



Nematicidal potential of Some Wild Weeds on Root-Knot Nematodes Infecting Tomato Plants

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ABSTRACT

Root knot nematodes (RKNs) are considered destructive plant parasitic nematodes of many host plants all over the world. Nematicidal potential of eight wild weeds i.e., *Atriplex lindleyi*, *Chenopodium album*, *Hyoscyamus muticus*, *Mesembryanthemum nodiflorum*, *Pluchea dioscoridis*, *Senecio squalidus*, *Zygopyllum qatarense*, and *Amaranthus ascendens* were tested against *Meloidogyne* spp. *in vitro* and *in vivo* on tomato plants. Results of *in vitro* tests indicated that *A. lindleyi* and *C. album*, recorded the highest percentage of egg hatching inhibition and larvae mortality by 97 and 100 % respectively at all exposure periods. Also, *A. lindleyi* at 4% of soil weight, recorded the highest reduction in nematode parameters i.e., number of galls, egg masses, females/root system and juveniles (J₂s)/250g soil, with 75, 80, 70 and 86%, respectively, Furthermore, tomato plant growth parameters i.e., root and shoot lengths, fresh root and shoot weights as well as plant dry weight were improved in treated plants compared with non-treated control. So that, weeds used in this study may be included in the plant parasitic nematode management programs.

Key words: Root-knot nematodes, *Meloidogyne* spp., safe management, wild weeds.

INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) considered a widely cultivated distributed annual vegetable crop which consumed as fresh, cooked, or processed: by canning, paste or juice. It is adapted with a wide tropical and subtropical region under protected system or field conditions. a wide tropical and subtropical region under protected system or field conditions. Egypt produces tomatoes by 6,751,856 million tons comes from 428182 feddan (FAO, 2020). Tomato plants are infected with several diseases such as fungi,

bacteria, virus and plant parasitic nematodes. Primary calculated annual yield losses tomatoes by plant parasitic nematodes in Egypt reached 12% with 1,753,17 million L.E. during 2011-2012 (Abd-El Gawad 2014). The root-knot nematodes (*Meloidogyne* spp.) belong to the most ten dangerous species with variable serious loss to tomato yield (Sasser, 1980). It can reduce annual world crop production by about 5% as reported by Sasser (1987). *Meloidogyne* spp. reported in nematological surveys in different crops at different locations

under Egyptian conditions such as tomato (Mousa,1997; Bakr *et al.*, 2011 and 2020; Abdel-Baset,2022). Symptoms usually were chlorosis, abnormal vegetative and physiological characters, root galling with final low yield production (Bakr *et al.*, 2017; Abd-Elgawad,2021). The total losses were probably affected by different factors including population of nematode in the soil, host plant cultivar, soil type, other soil microfauna plant pathogens, and weather conditions (Bakr and Ketta, 2018).

Due to different dangerous hazardous effects of chemical nematicides to humans, animals, plants and the environment atmosphere (Abdel-lateif and Bakr, 2018), most of plant nematologists all over the world thinking in innovation environmentally, friendly alternative approaches for control RKNs (Al-Hendy *et al.*, 2021). Recently, plants with nematicidal effect were advised as safety methods were used in control root-knot nematodes *Meloidogyne* spp. Treatments of wild weeds treatments as aqueous, acetic and methanolic extracts, dry or fresh were previously reported (Siddiqui *et al.*, 2004; Ibrahim *et al.*, 2018; Abdel-Mageed *et al.*, 2021; and Bakr,2021).

Therefore, the objective of this study is to evaluate eight naturally growing wild weeds against *Meloidogyne* spp. under laboratory tests and under greenhouse conditions on tomato plants.

MATERIALS AND METHODS

Stock culture and maintenance of *Meloidogyne* spp.

Stock culture of root-knot nematodes *Meloidogyne* spp. were done using tomato plants grown in plastic pots 30 cm in diameter contain sand-clay mixture soil (2:1, v/v) under greenhouse conditions, in the Faculty of Agriculture, Menoufia University, Egypt.

Extraction of *Meloidogyne* spp. eggs.

For RKN egg extraction, heavy galled tomato roots infected with *Meloidogyne* spp. were gently uprooted from pots and carefully washed with tap water to exclude the adherent soil particles. The roots were cut into small pieces (2-3cm), then using waring blender macerated at high speed for two times of 10 seconds each to allow release the highest eggs number from the roots. The macerated root solution was then placed into flask containing sodium hypochlorite (NaOCl) with final concentration of 0.5% following the technique described by Hussey and Barker (1973). Solution was vigorously shaken for 3 minutes to release the eggs from the gelatin matrix. After that solution was passed through different size sieves to remove the root tissue. Eggs were collected on the 26 micrometer (μm) (500 mesh) sieve and washed several times with tap water to remove the NaOCl residual. Eggs were transferred to a flask containing tap water and then counted under light microscope.

Collection of Wild Weeds and identification

Eight naturally growing wild weeds illustrated in Table 1 were collected from four different regions in El-Beheira and Menoufia governorates, Egypt. Collected weeds placed in bags and then transferred to the Laboratory of the

Department of Agricultural Botany, Faculty of Agriculture, Menoufia University, Shebin El-Kom, Egypt. Identification processes were done according to Boulos *et al.*, (1967) Tackholm (1974) and Boulos (1995).

Table (1): List of eight wild weed species screened for nematicidal activity.

Collection Site	Family	Botanical Name	Common Name
Wadi El-Natrun	<i>Amaranthaceae</i>	<i>Atriplex lindleyi</i>	Flat-top saltbush Lindley's saltbush
	<i>Chenopodiaceae</i>	<i>Chenopodium album</i>	Common lambsquarters Goosefoot, Fat-hen
	<i>Zygophyllaceae</i>	<i>Zygophyllum qatarense</i>	Bean caper Ratrayt
Edku City	<i>Aizoaceae</i>	<i>Mesembryanthemum nodiflorum</i>	Crystalline ice plant Slender-leaf ice plant
Al-Khatatbah	<i>Amaranthaceae</i>	<i>Amaranthus ascendens</i>	purplish amaranth
	<i>Asteraceae</i>	<i>Pluchea dioscoridis</i>	Pluchea
El-Sadat City	<i>Asteraceae</i>	<i>Senecio squalidus</i>	Oxford ragwort Oxford groundsel
	<i>Solanaceae</i>	<i>Hyoscyamus muticus</i>	Egyptian henbane

***In vitro* experiments:**

- Effect of extracts on *Meloidogyne* spp. egg hatching.

To evaluate the effect of aqueous extracts of eight different wild weeds on egg hatching, the fresh weeds (Vegetative part) were thoroughly soaked at 30g /100ml of distilled water in a beaker for 24 hrs, then filtered through filter paper Watman no.1. About 0.9 ml of each weed aqueous extract and 100 eggs of *Meloidogyne* spp. in 0.1 ml of distilled water were placed together in 5-cm Petri dishes. Petri dishes containing nematodes alone serve as control. Each treatment was

replicated 5 times. Separate sets of Petri dishes were incubated for each period of observation (24,48,72,120 and 168 hrs) under laboratory conditions at 25°C± 2. Fifty eggs were randomly examined using a light microscope for evaluating the nematicidal effect of the wild weeds aqueous extracts of wild weeds on hatching.

- Effect of extracts on *Meloidogyne* spp. juveniles' mortality.

To evaluate the effect of the aqueous extract of wild weeds aqueous extracts on juveniles' mortality, 0.9 mL of each weed aqueous extract (Previously prepared) and 100 eggs of *Meloidogyne*

spp. in 0.1 ml of distilled water were placed together in 5-cm Petri dishes. One hundred eggs of *Meloidogyne* spp. in 0.1 ml of distilled water and 0.9 mL of distilled water were placed together in 5-cm Petri dishes containing nematodes alone serve as control. Each treatment was replicated 5 times. Separate sets of Petri dishes were incubated for each period of observation (24,48,72,120 and 168 hrs) under laboratory conditions at $25 \pm 2^\circ\text{C}$. Thirty juveniles were randomly examined using light microscope. Inactive juveniles appeared rigid and elongated with head and tail sometimes slightly wrapped in total. Juveniles were washed with tap water to remove the residual culture filtrates then transferred into water for 24 hrs before vitality test is done. Still inactive juveniles were considered dead.

- Greenhouse experiment

Weeds were thoroughly washed several times with tap water and left in open air for 30 minutes then cut into small pieces. Treatments were added to plastic pots (15 cm-diameter) filled with 1.5 kg of sandy clay soil mixture (2:1, v/v) and mixed with the soil at the rate of (1, 2 and 4% of soil weight). Pots were regularly irrigated for one week to allow weeds decomposition then three weeks old tomato seedlings (*Salymia* 65010), were transplanted (one seedling/pot), each seedling was inoculated with about 3000 nematode eggs and larvae at the same time of seedlings transplanting by pipetting around the seedling's roots. Pots received nematodes only served as

control. Each treatment was replicated three times. Plants were arranged in a completely randomized block design in the greenhouse at approximately $25 \pm 2^\circ\text{C}$. Plants were regularly irrigated and weekly fertilized with 5 ml of 2g/L of N:P: K (20:20:20). Plants had been allowed to grow for 60 days of nematode inoculation.

In the termination of experiment, the plants were uprooted and transferred to laboratory. Nematode related parameters i.e. number of galls, egg masses, females and number of juveniles (J_{2s})/250g soil, gall index was assessed on a scale of 0-10 according to Bridge and page (1980), nematode final population (Pf) and reproduction factor (Rf) were recorded according to Goodey (1957) following the equation:

$$Rf = \frac{\text{Final population (Pf)}}{\text{Initial population (Pi)}}$$

Egg-masses were stained prior to counting by dipping the infected roots in phloxine-B solution (0.15 g/l tap water) for 20 minutes as described by Daykin and Hussey (1985). Population of females were counted by cutting the root system of each plant in 2 cm pieces and submerging the roots in a beaker full of tap water for 4 days at room temperature until they became soft, then the roots were then washed through 500 and 250 μm sieves to separate the females from the root debris (Mahdy, 2002). The soil nematode population was enumerated by extraction root-knot nematode juveniles (J_{2s}) using the tray

modification of Baermann funnel as described by Barker (1985).

Vegetative plant growth parameters i.e. shoot and root fresh weights (g), shoot and root lengths (cm) as well as plant dry weight were recorded. Dry weight was recorded after drying the roots in oven under 70°C overnight until the constant weight according to Agaba and Fawole (2016).

- High Performance Liquid Chromatography (HPLC) and phytochemical analysis of *Atriplex lindleyi*

High Performance Liquid Chromatography (HPLC) and phytochemical analysis were conducted at Analysis and Studies Component - Soil, Water and Environment Unit, Agricultural Research Center, Ministry of Agriculture, Egypt.

Statistical analysis

Data were statistically analyzed using analysis of variance (ANOVA) and comparisons of means at 5% level of significance using Costat 6.3 version program according to Duncan's multiple range test.

RESULTS

***In vitro* experiments**

- Effect on egg hatching of *Meloidogyne* spp.

The nematicidal potential of water extract of different eight weeds i.e., *A. lindleyi*, *C. album*, *H. muticus*, *M. nodiflorum*, *P. dioscoridis*, *S. squalidus*, *Z. qatarense*, and *A. ascendens*, were tested on egg hatching inhibition of *Meloidogyne* spp. at five different

exposure periods (24, 48, 72, 120 and 168h) under laboratory conditions.

Data in Figure (1) indicated that *A. lindleyi* followed by *C. album*, recorded the highest percentage of hatching inhibition at all exposure periods. The percentage of hatching recorded (97%) for both weeds. *Hyoscyamus muticus* and *Z. qatarense*, recorded the second rank in hatching inhibition with (93%) for both weeds. While the least percentage of hatching inhibition was recorded with *A. ascendens* at all exposure periods with (83%) after 168 h of exposure, comparing to the control (nematode only).

Results showed that among the treatments the nematicidal effect on egg hatching of *Meloidogyne* spp. gradually increased by increasing the exposure time of eggs to the weed extracts.

- Effect on juvenile's mortality of *Meloidogyne* spp.

Results illustrated in Figure (2) showed that the extracts of *A. lindleyi*, *C. album* and *H. muticus* recorded 100% mortality of inactive juveniles (J₂s) % after 168 h of exposure, followed by *S. squalidus* and *P. dioscoridis* that recorded (97%) of larvae mortality. Meanwhile, *A. ascendens* recorded the least mortality percentage by 90% of inactive juveniles (J₂s), comparing with the control (nematode only). Comparing the mortality percentages of juveniles (J₂s) achieved by each single treatment of weed extract solutions at the different exposure periods, results

demonstrated that effectiveness and percentage of juveniles (J_2s) mortality increased with increasing the exposure period. No significant differences were

recorded between the highest six effective percentages but differences with control were recorded.

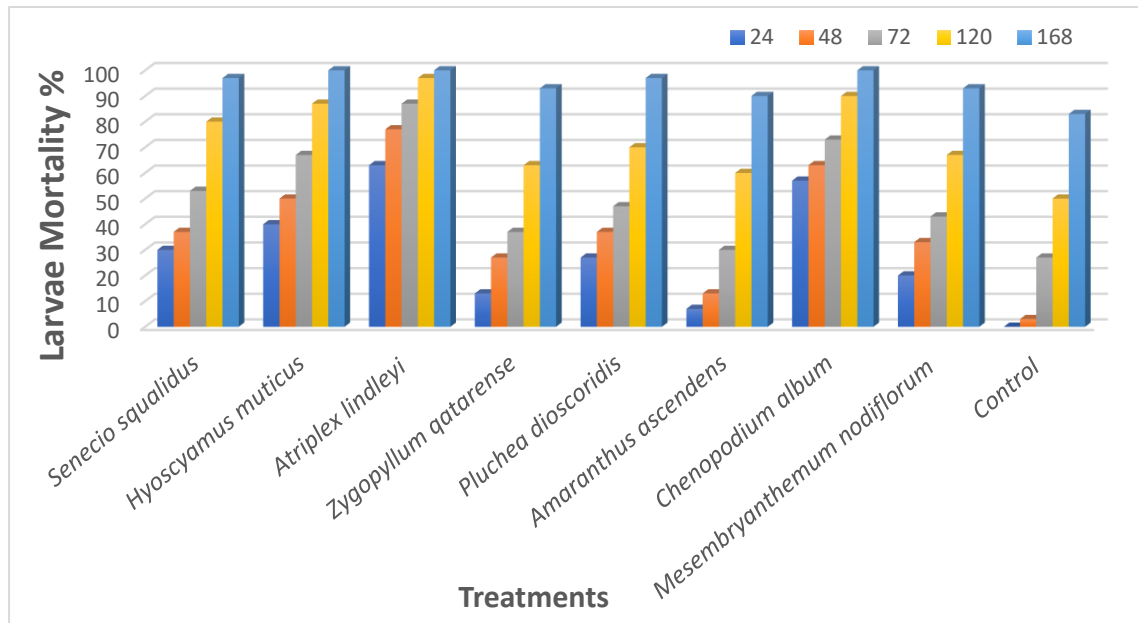


Figure (1): Effect of water extracts of different eight wild weeds on the egg-hatch of *Meloidogyne* spp. at different exposure periods.

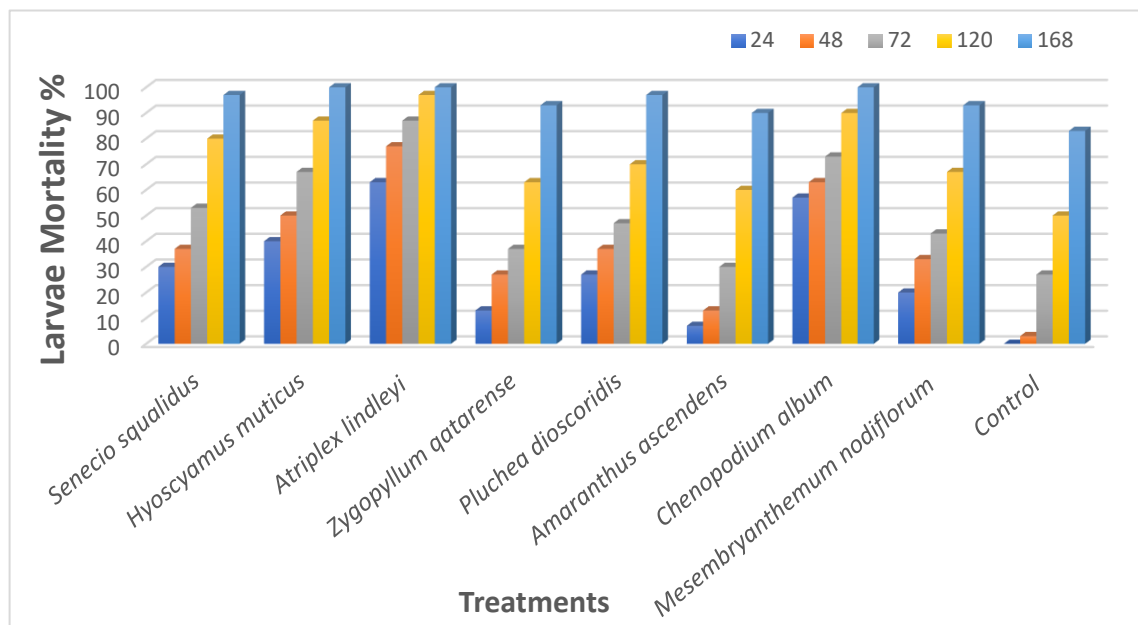


Figure (2): Effect of water extracts of eight wild weeds on mortality percentage of *Meloidogyne* spp. juveniles (J_2s) mortality at different exposure periods.

Greenhouse experiment

The obtained results in Table (2) proved that *A. lindleyi* at 4% of soil weight, recorded the highest reduction in nematode parameters i.e., number of galls, egg masses, females and number of juveniles (J_2s)/250g soil, with 75, 80, 70 and 86%, respectively, followed by *C. album* at concentration of 4% of soil weight, with 72, 62, 68 and 79%, respectively.

Meanwhile, *A. ascendens* at the concentration of 1% of soil weight recorded the least reduction in all nematode parameters with 9, 7, 12 and 8%, respectively, comparing with nematode alone.

Atriplex lindleyi at 4% of soil weight, achieved the highest reduction in gall index, nematode final population (Pf) and reproduction factor (Rf), followed by *C. album* at 4% of soil weight. Meanwhile *A. ascendens* at 1% of soil weight was the least effective one, compared with nematode alone.

Results in Table (3) reported that *A. lindleyi* at 4% of soil weight, recorded the highest increase in plant growth parameters i.e., root, shoot lengths, fresh root and shoot weights and plant dry weight with 108, 92, 122, 108 and 233%, respectively, followed by *C. album* at 4% of soil weight, with 92, 90, 77, 104 and 211%, respectively.

Meanwhile, *A. ascendens* at 1% of soil weight recorded the least increase in all plant growth parameters with 5, 3, 6, 9 and 0%, respectively, comparing with nematode alone.

- Phytochemical constituents of the most effective wild weed *Atriplex lindleyi*

High performance liquid chromatography (HPLC) analysis of phenolic acids fraction led to identification of three compounds i.e. gallic, tannic and benzoic acid as mentioned in Table (4).

- Organic acids separation of the weed *Atriplex lindleyi*

Total Organic acids analysis led to estimation of seven organic acids which were presented in Table (5). The most dominant one was formic acid by 309 mg/kg, followed by maleic acid by 301.5 mg/kg then salicylic acid was the third one by 53.1 mg/kg.

Phytochemical constituents of *A. lindleyi* revealed that three phytochemical components were the most dominant as illustrated in Figure (3). Total Amino Acids (TAA), free Amino Acids (FAA) and total oils (Oil) recorded 5.28, 3.71 and 3.29 % respectively.

Total phytohormones analysis showed that four plant hormones were detected. They were auxin such as: Indole-3-acetic acid (IAA), gibberellins (Gib), Cytokinins (Cyt) and abscisic acid (ABA) as shown in Table (7).

Table (2): Effect of fresh wild weeds amendments at different concentrations on nematode parameters of tomato plants infected with root-knot nematodes under greenhouse conditions.

Treatment	Conc. %	Nematode Parameters / root system						Number of J ₂ s / 250g	Reduction %	Gall index	PF	RF
		Number of Galls	Reduction %	Number of Females	Reduction %	Number of Egg mass	Reduction %					
<i>Senecio squalidus</i>	1	162.3	27	144.3	44	130.9	34	3413.3	19	5	3850	1.28
	2	151.3	32	132.3	49	128.9	35	3287.3	22	5	3699	1.23
	4	140.6	37	124.3	52	118.6	40	3118.3	26	5	3500	1.17
<i>Hyoscyamus Muticus</i>	1	124.3	44	114.3	56	109.3	45	1517.6	64	5	1864	0.62
	2	98.3	56	110.9	57	101.3	49	1475.3	65	4	1785	0.6
	4	80.3	64	98.6	62	95.3	52	1306.3	69	4	1579	0.53
<i>Atriplex lindleyi</i>	1	82.3	63	98.6	62	75.3	62	1010.9	76	4	1266	0.42
	2	73.6	67	90.3	65	71.3	64	843.3	80	4	1077	0.36
	4	55.6	75	77.3	70	40.3	80	590.3	86	4	762	0.25
<i>Mesembryanthemum nodiflorum</i>	1	153.3	31	128.9	50	113.3	43	2444.3	42	5	2839	0.95
	2	87.9	61	116.6	55	103.3	48	2191.3	48	4	2497	0.83
	4	80.3	64	155.3	60	91.3	54	1938.6	54	4	2264	0.75
<i>Pluchea Dioscoridis</i>	1	164.3	26	147.3	43	125.3	37	3287.6	22	5	3723	1.24
	2	118.3	47	137.3	47	113.3	43	3034.6	28	5	3402	1.13
	4	91.6	59	132.3	51	98.9	50	2823.3	33	4	3145	1.05
<i>Amaranthus ascendens</i>	1	202.3	9	227.6	12	184.3	7	3877.6	8	5	4490	1.5
	2	195.3	12	40.9	16	172.3	13	3357.9	9	5	3766	1.26
	4	184.3	17	52.3	20	162.3	18	3750.3	11	5	4148	1.38
<i>Chenopodium Album</i>	1	102.3	54	94.9	63	85.6	57	1306.6	69	5	1588	0.53
	2	87.6	61	93.3	64	79.3	60	1138.3	73	4	1397	0.47
	4	62.3	72	83.3	68	74.9	62	885.3	79	4	1105	0.37
<i>Zygopyllum Qatarse</i>	1	182.3	18	181.6	30	135.3	32	3877.6	8	5	4375	1.46
	2	173.3	22	175.3	32	149.3	25	3708.3	12	5	4205	1.4
	4	161.9	27	173.3	33	133.3	33	3624.3	14	5	4092	1.36
Nematode alone		222.3	-	258.3	-	198.3	-	4214.3	-	5	4892	1.63
Nontreated (control)		-	-	-	-	-	-	-	-	-	-	-
LSD 0.05		7.324	-	9.644	-	6.948	-	117.166	-	-	-	-

Table (3): Effect of fresh wild weed amendments at different concentrations on growth parameters of tomato plants infected with root-knot nematodes under greenhouse conditions.

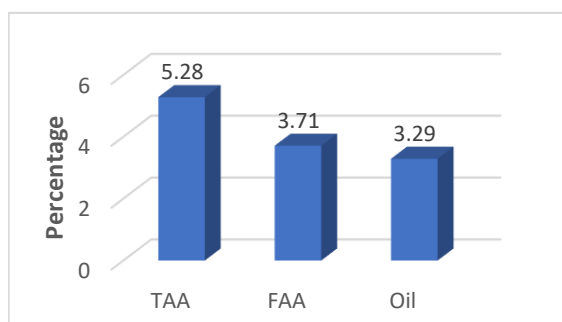
Treatment	Conc. %	Root length (cm)	% Efficacy	Shoot length (cm)	% Efficacy	Fresh root weight (g)	% Efficacy	Fresh shoot weight (g)	% Efficacy	Dry weight(g)	% Efficacy
<i>Senecio Squalidus</i>	1	7.6	29	28.3	28	1.9	6	6.9	32	1.0	11
	2	8.3	37	29.3	36	1.9	11	7.3	36	1.1	22
	4	8.3	37	30.6	39	2.3	17	7.3	25	1.14	27
<i>Hyoscyamus muticus</i>	1	8.6	44	34.3	55	1.9	11	8.3	53	1.7	89
	2	9.3	56	34.3	55	2.6	50	8.3	59	1.9	111
	4	9.9	96	34.6	58	3.3	83	8.6	60	2.0	122
<i>Atriplex lindleyi</i>	1	9.9	66	39.3	78	2.9	61	9.9	89	2.6	189
	2	10.3	96	37.6	71	2.3	33	9.6	79	2.4	167
	4	12.3	108	42.3	92	3.9	122	10.9	108	3.0	233
<i>Mesembryanthemum nodiflorum</i>	1	8.6	44	31.6	44	1.9	6	7.9	47	1.4	56
	2	8.9	47	32.3	47	1.9	11	7.9	51	1.5	67
	4	8.9	50	32.9	50	2.1	17	8.3	53	1.6	78
<i>Pluchea Dioscoridis</i>	1	7.9	35	29.9	36	2.6	56	7.9	49	1.4	56
	2	8.3	41	30.6	39	2.9	61	7.9	51	1.44	60
	4	8.3	42	31.3	42	3.3	72	8.3	55	1.5	67
<i>Amaranthus ascendens</i>	1	6.3	5	22.6	03	1.9	6	5.9	9	0.9	0
	2	6.6	10	23.9	09	1.9	11	5.9	13	0.91	1
	4	6.9	18	26.3	20	2.3	17	6.3	19	0.93	3
<i>Chenopodium Album</i>	1	7.6	31	36.6	67	1.9	11	8.9	68	2.2	144
	2	7.9	34	37.3	70	2.3	22	9.3	72	2.3	156
	4	11.3	92	41.6	90	3.3	77	10.9	104	2.8	211
<i>Zygopyllum Qatarense</i>	1	7.3	23	26.9	23	1.9	11	6.9	26	0.9	0
	2	7.3	25	27.3	24	2.3	17	6.9	30	0.91	1
	4	7.6	29	27.9	27	2.3	22	7.3	34	0.93	3
Nematode alone		5.9	-	21.9	-	1.9	-	5.3	-	0.9	-
Nontreated (control)		6.9	18	28.3	28	2.3	17	6.6	22	1.3	44
LSD 0.05		0.7915	-	2.7359	-	0.3985	-	0.4654	-	0.3413	-

Table (4): Phytochemical screening of phenolic constituents in dried leaves of *A. lindleyi*.

Compounds	Concentration (mg/kg)
Gallic acid	73.1
Tannic acid	122.6
Benzoic acid	436.2

Table (5): Organic acids separation analysis of *A. lindleyi*.

Organic acids	Concentration (mg/kg)
Ascorbic acid	41.7
Citric acid	3
Formic acid	309
Lactic acid	18.8
Maleic acid	301.5
Oxalic acid	25.6
Salicylic acid	53.1

**Figure (3): Percentage of Amino Acids (TAA), free Amino Acids (FAA) and oils in *A. Lindleyi*.****Table (7): Phytohormones concentration in *A. lindleyi***

Phytohormones	Concentration (mg/kg)
Indole-3-acetic Acid	713.4
Gibberellins	481
Cytokinin	1331
Abscisic Acid	5.5

Discussion

Results of the current study showed that the wild weed *A. lindleyi* was the most effective treatment in egg-hatching inhibition and juveniles (J₂s) mortality, followed by *C. album*, on the other hand weed *A. ascendens* was the least effective one and had a significant effect on nematode parameters. These results match Abdelnabby and Abdelrahman (2012), who revealed that extracts of *Atriplex halimus* and *Mesembryanthemum crystallinum* exhibited high nematicidal activity against second stage juveniles (J₂s) and egg hatching of *M. incognita*.

Weeds amendments reduced *Meloidogyne* spp. infection could be due to either (i) loss of mobility due to paralysis as revealed by results of *in vitro* experiments or (ii) disturbed orientation and host findings (linked to disturbance of chemoreception or injuries of sensory organs) disrupting the nematodes co-ordination in stimuli gradients and affecting the response to root attractants. The inhibitory effect of the extracts might be due to the chemicals present in the extracts that possess ovicidal and larvicidal properties. Shaukat and Siddiqui (2001) demonstrated the nematicidal potential of phenolic compounds such as benzoic acid, *p*-coumaric acid and caffeic acid against *M. javanica* larvae whereby they can suppress root-knot infection in mung-bean. Some of these extracts contained alkaloids, flavonoids, saponins, amides including benzamide

and ketones that singly and in combination inhibited hatching (Adegbite and Adesiyun, 2005). These chemicals either affected the embryonic development or killed the eggs or even dissolved the egg masses (Adegbite, 2003). Phytochemicals change nematode physiology by affecting ion uptake, membrane permeability, enzymatic activity, cell division and electron transport (Anaya, 2006). Nematicidal property of some phytochemical (saponins, flavonoids and glycosides) content extracted by these plant leaf or oxygenated compounds which have been characterized by their lipophytic properties that enable them to dissolve the cytoplasmic membrane of the nematode cells and their functional groups interfering with enzyme protein structures of nematodes (Trifonova and Atanasov, 2009; Bakr, 2021).

There are several defense enzymes that have been associated with systemic resistance including polyphenol oxidase (PPO), peroxidase (PO) and phenylalanine ammonia lyase (Pokhare *et al.*, 2012; Mhatre *et al.*, 2017). These enzymes increase plant defense level (Kamali *et al.*, 2015), and initiate the induction of resistance by producing phytoalexin and phenolic compounds (Viswanathan *et al.*, 2003). Moreover, the increase in the total phenolic content of nematode-infected plants was attributed to the stimulation of polyphenol oxidase activity (Nayak, 2015). Development of an antioxidant defense system in plants protect them

against oxidative stress damage by either partial suppression of reactive oxygen species (ROS) production or the scavenging of reactive oxygen species which are overproduced during plant pathogen interactions (Ye *et al.*, 2006; Cavalcanti *et al.*, 2007), and become dangerous because ROS impair the normal functions of cells due to their oxidative reaction with membrane proteins, lipids, deoxyribonucleic acid, as well as the inactivation of enzymes (Ashraf, 2009).

Peroxidases participate into cell-wall reinforcement. They are involved in the final steps of lignin biosynthesis and in the cross-linking of cell wall proteins (Kombrink and Somssich, 1995). Peroxidases are involved in the regulation of the level of hydrogen peroxide (H_2O_2), which stimulates a great activity of phenylalanine ammonia lyase, responsible for the synthesis of phenolic compounds in plant tissues, specially the infected, (Wojtaszek, 1997; Hao *et al.*, 2014).

Polyphenol oxidase is a copper containing enzyme, oxidizing phenolics to highly toxic quinines, which are frequently more toxic to pathogens than the original phenol, and involved in the terminal oxidation of diseased plant tissues which was attributed for its role in disease resistance (Safdarpour and Khodakaramain, 2018). Therefore, these enzymes may be directly involved in stopping pathogen development (Melo *et al.*, 2006; Shimzu *et al.*, 2006), accelerating the cellular death of cells close to the infection site, preventing

the advance of infection, and /or by generating a toxic environment inside the cells which will inhibit the pathogen development (Bi and Felton, 1995).

High performance liquid chromatography (HPLC) analysis of phenolic acids fraction of the most effective weed, *Atriplex lindleyi* indicated presence of three major phenolic constituents, gallic acid, tannic acid and benzoic acid (the predominant one). Similarly, Matloub et al., (2014) found that *Atriplex lindleyi* contains forty-two compounds as well as sixteen fatty acids. Benzoic acid has a great biotic role in nematode susceptible plants (Fiaz et al., 2018). Tannins and other polyphenolic compounds may be chemical cues that *Meloidogyne* spp. use to navigate toward roots, recognize plant hosts, or locate areas for root penetration.

Tannins may react directly with surface proteins of J₂s causing physiological dysfunctions with regard of the mobility and the absorption of nutrients, leading to the death of worms as observed by Massamha et al., (2010). Another possible anthelmintic effect of tannins is that they can bind to glycoproteins on the cuticle of the parasite and can indirectly cause death (Thompson and Geary, 1995; Iqbal et al., 2007) or cause protein coagulants which could result in a broad-spectrum worm killing activity (Yin, 2010; Jeon et al., 2009).

Gallic acid is a compound derived from the secondary metabolism of

various plants. Previous studies have shown that this compound and its alkyl ester derivatives (gallates) possess antioxidant and antimicrobial activities (Savi et al., 2005; Krol et al., 2015). Gallic acid is toxic to the young stages (eggs and young juveniles) of nematode and induces apoptosis (Singulani et al., 2017; Santhi et al., 2019). Analysis of *A. lindleyi* subsp. *inflata* indicated presence of seven major organic acids, citric acid, lactic acid, oxalic acid, ascorbic acid, salicylic acid, maleic acid and formic acid which may be involved in many biochemical pathways such as the production of energy, amino acid biosynthesis, and plant defense mechanisms. They are mainly produced in mitochondria through tricarboxylic acid or the Krebs cycle (EPA, 2013).

The levels of oxalic acid, citric acid, acetic acid, formic acid, butyric acid, propionic acid, valeric acid, salicylic acid and ascorbic acid production increased in *M. incognita* infected tomato plants that were able to suppress the proliferation of nematodes (Radwan et al., 2017). Organic acids are implicated in adjusting physiological processes of plants, including decreasing lipid peroxidation and boosting antioxidant enzyme activity (Apel and Hirt, 2004).

Malic acid was shown to have nematicidal activity against plant-parasitic nematodes (Lee et al., 2014). It is the most effective nematicides among dicarboxylic acids metabolites (Abdel-Rahman et al., 2008). It exhibited great nematicidal activity against *Meloidogyne incognita* (Kim et al.,

2016). Malic acid, lactic acid, and citric acid have shown promising antimicrobial properties (Dickson, 1992; Eswaranandam *et al.*, 2004).

Ascorbic acid (AA) has a well-documented function for enhancing the resistance of plant against pathogenic stress especially against root-knot nematodes during the development of plant systems (Osman *et al.*, 2013). It modulates the complex of biochemical pathway reactions such as enzymatic reactions, induction of proteins synthesis, and the production of different defense metabolites that tolerate the plant against stress response (Khan *et al.*, 2011). Another function of AA is to perform as an effective radical scavenger to eliminate the reactive oxygen species (ROS) in plants which consequently lead to plant protection against oxidative stress (Pastori *et al.*, 2003).

Oxalic acid was found to be effective against root-knot nematodes and its nematicidal potential is most related to the strong acidity of the acid which may cause rapid destruction of cells and tissues of the nematode bodies and eggs by osmoregulation disruption and then fluid accumulation (Seo and Kim, 2014; Jang *et al.*, 2016). Lactic acid was shown to have nematicidal effects on the egg hatching of *Meloidogyne incognita* (Abdel-Rahman *et al.*, 2008; Lee *et al.*, 2014).

The potential use of salicylic acid (SA) and its derivatives as chemical inducers in defense responses of tomato

to *M. incognita* and *M. javanica* in terms of reducing tomato galls and nematode population in soil are well agreement with those obtained by (Osman, 1993, Mukherjee *et al.*, 2012, Radwan *et al.*, 2017). This effect was likely to be due to the signaling role of SA in inducing plant resistance to pathogen. It mediates the oxidative stress precedes the systemic acquired resistance (SAR) development which closely related to the formation of pathogenesis-related proteins and enhancement the activities of antioxidant enzymes (Shirasu *et al.*, 1997). Also, Salicylic acid is contributed to the Mi-1 resistance gene of tomato that confers resistance to root-knot nematodes (Branch *et al.*, 2004).

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