueous Plant Extracts for Controlling *Meloidogyne incognita* and Modulating Chemical Constituents in Tomato under Greenhouse Conditions

Doaa Khairy[1], Mohamed Ali Osman[2] and Fatma Abdel Mohsen Mostafa[1]*

[1]Agricultural Zoology Department, Faculty of Agriculture, Mansoura University, Mansoura, Egypt; [2]Biology Department, Faculty of Science, Imam Abdul Rahman bin Faisal University, Dammam, Saudi Arabia.

Abstract | The present study focuses on the potential of aqueous leaf extracts of moringa (*Moringa oleifera* Lam), or neem (*Azadirachta* indica A. Juss) singly or in integration with different parts of canola (*Brassica napus* L.) extracts to alleviate the deleterious effect of *Meloidogyne incognita* as well as ameliorate tomato growth *in vivo.* A mixture of moringa or neem aqueous leaf extracts with different parts of canola viz. leaf, stem and root gave better results than did single ones. Dual application of neem leaf extracts and canola parts extracts exhibited detectable augmentation in plant biomass better than other treatments. However, triple application of canola, moringa and neem leaf extracts (41.0%) surpassed all treatments and improved tomato length. All treatments significantly (P<0.05) suppressed nematode population, root galling and number of egg masses. The highest nematicidal activity was performed by leaf extracts mixture of moringa, neem and canola. NPK, chlorophyll Aand B, salicylic acid, phenols and phenylalinine enzyme (PAL) in tomato leaves were significantly (P<0.05) induced by plant extracts mixture. Phytochemical analysis of aqueous neem leaf extract demonstrated the occurrence of a number of flavonoids (21) and phenolic compounds (23) including Hespiridin, Naringin, Rosmarinic acid, Pyrogallol, Salicylic acid and Gallic acid which are implicated in plant preservation and hence induce strength against nematode infection. The results of current study indicated that aqueous leaf extracts of neem, moringa and canola parts in mixture can act as plant growth promoters and potentially be developed into a commercial nematicide to be used in sustainable and organic farming systems.

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***Correspondence** | Fatma Abdel Mohsen Mostafa, Agricultural Zoology Department, Faculty of Agriculture, Mansoura University, Mansoura, Egypt; **Email:** mohsenfatma@hotmail.com
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Introduction

Root-knot nematodes, *Meloidogyne* spp. are an ubiquitous pathogens causing major problems to economic vegetable crops globally grown in tropics and subtropics. *Meloidogyne incognita* (Kofoid and White) Chitwood is among the most widespread pathogen infecting solanaceous plant and considered a main obstacle of tomato (*Solanum lycopersicum* L.) production. Egypt ranked the fifth worldwide in tomato cultivation producing 6.9 million tons in 2019 (FAOSTAT, 2020). Minimizing the negative influence of root-knot nematodes, *Meloidogyne* spp., could be accomplished by chemical nematicides. However, the toxic nature of such chemicals to ecosystem, human, plant and animal life has compelled the researchers to use economically and ecofriendly alternatives strategies.

A strong impulse has given to the study of the botanical nematicides because of the presence of different metabolites with an activity against phytoparasitic nematodes (El-Deriny *et al*., 2020). Nematicidal activities of various botanical extracts have been evaluated by many researchers (Saeed *et al*., 2015; Kepenekci *et al*., 2016; Haroon *et al*., 2018) against *Meloidogyne* spp. and still increasing since botanical species produce a massive secondary metabolite i.e. alkaloids, terpenoids and phenolic compounds which play a vital role in resistance mechanism against pests and diseases.

Neem trees (*Azadirachta indica* Juss) are known to have biocide potential against insects, fungi and some plant parasitic nematodes and many neem-based pesticides formulations have been evolved and registered in several countries with commercial names i.e., Bioneem, Neem Azal-T, etc. (Haroon *et al*., 2018; Metwally *et al*., 2019). Azadirachtin is the staple compound present in neem with strongest nematicidal activity (Ntalli *et al*., 2009; Khalil, 2013) and may act as environmentally friendly favorable option in integrated nematodes management programs.

Various parts of moringa (*Moringa oleifera* Lam) possess several biological activities i.e. antioxidant, antimicrobial and antibacterial, antifungal and anti -inflamatory properties. Phytochemical profiling of Moringa revealed its richness in alkaloids, steroids, tannins, flavonoids, terpenoids, saponins, anthraquinones, reducing sugars along with glucosinolates and isothiocyanates (Izuogu *et al*., 2013). The toxicity of moringa leaves and seeds against plant parasitic nematodes has been appraised by a (Izuogu *et al*., 2013; Khairy, 2016; Haroon *et al*., 2018; Khairy *et al*., 2021).

Canola plant (*Brassica napus*) belongs to family cruciferae also received considerable concern as a substitutional approach for controlling plant parasitic nematodes. Glucosinolates found in cruciferous, are naturally secondary metabolites which could be hydrolyzed by myrosinase releasing a number of compounds essentially isothiocyanates (Mojtahedi *et al*., 1991; Asaduzzaman *et al*., 2014; Ntalli *et al*., 2020). The nematicidal properties of root extracts of various plants against parasitic nematodes have been conducted by many investigators (Goswami and Vijayalakshmi, 1986; Onifade and Egunjobi, 1994).

Compatibility of plant extract mixtures against *M. incognita* had rarely done therefore the current study was carried out to evaluate extracts of some plant parts as potential inducers for controlling *Meloidogyne incognita*, as well as, improving tomato plant growth and modulating chemical components.

Materials and Methods

Collection of plant parts

Leaves of moringa plant (*Moringa oleifera* Lam, Fam. Moringaceae) and neem plant (*Azadirachta indica* A. Juss, Fam. Meliaceae) as well as leaves, stems and roots of canola plant (*Brassica napus* L., Fam. Cruciferae) were collected from the experimental farm of Faculty of Agriculture, Mansoura University, Mansoura, Egypt and transferred to the Nematological laboratory for plant extracts preparation.

Botanical extracts

Leaves, stems and roots of canola as well as moringa and neem leaves were thoroughly washed and chopped. Twenty-five grams of each plant part were separately ground to fine particles in 100 ml distilled water using an electric blender. Solutions were then centrifuged at 5000 rpm for five minutes, filtered through filter paper Whatman No. 1 and considered as undiluted solutions (Nimbalkar and Rajurkar, 2009). Aqueous leaf extracts of moringa and neem were prepared at 5% concentration. Whereas aqueous extracts of canola parts were prepared at 10% concentration.

Nematode inoculum

Eggs of *M. incognita* extracted from coleus roots heavily infested with *M. incognita* (Hussey and Barker, 1973) were used as inoculum.

Experimental design

The experiment was carried out in plastic pots (13 cm-d) filling with 850 g steam sterilized clay loamy soil (Coarse sand 2.88; Fine sand 23.41; Silt 35.09; Clay 38.62%). Seedlings of tomato cv. Kesmat (25 days old) were separately transplanted in each pot and simultaneously soil was drenched with aqueous leaf extracts of moringa and neem singly and in combination with different parts of canola i.e. leaves, stems and roots at the rate of 2 ml/plant/pot. Five days later, 1400 eggs and juveniles of *M. incognita* were applied to each seedling. Oxamyl (0.3g/ plant/pot), a carbamate systemic nematicide, was introduced two days after nematode inoculation. Uninoculated and untreated seedlings served as negative control, however those received nematode inoculum served as positive control. The experiment was set up in a randomized complete block design (RCBD) system with four replicates for each treatment (25±4°C). Plants were regularly irrigated. Treatments were as follows: 1- Moringa leaf (Ml); 2-Neem leaf (Nl) 3- Canola leaf (Nl); 4- Canola shoot (Cs); 5- Canola root (Cr); 6- Ml+ Cl; 7- Ml + Cs; 8- Ml+ Cr; 9- Nl + Cl ; 10- Nl + Cs; 11- Nl+Cr; 12- Ml+ Nl+ Cl; 13- Ml+Nl+ Cs; 14- Ml+Nl+ Cr; 15- Oxamyl; 16-Healthy plants and 17- Nematode alone. The experiment was terminated 42 days after inoculation. Plants were harvested and observations on growth criteria were undertaken. Nematode population (J2) was extracted from soil using sieving and modified Baermann-technique (Goodey, 1957). Roots were stained in acid fuchsin lactic acid as described by Byrd *et al*. (1983) and measured for root galling, number of egg masses, females, and developmental stages. Root gall and egg masses indices (EI) were determined on a scale of 1-5 (Taylor and Sasser, 1978).

Chemical components

Dried weight of tomato leaves (0.2 g) was subjected to chemical analysis for nutrient contents (N, P and K). Total nitrogen (N) was determined according to Kjeldahl method (AOAC, 1980). Total phosphorus(P) was estimated by Fiske and Subbarow (1925), while total potassium (K) was flam photometry estimated (Jackson, 1967). The leaf photosynthesis pigments (chlorophyll A, B) were spectrophotometrically measured using Fadeel's method (1962).

Defense related compounds

Total salicylic acid (SA) and total phenol (TP) were estimated in dried leaves of tomato according to the method of Javaheri *et al*. (2012).

Enzymes activity

Phenylalanine ammonia-lyase (PAL) activity was estimated as described by Chen *et al*. (2000).

Flavonoides and phenolic active ingredients

The crude aqueous leaf extract of neem was analysed for the estimation of flavonoids and phenolic active ingredients using High Pressure Liquid Chromatography (HPLC). Extraction was carried out at Food Technology Institute, Agric. Research Center, Giza, Egypt.

Statistical analysis

The data were statistically analysed (ANOVA) according to Gomez and Gomez (1984), followed by Duncan's multiple range test to compare differences between means at P >0.05 (Duncan, 1955).

Results and Discussion

Screened plant extracts of moringa, neem and canola parts combined in mixtures to expand their nematicidal properties against *M. incognita* as well as to increase tomato growth are shown in Tables (1 and 2). The obtained data indicated that *M. incognita* infection caused a significant decrement in plant growth parameters with reduction percentage in plant length and total plant fresh weight reached 37.7 and 49.6%, respectively (Table 1). Aqueous leaf extract of neem and canola were the best amongst individual applications and significantly (P<0.05) improved shoot length and weight of tomato infected with *M. incognita*, respectively. However, dual and triple applications showed synergistic impact on plant growth compared with single application. Hence, triple application of canola (Cl), moringa (Ml) and neem leaf (Nl) extracts significantly (P<0.05) improved tomato shoot length with percentage of increase over control reached 37.5% followed by Ml+Nl+Cr (30.2%) in par with NL+Cs (30.2%) and Nl+Cr (30.2%). All treatments significantly suppressed nematode population whether in soil or root as compared to control (Table 2). The greatest suppression in nematode parameters was recorded with pots received oxamyl (Rf= 0.25). Among dual applications, Nl integrated with Cl extracts were found to have synergistic effect and non-significantly suppressed soil nematode population (Red.= 77.1%, Rf= 0.91) compared to single application of aqueous Cl (Red.=74.07%, Rf= 0.95) or Nl (Red.= 73.46%, Rf= 0.98) extracts.

Table 1: *Effect of aqueous plant extracts singly and in mixture as potential inducers on growth of tomato infected with Meloidogyne incognita under greenhouse conditions.*

Treatments	Plant Growth Response							
	Length (cm)		Total Length	% dec. / inc	Fresh weight (g)		Plant F. wt	% dec/inc.
	Shoot	Root			Shoot	Root		
Ml	18.5 ab	16.3 b-d	34.8	16.8	4.9 ab	1.8a-c	6.7	9.9
Nl	19.3 ab	15.5 b-d	34.8	16.8	5.0 ab	1.8a-c	6.8	11.8
Cl	13.3 c	15.0 b-d	28.3	-5.0	6.2 a	1.0bc	7.2	17.2
Cs	15.0 bc	12.0cd	27.0	-9.3	4.5 ab	1.3bc	5.8	-4.9
Cr	15.0 bc	12 .0cd	27.0	-9.3	5.6 ab	1.8a-c	7.4	21.3
Ml+ Cl	16.3 a-c	21.5bc	37.8	26.8	4.0 ab	3.6a	7.6	24.6
Ml+Cs	15.3 bc	24.3 ab	39.6	32.8	4.2ab	3.9a	8.1	32.8
Ml+Cr	17.3 a-c	21.3 bc	38.5	29.1	5.0 ab	3.2a	8.2	34.4
Nl + Cl	17.1 a-c	19.7 bc	36.8	23.4	5.4 ab	3.4a	8.8	44.3
Nl + Cs	16.5 a-c	22.3 ab	38.8	30.2	5.1ab	4.0a	9.1	49.2
Nl + Cr	16.8 a-c	22.0 ab	38.8	30.2	5.6 ab	3.6a	9.2	50.8
Ml+ Nl + Cl	19.0 ab	22.0 ab	41.0	37.5	4.5ab	3.7a	8.2	34.4
Ml+ Nl + Cs	17.0 a-c	19.3 bc	36.3	21.8	4.5a b	4.0a	8.5	39.3
Ml+ Nl + Cr	15.5 bc	23.3 ab	38.8	30.2	3.8 b	4.4a	8.2	34.4
Oxamyl	20.8 a	27.5 a	48.3	62.0	9.6 a	4.2a	13.8	126.2
Healthy plants	19.3 ab	28.5 a	47.8	60.4	8.0 a	4.1a	12.1	98.4
Nematode only	13.6 c	16.2 b-d	29.8	-	3.3 b	2.8 a	6.1	-

Each value is a mean of four replicates. Means marked with the same letter (s) in each column did not significantly differ at P< 0.05 according to Duncan's multiple- range test. Ml: moringa leaf; Cl: canola leaf; Cs: canola stem; Cr: canola root and Nl: neem leaf.

Table 2: *Effect of aqueous plant extracts singly and in mixture as potential inducers on Meloidogyne incognita population and reproduction on tomato under greenhouse conditions.*

Treatments	Nematode population in		No .eggs/ egg mass	Total final population (Pf)	Rf* (Pf/Pi)	**RGI	**EI
	Root (females and developmental stages)	Soil (J_2s)					
Ml	10 de	799 bc	192 b	2346	1.68	3.0	2.0
Nl	9 ef	735 b-d	90 b-e	1375	0.98	2.0	2.0
Cl	8 f	718 b-d	100 b-e	1327	0.95	2.0	2.0
Cs	10 de	633 c-e	129 b-e	1547	1.11	2.0	2.0
Cr	10 de	726 b-d	170 bc	1928	1.38	3.0	2.0
Ml+ Cl	9 ef	894 bc	147 b	1346	0.96	2.0	2.0
Ml+Cs	7 f	993 b	172 b	1690	1.21	2.0	2.0
Ml+Cr	12 bc	944 bc	166 b	1787	1.28	2.0	2.0
Nl + Cl	9 ef	635 cd	158 b	1280	0.91	2.0	2.0
Nl + Cs	11 cd	707 b-d	167 b	1722	1.23	2.0	2.0
Nl + Cr	12 bc	809 bc	149 b	1866	1.33	3.0	2.0
Ml+ Nl + Cl	10 de	434 de	151 b	1353	0.97	3.0	2.0
Ml+ Nl + Cs	13 b	611 cd	174 b	2369	1.69	3.0	2.0
Ml+ Nl + Cr	10 de	748 b-d	169 b	1608	1.15	3.0	2.0
Oxamyl	3 g	291 ef	51 c	345	0.25	1.3	0.5
Nematode only	68a	2769 a	364 a	12671	9.05	4.0	3.8

*Each value is the mean of four replicates. Means marked with the same letter(s) in each column did not significantly differ at P< 0.05 according to Duncan's mulltiple range test. *Rf: Reproduction factor; Pf: Nematode population in soil+ No. developmental stages +No. females+ (No. eggs/ egg mass x No. egg masses), Pi:1400 eggs and juveniles of M. incognita. Ml: moringa leaf; Cl: canola leaf; Cs: canola stem; Cr: canola root and Nl: neem leaf. *Root gall index (RGI) or egg masses index (EI) was determined according to the scale given by Taylor and Sasser (1978) as follows : 0= no galls or egg masses; 1= 1-2; 2= 3-10; 3= 11-30; 4=31-100 and 5= more than 100 galls or egg masses.*

However, the highest synergistic action was postulated with the introduction of Nl with both Ml leaf and Cl extracts that significantly (P<0.05) suppressed nematode population in soil (84.33%) with Rf equal 0.97 compared to nematode alone (Rf= 9.05) followed by Ml+Nl+Cs (77.9%) then Ml+Nl+Cr (73.0%). However, aqueous leaf extracts of canola and neem showed more lethal effect on total population (Pf) and number of eggs/ egg mass in comparison to stem and root extracts of canola and moringa leaf.

Root galling and egg mass numbers of tomato plants were significantly (P<0.05) decreased by all treatments of plant extracts (Table 2). Root gall and egg masses indices ranged from 1.3 to 3.0 and 0.5 to 2.0, respectively compared to control plants (RGI=4.0; EI=3.8, respectively). The highest reduction in root galling (95.7%) or egg masses (96.3%) was recorded with oxamyl (RGI=1.3 and EI = 0.5.) Even, significant difference in root galling or number of egg masses in most treatments was not detected (Table 2).

NPK and photosynthesis pigments

Chemical constituents in dried tomato leaves were significantly altered as a result of mixture plant extracts (Table 3). Hence, aqueous leaf extracts of moringa and neem integrated with different botanical part extracts of canola showed better results than did single or dual applications. Meanwhile, Ml + Nl + Cr extract registered the most effective and exhibited the highest percentage of increase in N (29.88%), P (41.37%) and K (40.66%) and total chlorophyll (12.52%).

Resistance related compounds

Herein, phenylalanine ammonia lyase (PAL), total phenol and salicylic acid (SA) activities were significantly raised in tomato plants as a result of root-knot nematode, *M. incognita*. Plant extracts applied singly or in mixture showed significantly lower activities in such compounds compared to nematode alone. Irrespective to oxamyl, the highest suppression in such criteria was demonstrated with Ml + Nl + Cr extract (Figure 1).

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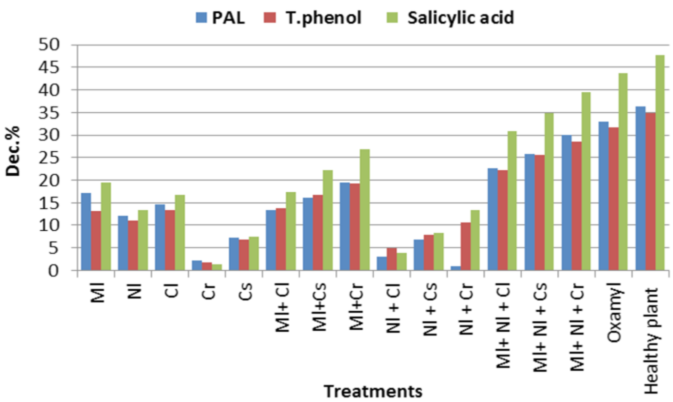

Figure 1: *Effect of aqueous plant extracts singly and in mixture as potential inducers on PAL, Total phenol and salicylic acid in dried leaves of tomato infected with Meloidogyne incognita.*

Estimation of flavonoids and phenolic compounds in aqueous extract of neem leaves

Flavonoids fractioned using HPLC (Table 4) showed the presence of 21 compounds in aqueous leaf extract of neem i.e. Hespiridin, Naringin, Hespirtin and Rosmarinic acid. Also, chemical analysis of aqueous neem leaf extract also revealed the presence of 23 phenolic compounds including e-Vanillic, pyrogallol, benzoic, salicylic acid and gallic acid (Table 4). The chemical structure of selected flavonoids and phenolic compounds is presented in Figures 2 and 3.

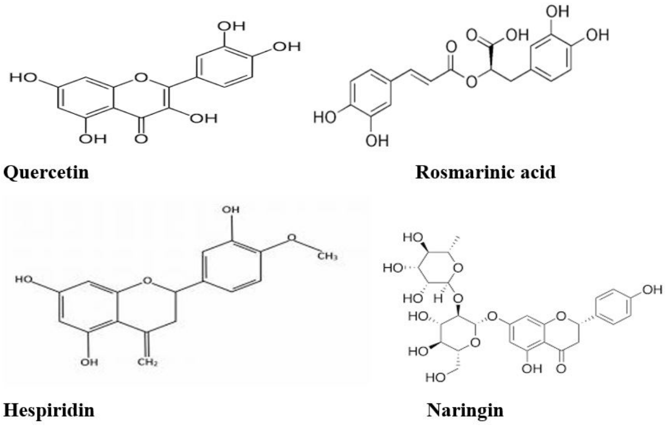

Figure 2: *Chemical structure of selected flavonoids compounds in aqueous extract of neem leaf.*

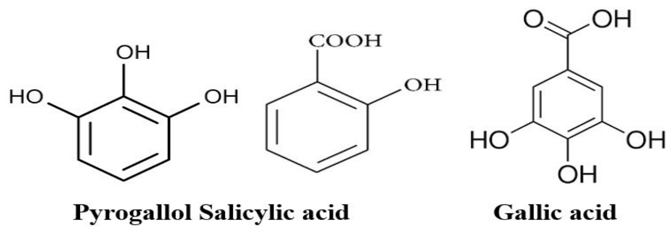

Figure 3: *Chemical structure of selected phenolic compounds in aqueous extract of neem leaf.*

Table 3: *Effect of aqueous plant extracts singly and in mixture as potential inducers on chemical constituents in dried leaves of tomato infected with Meloidogyne incognita under greenhouse conditions.*

Treatments	Chemical constituents					
	N	P	K	Chlorophyll content (m/g)		
				Chlo A	Chlo B	Total (A+B)
Ml	2.95fg	0.277 c	2.51 h	0.629 j	0.446 j	1.075
Nl	2.80 hi	0.261 de	2.33 l	0.618 m	0.434 m	1.052
Cl	2.87 gh	0.288 b	2.42 j	0.625 j	0.440 l	1.065
Cs	2.47 m	0.226 hi	1.98 q	0.601 p	0.416 p	1.017
Cr	2.63 k	0.243 fg	2.15no	0.609 o	0.425 o	1.034
Ml+ Cl	2.85 g-i	0.241 gh	2.49 i	0.645 h	0.445 k	1.090
Ml+Cs	2.93 fg	0.252 ef	2.58 g	0.654 g	0.452 g	1.106
Ml+Cr	3.02 ef	0.263 d	2.66 f	0.663 f	0.458 f	1.121
Nl + Cl	2.59 kl	0.212 ij	2.18 n	0.621 l	0.429 n	1.050
Nl + Cs	2.68 jk	0.221 i	2.29 m	0.630 k	0.434 m	1.064
Nl + Cr	2.76 ij	0.232 h	2.37 k	0.638 i	0.440 l	1.078
Ml+ Nl + Cl	3.1 de	0.270 cd	2.79 e	0.671 e	0.464 e	1.135
Ml+ Nl + Cs	3.17 cd	0.279 bc	2.87 d	0.682 d	0.471 d	1.153
Ml+ Nl + Cr	3.26 bc	0.287 b	2.94 c	0.689 c	0.479 c	1.168
Oxamyl	3.34 ab	0.298 a	3.03 b	0.695 b	0.485 b	1.180
Healthy Plant	3.43 a	0.306 a	3.12 a	0.703 a	0.489 a	1.192
Nematode only	2.51 lm	0.203 j	2.09 p	0.613 n	0.425 o	1.038

Each value is the mean of four replicates. Means marked with the same letter(s) in each column did not significantly differ at P< 0.05 according to Duncan's mulltiple range test. Ml: moringa leaf; Cl: canola leaf; Cs: canola stem; Cr: canola root and Nl: neem leaf; N: nitrogen, P: phosphorus; K: potassium.

Table 4: *Flavonoids and phenolic compounds in aqueous neem leaf extract.*

Flavonoids compounds	Contents (µg/100g)	Phenolic compounds	Contents (µg/100g)
Luteo.6-arabinose8-glucose	4250.63	Gallic acid	51.45
Luteo.6-glucose 8- arabinose	1046.68	Pyrogallol	902.16
Apig.6-arabinose8-glucose	1032.97	4-Amino-Benzoic	3.29
Apig.6-rhamnose 8-glucose	2022.61	Protocatechuic	162.29
Apig.6-glucose 8-rhamnose	1764.94	Catechein	39.94
Luteo.7- glucose	390.23	Chlorogenic	100.55
Naringin	796.59	Catechol	76.14
Rutin	93.84	Epicatechin	122.30
Hespiridin	6494.52	Caffien	46.58
Rosmarinic acid	126.88	Caffeic	10.77
Apig.7-0-o-neohespiroside	222.80	Vanillic	111.75
Kamp.3,7-dirhamoside	195.44	p-Coumaric	231.68
Apigenin-7-glucose	203.15	Ferulic	92.36
Quercetrin	45.45	Iso- Ferulic	51.37
Quercitin	26.40	Reversetrol	17.62
Naringenin	43.33	Ellagic	386.71
Hespirtin	302.16	e- Vanillic	4699.53
Kampferol	114.51	α –Coumaric	260.58
Rhamnetin	79.88	Benzoic	387.42
Apigenin	8.80	3,4,5 methoxy Coumaric	59.70
Acacetin	1.24	Coumaric	21.86
		Salicylic acid	236.20
		Cinnamic	28.76

Synergistic effects of plant extracts mixture have been widely documented on bacteria and soil borne fungi (Begum and Navaraj, 2012; Al-Terehi *et al*., 2013; Riveral *et al*., 2014). However, little attempt has been given to nematodes (Yen *et al*., 2005; Ibrahim *et al*., 2007; Khairy, 2016). In the present study, screened plant extracts are combined in mixture to widen the spectrum of nematicidal activity against root-knot nematode, *M. incognita* thereby ameliorating tomato plant growth criteria. Aqueous leaf extracts of neem and moringa have been previously reported to induce remarkable improvement in plant infected with *M. incognita* (Nwankwo *et al*., 2016; Kankam *et al*., 2018; Khairy *et al*., 2021) which support the present findings in that aqueous leaf extract of neem and canola were the best amongst individual applications and significantly (P<0.05) improved shoot length and weight of tomato infected with *M. incognita*, respectively. In all treatments of mixture extracts, dual and triple applications showed synergistic impact on tomato growth compared with single application. Hence, triple application of canola (Cl), moringa (Ml) and neem leaf (Nl) extracts significantly (P<0.05) improved tomato shoot length followed by Ml+Nl+Cr and dual application of Nl+Cs and Nl+Cr. However, the mixture of neem leaf and different parts of canola showed significant augmentation in plant biomass better than did other treatments with a range of 44.3-50.8%. even no significant differences (P<0.05) between treatments were noticed.

In some treatments, the nematicidal efficacy of mixture of aqueous plant parts was considerably increased related to the low activities indicated with singly parts extracts. Individual applications of aqueous leaf extract of canola and neem were found to be more effective in suppressing total nematode population and number of eggs/ egg mass in comparison to stem and root extracts of canola and moringa leaf. These observations are in conformation with the findings of Haroon *et al*. (2018) who reported. that methanolic neem extract (leaf and root) was the most effective in inhibiting egg hatching of *M. incognita,* followed by *M. oleifera* extract. However, the highest synergistic action was accomplished with the combination of leaf extracts of neem, moringa and canola that significantly (P<0.05) suppressed nematode reproduction (Rf =0.97) compared to nematode alone (Rf= 9.05). These findings are consistent with published literature citing the nematicidal activity of Sincocin, a mixture of neem+mustard against *M.incognita* (Yen *et al*., 2005; Youssef, 2008). Similarly, root gall and egg masses indices were obviously reduced by all treatments.

Generally, it has been stated that ethanolic extracts of moringa possess growth enhancing compounds that belong to the group of cytokinin which is functional in controlling nematode population density and improving in plant growth and vigor (Guzman, 1984). Also, cultivating rapeseed (canola) cv. Jupiter in *M. chitwood* infested soil for 2 months and then incorporating shoots into soil resulted in significant (P < 0.05) reduction in final population (Mojtahedi *et al*., 1991). However, glucosinolates, found in all parts of canola plants, were hydrolyzed by myronase to is thiocyanate which is considered an effective biocide (Ntalli *et al*., 2020).

Plant extracts were found to induce NPK, chlorophyll and defense related enzymes which lead to systemic resistance in tomato plant infected with *M.incognita* (Table 3). Meanwhile, Ml + Nl + Cr extract being the most effective and exhibited the highest percentage of increase in N (29.88%), P (41.37%) and K (40.66%) and total chlorophyll (12.52%). A significant increment in NPK and total chlorophyll has been reported in economic plants infected with *M. incognita* treated with plant extracts and botanical biopesticides (Khairy, 2016; Metwally *et al*., 2019).

Current study implies that salicylic acid (SA), total phenols and phenylalanine ammonia lyase (PAL) were significantly (P<0.05) fostered in leaves of tomato infected with target nematode related to healthy plants. Phenolic compounds form one of the most significant groups of plant secondary metabolites which render as defense components against plant pathogens (Bahattacharya, 2010). Phenol increment induced by pathogen infestation trigger the RNA transcription; increasing amounts of PAL gives rise to the composition of phenolic compounds (Taiz and Zeiger, 2002).

Phenylalanine (PAL) is an important enzyme for trans-cinnamic acid formation, that is the prospector of phenolic compounds where lignin is the final product (Campbell and Sederoff, 1996). The activity of resistance related compound i.e. phenylalanine ammonia lyase (PAL) was significantly raised in tomato seedlings as a result of *M. incognita* infection. This finding is consistent with published literature citing that the high PAL activity at control plants treatment may reflect a retard in the host defense, which simplifies any hazards effects induced by the pathogenic organism (Kyndt *et al*., 2013). Salicylic acid was also found to act as growth promoters and an inducer of resistance to *Meloidogyne* spp. in tomato (Molinari, 2008; Mukherjee *et al*., 2012).

In the current investigation, plant extracts applied singly or in mixture showed significantly lower levels of SA, phenols and PAL activity in tested plants infected with the target nematode, *M. incognita* compared to untreated uninoculated plant. Among plant extracts the greatest suppression in such criteria was accomplished with Ml + Nl + Cr extract. According to Ahmed *et al*. (2009) the lower levels of phenols in mung bean infected with *M. javanica* could be attributed to oxidation reaction of phenolic compounds by polyphenoloxidase (PPO), and formation of quinones, which are extremely toxic to nematodes.

The nematicidal properties of moringa leaf (Ml) and neem leaf (Nl) extract against *M.incognita* could be imputed to the presence biological compounds as tannins, flavonoids, saponins, steroids, triterpenoids alkaloids and reducing sugars. Some of these phytochemicals such as tannins, saponins and flavonoids are reported to cause disruption of membranes in organisms thereby facilitating penetration of toxic principles to the detriment of such organism (Izuogu *et al*., 2013; Chin *et al*., 2018; Olajidei, 2018). Flavonoids have been known to exert enormous biological effects including antioxidant and antimicrobial activity against a wide array of pathogens through inhibiting the membrane bound enzymes (Li-Weber, 2009; Dash *et al*., 2017). Moreover, alkaloids and flavonoids present in botanical extracts showed ovicidal activity against *Meloidogyne* eggs (Adegbite, 2003).

Also variation in chemical components was observed between canola organs (root extracts, shoot extracts) and between canola genotypes (Asaduzzaman *et al*., 2014). Root extracts sustained more secondary metabolites compared to shoot extracts. Fourteen secondary metabolites, were identified in such organs including quercitin, rutin, jasmonic acid, vanillc acid, methyl-jasmonate, and 2-phenylethyl glucosinolates. The flavonols i.e. kaempferol, quercetin, and myricetin have been reported to repel and slow second stage juveniles of *M. incognita* (Wuyts *et al*., 2006). Whereas, Patuletin, patulitrin, quercetin, and rutin showed lethal impact on *Heterodera zeae* juveniles (Faizi *et al*., 2011).

Twenty one flavonoids and twenty three phenolic compounds were evidenced using HPLC in aqueous leaf extract of neem. Hespiridin, Naringin, Hespirtin and Rosmarinic acid have been reported to have nematicidal properties against plant parasitic nematodes (Wuyts *et al*., 2006; Wang *et al*., 2012). Also phenolic compounds including e-Vanillic, pyrogallol, benzoic, salicylic acid and gallic acid. Pyrogallol and gallic acid were found to be highly toxic against *M.incognita* (Ohri and Pannu, 2010).

Conclusions and Recommendations

In conclusion mixtures of plant extracts have shown potentiality to reduce *M. incognita* reproduction and alter chemical constituents in tomato and could be recommended for sustainable management of *Meloidogyne* spp. However, isolation and identification of the nematicidal compounds is fundamental for further promotion.

Novelty Statement

The current study indicated the nematicidal properties of combined use of plant extracts against root-knot nematode, *M .incognita* . Screened plant extracts can act as resistance inducers, plant growth promoters and biocontrol agents within sustainable management.

Author's Contribution

Doaa Khairy: Conducted the experiment, performed material preparation, data analysis and drafted the manuscript.
Mohamed Ali Osman and Fatma Abdel Mohsen Mostafa: Contributed to the study conception and design, supervised and approved the final manuscript.

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

References

Asaduzzaman, M., Pratley, J.E., An, M., Luckett, D.J., and Lemerle, D., 2014. Metabolomics differentiation of canola genotypes: toward an understanding of canola allelochemicals. Front

Plant Sci., 5: 765. https://doi.org/10.3389/fpls.2014.00765

AOAC, 1980. Official methods of analysis. Twelve Ed. Published by the Association of Official Analytical chemists, Benjamin, France line station, Washington. DC.

Adegbite, A.A., 2003. Comparative effects of Carbofuran and water extract of *Chromolaena odorata* on growth, yield and food components of root-knot nematode-infested soybean (*Glycine max* (L.) Merrill). Ph.D. thesis, University of Ibadan, Ibadan, Nigeria. pp. 217.

Ahmed, N., Abbasi, M.W., Shaukat, S.S., and Zaki, M.J., 2009. Physiological changes in leaves of mungbean plants infected with *Meloidogyne javanica*. Phytopathol. Medit., 48(2): 262-268.

Al-Terehi, M., Al-Saadi, A.H., Al-Musawi, H.T., Zaida, H.K., Behjet, R.H., and Haleem, Z., 2013. Synergism effect of antibacterial activity of some medicinal plants. J. Biol. Med. Sci., 1: 46-53.

Bahattacharya, A., Soodi, P., and Citovsky, V., 2010. The roles of plant phenolics in defence and communication during *Agrobacterium* and *Rhizobium* infection. Mol. Plant Pathol., 11(5): 705–719. https://doi.org/10.1111/j.1364-3703.2010.00625.x

Begum, S.S., and Navaraj, P.S., 2012. Synergistic effect of plant extracts supplemented diets on immunity and resistance to *Aeromonas hydrophila* in *Mystus keletius*. IOSR J. Pharm. Biol. Sci., 2(4): 30-36. https://doi.org/10.9790/3008-0243036

Byrd, D.W., Kirapatrick, T., and Barker, K., 1983. An improved technique for clearing and staining plant tissues for detection nematodes. J. Nematol., 15(3): 142-143.

Campbell, M.M., Sederoff. 1996. Variation in lignin content and composition (Mechanisms of Control and Implications for the Genetic Improvement of Plants. Plant Physiol., 110 (1):3-13. https://doi.org/10.1104/pp.110.1.3

Chen, C., Belanger, R.R., Benhamou, N., and Paulitz, T.C., 2000. Defense enzymes induced in cucumber roots by treatment with plant growth-promoting rhizobacteria (PGPR) and *Phytium aphanidermatum*. Physiol. Mol. Plant Pathol., 56: 13-23. https://doi.org/10.1006/pmpp.1999.0243

Chin, S., Abehm, C.A., and Mathesius, U., 2018. Functions of flavonoids in plant nematode interactions. Plants, 7(4): 85. https://doi.org/10.3390/plants7040085

Dash, S.P., Dixit, S., and Sahoo, S., 2017. Phytochemical and biochemical characterizations from leaf extracts from *Azadirachta indica*: An important medicinal plant. Biochem. Anal. Biochem., 6: 2. https://doi.org/10.4172/2161-1009.1000323

Duncan, D.B., 1955. Multiple range and multiple, f-test biometrics. 11: 1-42. https://doi.org/10.2307/3001478

El-Deriny, M.M., Ibrahim, D.S.S., and Mostafa, F.A.M., 2020. Organic additives and their role in phytoparasitic nematodes management. Springer nature management of phytonematodes: Recent advances and future challenges. pp. 73-93. https://doi.org/10.1007/978-981-15-4087-5_4

Fadeel's, A.A., 1962. Location and properties of chloroplasts and pigment determination in roots. Physiol. Plant, 15: 130-147. https://doi.org/10.1111/j.1399-3054.1962.tb07994.x

Faizi, S., Fayyaz, S., Bano, S., Yawar, Iqbal, E., Siddiqi, Lubna, H., and Naz, A., 2011. Isolation of nematicidal compounds from *Tagetes patula* L. yellow flowers: Structure–activity relationship studies against cyst nematode *Heterodera zeae* infective stage larvae. J. Agric. Food Chem., 59: 9080–9093. https://doi.org/10.1021/jf201611b

FAOSTAT, 2020. Tomato production in 2019. https://en.wikipedia.org/wiki/Tomato.

Fiske, C.H., and Subbarow, Y., 1925. The colorimetric determination of phosphorus. J. Biol. Chem., 66: 375–400. https://doi.org/10.1016/S0021-9258(18)84756-1

Gomez, K.A., and Gomez, A.A., 1984. Statistical procedures for agriculture research. 2nd Ed., June Wiley and Sons. Inc. New York.

Goodey, J.B., 1957. Laboratory methods for work with plant and soil nematodes. Tech. Bull. 2 Min. Agric. Fish Ed. London. pp. 47.

Goswami, B.K., Vijayalakshmi, K. 1986. Nematicidal properties of some indigenous plant materials against root-knot nematodes, *Meloidogyne incognita* on tomato. Indian J.Nematol.,16:65-68.

Guzman, R.S., 1984. Toxicity screening of various plant extracts, *Anthocephalus chinensis* (Lamb) Rich ex Walp, *Desmodium gangeticum*(Linn) DC, *Artemisia vulgaris* Linn, *Eichornia crassipes* (Mart) Solms, *Leucaena leucocephala* (Lam) de

Wit, *Allium cepa* Linn, *Allium sativum* Linn and *Moringa oleifera* Lam] against *Meloidogyne incognita* Chitwood and *Radopholus similis* Cobb and characterization of their nematicidal components. Ph.D. thesis, University of the Philippines, Los Banos, Philippines.

Haroon, S.A., Hassan, B.A.A., Hamad, F.M.I., and Rady, M.M., 2018. The efficiency of some natural alternatives in root-knot nematode control. Adv. Plants Agric. Res., 8(4): 355-362. https://doi.org/10.15406/apar.2018.08.00337

Hussey, R.S., and Barker, K.R., 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Dis. Reptr., 57: 1025–1028.

Ibrahim, I.K.A., EL-Saedy, M.A.M., and Mokbel, A.A., 2007. Control of the root–knot nematode *Meloidogyne incognita* on sunflower plants with certain organic plant materials and biocontrol. Egypt. J. Phytopathol., 35(1): 13-24.

Izuogu, N.B., Badmos, A.A., and Raji, S.O., 2013. The potency of *Moringa oleifera* and *Jatropha curcas* leaf extracts as control for root- knot nematode in maize (*Zea mays*). Int. J. Phytofuels Allied Sci., pp. 116-124.

Jackson, M.L.,1967. Soil chemical analysis. Printic Hall of India, New Delhi. pp. 144-197.

Javaheri, M., Mashayekhi, K., Dadkhah, A., and Tavallaee, F.Z., 2012. Effects of salicylic acid on yield and quality characters of tomato fruit (*Lycopersicum esculentum* Mill.). Int. J. Agric. Crop Sci., 4(16): 1184-1187.

Kankam, F., Sowley, E., and Dasmana, H., 2018. Effect of time of application of moringa (*Moringa oleifera* L.) leaf powder on root-knot nematode (*Meloidogyne* spp.) infecting cowpea (*Vigna unguiculata* L. walp). Adv. Agric. Sci., 6(4): 32-41.

Kepenekci, L., Erdoğuş, D., and Erdogan, P., 2016. Effects of some plant extracts on root-knot nematodes *in vitro* and *in vivo* conditions. Turk. J. Entomol., 40(1): 3-14. https://doi.org/10.16970/ted.84688

Khairy, D., 2016. Management of root-knot nematode *Meloidogyne incognita* by the use of certain bioagents. M.Sc. thesis, Fac. Agric., Mansoura Univ. Egypt. pp. 110.

Khairy, D., Refaei, A.R., and Mostafa, F.A.M., 2021. Management of *Meloidogyne incognita* infecting eggplant using moringa extracts, vermicompost, and two commercial bio-products. Egypt. J. Agronematol., 20(1): 1-16. https://doi.org/10.21608/ejaj.2021.134910

Khalil, M.S., 2013. Abamectin and azadirachtin as eco-friendly promising biorational tools in integrated nematodes management programs. Plant Pathol. Microbiol., 4(4): 1-7. https://doi.org/10.4172/2157-7471

Kyndt, T., Vieira, P., Gheysen, G., and Almeida-Engler, J., 2013. Nematode feeding sites: Unique organs in plant roots. Planta, 238: 807-818. https://doi.org/10.1007/s00425-013-1923-z

Li-Weber, M., 2009. New therapeutic aspects of flavones: The anticancer properties of *Scutellaria* and its main active constituents wogonin, baicalein and baicalin. Cancer Treat. Rev., 35(1): 57-68. https://doi.org/10.1016/j.ctrv.2008.09.005

Metwally, W.E., Ashraf, E.K., and Mostafa, F.A.M., 2019. Biopesticides as ecofriendly alternatives for the management of root-knot nematode, *Meloidogyne incognita* on cowpea (*Vigna unguiculata* L.). Egypt. J. Agronematol., 18(2): 129-145. https://doi.org/10.21608/ejaj.2019.51846

Mojtahedi, H., Santo, G.S., Hang, A.N., and Wilson, J.H., 1991. Suppression of root-knot nematode populations with selected rapeseed cultivars as green manure. J. Nematol., 23(2): 170-174.

Molinari, S., 2008. Salicylic acid as an elicitor of resistance to root-knot nematodes in tomato. Acta Hortic., 789: 119-125. https://doi.org/10.17660/ActaHortic.2008.789.15

Mukherjee, A., Babu, S.P.S., and Mandal, F.B., 2012. Potential of salicylic acid activity derived from stress-induced (water) tomato against *Meloidogyne incognita*. Arch. Phytopathol. Plant Protect., 45: 1909-1916. https://doi.org/10.1080/03235408.2012.718220

Nimbalkar, R.K., and Rajurkar, S.K., 2009. Effect of plant root extracts to control root-knot nematode (*Meloidogyne* spp.) of soybean (*Glycine max*). Biol. Forum Int. J., 1(1): 65-68.

Ntalli, N., Adamski, Z., Doula, M., and Monokrousos, N., 2020. Nematicidal amendments and soil remediation. Plants, 9: 429. https://doi.org/10.3390/plants9040429

Ntalli, N.G., Menkissoglu-Spiroudi, U., Giannakou, I.O., and Prophetou-Athanasiadou, D.A., 2009. Efficacy evaluation of a neem (*Azadirachta indica* A. Juss) formulation against root-knot nematodes, *Meloidogyne incognita*. Crop Protect., 28: 489-494. https://doi.org/10.1016/j.cropro.2009.01.011

Nwankwo, E.N., Onuseleogu, D.C., Ogbonna Confidence, U., and Okorocha, A.O.E., 2016. Effect of neem leaf extracts (*Azadirachta indica*) and synthetic pesticide (Carbofuran) on the root-knot nematode (*Meloidogyne* spp.) of cowpea (*Vigna unguiculata* L. Walp). Int. J. Entomol. Res., 1(3): 01-06

Ohri, P., and Kaur, S., 2010. Effect of phenolic compounds on nematodes. A review. J. Appl. Nat. Sci., 2(2): 344-350. https://doi.org/10.31018/jans.v2i2.144

Olajidei, M.C., Izuogui, N.B., Apalowoi, R.A., and Baba, H.S., 2018. Evaluation of the nematicidal and antifungal activity of aqueous extract of *Moringa oleifera* leaves and seed in cucumber field. Cercet. Agron. Mol., LI 4 (176): 47-59. https://doi.org/10.2478/cerce-2018-0035

Onifade, A.K., Egunjobi, O.A. 1994. Suppression of *Meloidogyne incognita* populations with water hyacinth and water lettuce in Nigeria. Afro-Asian J. Nematol., 4: 96-100.

Riveral, S.E.V., Escobar-Saucedo, M.A., Morales, D., Aguilar, C.N., and Rodríguez-Herrera, R., 2014. Synergistic effects of ethanolic plant extract mixtures against food-borne pathogen bacteria. Afr. J. Biotechnol., 13(5): 699-704. https://doi.org/10.5897/AJB2013.12273

Saeed, M.R.M., Awadh, G.A.M., Al-Thobhani, M.A., and Taj Al- Deen, Aziza. 2015. *In vitro* nematicidal activity of ten plant extracts against juveniles of *Meloidogyne incognita*. Egypt. J. Agronematol., 14(1): 78-90. https://doi.org/10.21608/ejaj.2015.60403

Taiz, L., and Zeiger, E., 2002. Plant Physiology (Third Edition). Sinauer Associates, Inc., Publishers, Sunderland, pp. 67-86.

Taylor, A.L., and Sasser, J.N. 1978. Biology, identification and control of root-knot Nematodes (*Meloidogyne* spp.) Coop. Pub. Dept. Pl. Pathol. North Carolina State Univ. and U.S. Agency Int. Dev. Raleigh, N.C. pp. 111

Wang, J., Pan, X., Han, Y., Guo, D., Guo, Q., and Li, R., 2012. Rosmarinic acid from eelgrass shows nematicidal and antibacterial activities against pine wood nematode and its carrying bacteria. Mar. Drugs, 10(12): 2729-2740. https://doi.org/10.3390/md10122729

Wuyts, N., Swennen, R., and De Waele, D., 2006. Effects of plant phenylpropanoid pathway products and selected terpenoids and alkaloids on the behaviour of the plant-parasitic nematodes *Radopholus similis, Pratylenchus penetrans* and *Meloidogyne incognita*. Nematology, 8: 89–101. https://doi.org/10.1163/156854106776179953

Yen, J.H., Wang, H.Y., Chen, D.Y., Tsai, S.P., and Tsay, T.T., 2005. Efficacy of sincocin and ditera for controlling of southern root-knot nematode, *Meloidogyne incognita*. Plant Pathol. Bull., 14(4): 275-278.

Youssef, M.M.A., 2008. Role of biotechnology in the management of plant parasitic nematodes. A review. Pak. J. Nematol., 26(1): 1-12.