

Research Article

In Vivo and *In Vitro* Inhibition of Three Plants Water Extracts on *Meloidogyne incognita (Meloidogynidae*)

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Abstract

A trial experiment was conducted in order to study the effectiveness of three concentrations (ml/ml: in ratio 25%, 50% and 75%) of water leave extracts of *Punica granatum, Conocarpus lancifolius* and *Citrullus colocynthis* singly or integrated in comparison with oxamyl on reproduction of *M. incognita* at the level of 1000 juveniles infecting eggplant under greenhouse conditions. All treatments showed remarkable increase in plant growth parameters as well as reduce nematode criteria. There was a positive correlation between the concentration level and second stage juvenile mortality. The highest water extract concentration of 75% recorded the highest rate of nematodes paralysis for the three extracts used i.e. *Conocarpus* water dried-leaf extract at the highest conc. 75% ranked first in increase percentage of nematode paralysis. On the other hand, the application of *Conocarpus* water extract as a dual application with oxamyl achieved the highest values in the improvement of plant growth criteria with the increase of 99.8%, 84.5%, and 69.4% for plant height, total plant fresh weight, and shoot dry weight, as well as recorded the highest reduction percentages of nematodes final population, galls and egg masses numbers with values 87.4%, 78.2% and 85.4%, respectively as compared with nematode alone.

Keywords: Eggplant; *Meloidogyne incognita*; Control; Oxamyl; Induced resistance; Plant; Powder; Leaves

Introduction

Eggplant, Solanumm longena L, is a one of the plant family, Solanaceae that grows between August and October in Iraq, is the richest species in terms of nutritional value and taste. It contains, carbohydrates and vitamins A, B. Black eggplant, in particular, is rich in some useful compounds such as zinc and potassium that helps in prevent heart disease, maintains urinary tract, strengthens memory and prevents some types of cancers. The eggplant infected with many pests, including *Meloidogyne incognita*, which is the most important plant nematode because of its wide host range and not generally specialized with at least 2500 plant species of almost all cultivated crops. It is one of the largest pests disturbing the world agricultural production, causing damage of about hundred billion dollars all over the world [1]. Root-knot nematodes are obligate parasites and very harmful plant pests for restrictive the agricultural yield. The majority cultivated plant species are not resistance to root-knot nematode disease. During the last two decades, the control of phytonematodes based on the use of nematicides but this caused environmental toxicity in addition to high chemical control costs. These have led to the search for other techniques to control these pests by using safe and environmentally friendly means [2]. Since the require of resistance in plants to most species of root-knot nematode as well as the ecological limits on nematicidal utilize for controlling phyto-nematodes, recently further eco-friendly disease management examination have earned increasing attention. On the other hand, it recommended that rising induced resistance in plants might propose a significant latent for biological control of phytonematodes. A novel approach for adjusting plant parasitic nematodes based on the activation of the plant's own

resistance system through different biotic and abiotic agents. It was extremely vital to establish naturally nematicides that might be safely to human being health and environment as well as efficient adjacent to nematode as artificial nematicides. There are many references in using compounds derived from different plant parts and their use as alternatives methods to synthetic pesticides [3-6]. Some plants contain natural materials that have no long soil effect and low toxicity to the plants as well as to reduce pest dispersal in agricultural lands [7]. These materials such as organic acids, aldehydes, aromatic acids, simple unsaturated lactose, courmains, quinones, flavonoids, tannins, alkaloids, terpenoids and steroids as well as some toxic gases [8]. There is no literature review verified the effectiveness of certain plant leaves i.e. Punica granatum, Conocarpus lancifolius and Citrullus colocynthis against plant parasitic nematodes. The objective of this study is to determine the toxicity of the water leaf extracts of some wild and cultured plants in Qadisiyah Governorate, Iraq on the second larval stage (J₂) of the root knot nematode, *M. incognita* under laboratory condition in addition their effect on *M. incognita* infecting eggplant cv. Blanc Long under greenhouse condition.

Materials and Methods

The nematode inocula

Indentified (J_2) of *M. incognita* (Kofoid and White) Chitwood were obtained from a pure culture of *M. incognita* initiated by a single egg mass propagated on coleus plants, Coleus blumei in the greenhouse [9]. Nematode inocula extracted by sieving and modified Baermann method counted in a Hawksely counting slide under 10x magnification by optical microscope [10].

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Preparation plant extracts

The Citrullus colocynthis (Cucurbitaceae), Punica granatum (Punicaceae) and Conocarpus lancifolius (Combretaceae) plant leaves werecollected from the gardens of the Faculty of Agriculture, Kadsia University Iraq. The leaveswere washed by tap water and then cut into small pieces then were sun dried. Plant leaves were grilled by electric blender then about 250 g of the grilled plant parts were dissolved in 500 ml distilled water at 60°C with mix the mixture for about 5 minutes, the solution left 24 h. for deposition of heavy plant parts El-Demeer et al. Each extract was then filtered through a sieve (250 meshes) and the obtained liquid was separated by a vacuum discharge device using the appropriate filter paper and completes the volume up to 500 ml distilled water. This solution is considered the stock solution (100%) of the plant product extract. Each plant extract was placed separately in a sterilized, filtered sealed container and stored in the refrigerator at a temperature of 5°C until use. Three concentrations 25%, 50% and 75% of each tested plant extracts were prepared. Two Tween 20 droplets added as a diffusion material. The distilled water was also present for comparison between treatments.

In vitro test

Three plant extract concentrations were added (10ml) in a Petri dish (10 cm dam-1.5 cm high) then 100 individuals of the second stage root knot nematode juveniles, *M. incognita* were added. Each plant extract concentrate were replicated five times as well as the trail were done twice in the same time. The percentages of non-moving nematodes were recorded every day for three consecutive days from nematode inculcation. The immobile nematodes transferred to Petri dishes containing 2 ml of distilled water, the number of nematodes recovered and the number of non-moving nematodes separately recorded. The vitality of non-moving nematodes assessed by the blue vital stain guide [11]. The black-colored larvae considered dead, while the non-colored nematodes were still alive. Non-colored nematodes considered paralyzed. The mortality rate calculated according to the following formula:

Nematode recovery movement %

$$= \left(\frac{Number of nematode recovery movement}{Number of paralysis nematode}\right) \times 100$$

Mortality rate % = (100 - % of recovery nematode rate).

Nematicides

Oxamyl (Vydate 24% L) Methyle-N-N-dimethyl-(N-(methyl) carbomycocyl)-1-Thioxamidate.

In vivo test

The experiment was conducted under greenhouse condition $(25 \pm 3^{\circ}\text{C})$ using the Randomized Complete Block Design with five replications (Repeated twice) for 10 treatments including: *Citrullus colocynthis, Punica granatum* and *Conocarpus lancifolius* dried leaf extracts (25%, 50% and 75%); nematode+oxamyl (0.3 mL/pot); nematode check (nematode alone); and plant without nematode (plant free-nematode). Fifty plastic pots (10 cm diam.) filled in individually with one kg autoclaved soils (clay: sand; 1:1, v/v) and transplanted with eggplant CV Black Long at age of 30 days of growth. One week later,

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1000 eggs of *M. incognita* were added for 8 treatments with consist of 45 pots, also, one week later, all plant extracts as well as the nematicide (oxamyl) were applied as soil drenching.

Evaluation parameters for treatment efficacy

All plants related to each treatment were harvested up-rooted 45 days after beginning of nematode inoculation, and both of vegetative and root systems were used as fresh and dried tissues for the following efficacy evaluation analyses.

Plant growth parameters

The tomato plant growth parameters including; fresh shoot lengths; fresh shoot and root weights (FWt) and shoot dry weight (DWt) were measured and recorded.

Determination of nematode parameters

Tomato plants roots were stained in 0.01 acid fuchsin in lactic acid [12] and examined under stereo microscope for galls and egg masses numbers. Root galling (RGI) and egg masses (REI) were rated on a scale of 0 to 5 as follow: 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 per root system according to Taylor and Sasser. Vermiform stages of *M. incognita* were extracted from soil according to the method of Goodey and then reproduction factor (RF) was calculated [10].

Statistical analysis

Data were analyzed to variance according to then compare means [13].

Results and Discussion

In vitro test

Data represented in Table 1 and in Figures 1 and 2 documented the nematicidial activity of three water extracts at the cons against the second stage larvae (J₂) of *M. incognita in vitro* for three consecutive days under the laboratory temperature. In general, there was a positive correlation between the rate of concentrations and the nematodes mortality rate as the highest water extract concentration of 75% recorded the highest rate of nematodes paralysis for the three extracts used i.e. Conocarpus water dried-leaf extract at the highest conc. (75%) ranked first in increase percentage of nematode paralysis, which amounted to 24.5%, 28.5%, 39.75% and 92.75%, for the three consecutive days and total nematode paralysis rate, respectively. Moreover, Citrullus leaf water extract 75% ranked second with value of 85.75% for total nematode paralysis rate whereas, the least rate of nematode paralysis achieved by *Punica* water extract at the lower conc. (25%) with values of 1.25%, 3.75%, 5.0% and 10.0% for the three consecutive days and total nematode paralysis rate, respectively. Data in Figure 3 documented number of nematodes that recovered their movement or dead after one day of transferred to distilled water of application with at thetested three water extracts three different concs in vitro. It was clear that Conocarpus water extract recorded the highest value of dead nematodes (84.1%) as well as, Punica water extract achieved the lowest value (5.0%) in this respect.

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Treatment		Nematode number							
		1 st day	I st day 2 nd day 3 rd day Total		Total	% paralysis nematode			
1	Punica water extract 25%	1.25	3.75	5	10	0.1			
2	Punica water extract 50%	8.25	11	18.5	37.75	0.3775			
3	Punica water extract 75%	22.25	27.5	31.5	81.25	0.8125			
4	Conocarpus water extract 25%	21	24	27.5	72.5	0.725			
5	Conocarpus water extract 50%	18.5	27.5	34.75	80.75	0.8075			
6	Conocarpus water extract 75%	24.5	28.5	39.75	92.75	0.9275			
7	Citrullus water extract 25%	14	17	37.5	68.5	0.685			
8	Citrullus water extract 50%	23	27	28.5	78.5	0.785			
9	Citrullus water extract 75%	21.25	27.5	37	85.75	0.8575			

Table 1: Nematicidial activity of three water plant leaf extracts at three cons against the second stage larvae (J_2) of *M. incognita in vitro* for three consecutive days under the laboratory temperature.



Figure 1: Nematicidal activity of three water plant leaf extracts at three concs against the second stage larvae (J_2) of *M. incognita in vitro* for three consecutive days.

In vivo test

The data in Tables 2 and 3 revealed the effect of leaf water extracts of *Citrullus, Punica* and *Conocarpus* on *M. incognita* infecting eggplant cv. Black Long as integrated application in comparing with oxamyl at the recommended dose under greenhouse condition $(25 \pm 3^{\circ}C)$. In general, all tested materials significantly ($p \le 0.05$) showed a marked improvement in vegetative parameters of eggplant and reduced nematode rate of reproduction as well. The application of *Conocarpus* water extract as a dual application with oxamyl achieved the highest values in the improvement of plant growth criteriawith the increase of 99.8%, 84.5% and 69.4% for plant height, total plant fresh weight, and shoot dry weight, respectively. The application of *Citrullus* aside from oxamyl recorded the next rank with an increase of 92.4, 86.7, and 47.2 for the same measurements, respectively.



Figure 2: Number of immobility stages of *M. incognita* after three consecutive days of application with three water extracts under the laboratory temperature.

Among the triple treatments, the application of *Punica* water extract 75%+Citrullus and 130.36%, respectively. At the same time, the treatment with *Conocarpus* water leave extract 75%+Punica water extract 75%+Citrullus water (106.7\%, 108.9\%, and 105.6\%), for the same parameters, respectively. However, the quadrant treatment of (*Conocarpus* water leave extract 75%+Punica water extract 75%+Citrullus water extract +Citrullus water +Citrullus +Citrullus water +Citrullus +Citrull

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Figure 3: Number of nematode, M. incognita recovered movement or dead after one day of transferred to distilled water of application with three water extracts under the laboratory temperature.

Data in Table 3 showed nematicidial activity of water extracts of Citrullus, Punica and Conocarpus (dried leaves) on M. incognita infecting eggplant cv. Black Long integrated with oxamyl under greenhouse condition ($25 \pm 3^{\circ}$ C). The root knot nematode criteria i.e. number of galls, egg masses, females and larval stage in the soil were significantly ($p \le 0.05$) affected by the tested three water extracts mixed with the pesticide compared to the oxamyl at under agricultural greenhouse conditions. Among the dual treatments with the oxamyl, it was clear that the plant treated with (Conocarpus water extract 75% +oxamyl) recorded the highest reduction percentages of nematodes final population, galls and egg masses numbers with values 87.4%, 78.2% and 85.4% followed by that of Citrullus water extract 75%+ oxamyl that amounted to be 86.4, 69.9 and 83.1% for the same nematode criteria, respectively. In addition, the triple treatment (Citrullus extract 75%+Conocarpus water extract 75%+oxamyl) ranked first and represented the maximum values for the final nematodes population (91.6%) galls (78.8%) and egg masses numbers (86.6%) respectively.

Treatments	Plant Growth response									
	Plant length (cm)		Total Plant Length	%Increase	Plant weight (g)		Total Plant weight	% Increase	Shoot dry weight	% Increase
	Shoot	Root			Shoot	Root				
T ₁	58.5 ^b	28.6 ^e	87.1 ^c	99.8	10.6 ^{bc}	22.6 ^d	33.2 ^d	84.5	6.1 ^d	69.4
T ₂	51.2 ^d	25.7 ^h	76.9 ^e	74.5	8.3 ^e	20.6 ^e	28.9 ^e	60.6	5.2 ^e	44.4
T ₃	56.0 ^c	27.2 ^g	83.2 ^d	92.4	10.9 ^b	22.5 ^d	33.6 ^d	86.7	5.3 ^e	47.2
T ₄	60.7 ^a	34.8 ^a	95.5 ^a	119	11.7 ^a	29.1 ^a	40.8 ^a	126.7	8.3 ^a	130.6
T ₅	56.3 ^c	30.5 ^d	86.8 ^c	99.1	10.4 ^c	24.1 ^c	34.5 ^c	91.7	5.5 ^e	52.8
T ₆	56.4 ^c	33.7 ^b	91.1 ^b	106.7	10.5 ^c	27.1 ^b	37.6 ^b	108.9	7.4 ^b	105.6
T ₇	48.0 ^e	27.7 ^f	75.7 ^f	73.7	9.3 ^d	24.3 ^c	33.6 ^d	86.7	6.6 ^c	83.3
T ₈	25.5 ^g	32.3 ^c	57.8 ^h	32.7	10.6 ^{bc}	16.3 ^f	26.9 ^f	49.4	6.4 ^c	77.8
T ₉	20.5 ^h	23.1 ⁱ	43.6 ⁱ		7.8 ^f	10.2 ^h	18.0 ^h		3.6 ^g	
T ₁₀	28.5 ^e	31.9 °	60.4 ^g	38.7	8.1 ^e	11.3 ^g	19.4 ^g	7.8	4.4 ^f	22.2
L.S.D 0.05%	0.34	0.45	0.45		0.24	0.2	0.35		0.25	

N=1000 J₂ of *M. incognita* (Each value is the mean of four replicates)

Means in each column followed by the same letter (s) did not differ at p < 0.05 according to Duncan multiple- range test

Number between parentheses represented the percentage of increase or decrease in plant growth response.

T1=1/2 dose of Punica water extract 75%+oxamyl

T₂=1/2 dose of Conocarpus water extract 75%+oxamvl

T₃=1/2 dose of *Citrullus* water extract 75%+oxamyl

 $T_4=1/3$ dose of *Punica* water extract 75%+*Citrullus* water extract 75%+oxamyl $T_5=1/3$ dose of *Citrullus* extract 75%+*Conocarpus* water extract 75%+oxamyl

 $T_{6=}^{-1/3}$ dose of Conocarpus water leave extract 75%+Punica water extract 75%+oxamyl

T₇=1/4 dose of Conocarpus water leave extract 75%+ Punica water extract 75%+Citrullus water extract +oxamyl

T₈= Recommended dose of Oxamvl 0.3 ml/plant

To=Nematode alone

T₁₀=Plant free of nematode and any treatments

Table 2: Plant growth response of eggplant cv. black long infected with M. incognita under the stress of three water extracts Citrullus, Punica and *Conocarpus* integrated with oxamyl under greenhouse conditions ($25^{\circ}C \pm 3^{\circ}C$).

The application of *Punica* water extract 75%+*Citrullus* water extract 75%+oxamyl is achieved moderate reduction percentage with values of 89.0%, 76.9% and 84.3% for the tested nematode characters,

respectively. However, the quadrant treatment of (Conocarpus water leave extract 75%+Punica water extract 75%+Citrullus water extract +oxamyl)surpassed other tested materials and recorded highest values

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Treatments	Number of nematode soil		J_2 in soil	Total population	Red%	RF	No. of Galls	RGI	Red%	No. of egg	EI	Red%
	Develomental stages	Females	o.									
T ₁	20.0 ^e	9.0 ^{cd}	252.3 ^e	281.3 ^e	87.4	0.28	24.0 ^e	3.0 ^c	78.2	13.1 ^{de}	3.0 ^b	85.4
T ₂	29.2 ^b	12.5 ^b	295.6 ^b	337.3 ^b	84.9	0.34	35.3 ^b	4.0 ^b	67.9	16.3 ^b	3.0 ^b	81.9
T ₃	25.1 ^c	12.1 ^b	266.1 ^c	303.3 ^c	86.4	0.3	33.1 ^c	4.0 ^b	69.9	15.2 ^c	3.0 ^b	83.1
T ₄	20.0 ^e	10.3 ^c	216.5 ^g	246.8 ^g	89	0.25	25.4 ^d	3.0 ^c	76.9	14.1 ^d	3.0 ^b	84.3
T ₅	23.2 ^d	8.1 ^d	155.6 ^h	186.9 ^h	91.6	0.19	23.3 ^e	3.0 ^c	78.8	12.1 ^e	3.0 ^b	86.6
T ₆	20.3 ^e	13.5 ^b	235.2 ^f	269.0 ^f	88	0.27	24.2 ^e	3.0 ^c	78	16.3 ^b	3.0 ^b	81.9
T ₇	15.4 ^f	10.1 ^c	260.9 ^d	286.4 ^d	87.2	0.29	25.1 ^d	4.0 ^b	77.2	12.2 ^e	3.0 ^b	86.4
T ₈	15.6 ^f	0.0 ^e	145.2 ⁱ	160.8 ⁱ	92.8	0.16	13.1 ^f	3.0 ^c	88.1	50.0 ^f	3.0 ^b	94.4
T ₉	80.1 ^a	55.0 ^a	2100.1 ^a	2235.2 ^a		2.24	110.0 ^a	5.0 ^a		90.0 ^a	2.0 ^c	
L.S.D 0.05%	0.82	1.62	0.63	2.2			0.82			0.82	4.2 ^a	

for reduction percentage of the final nematodes population (92.8%) galls (88.1%) and egg masses numbers (94.4%) and respectively.

N=1000 J₂ of *M. incognita* (Each value is the mean of four replicates)

(RGI)=Root gall index, (EI)=Egg mass index

Means in each column followed by the same letter (s) did not differ at p< 0.05 according to Duncan multiple- range test

Number between parentheses represented the percentage of increase or decrease in plant growth response.

T₁=Punicawater extract 75%+oxamyl

T₂=Conocarpus water extract 75%+oxamyl

 T_3 =*Citrullus*water extract 75%+oxamyl

T₄=Punicawater extract 75%+Citrullus water extract 75%+oxamyl

T₅=Citrullus extract 75%+Conocarpus water extract 75%+oxamyl

 $\mathsf{T}_6 \texttt{=} \textit{Conocarpus} \text{ water leave extract 75\%} \texttt{+} \textit{Punicawater extract 75\%} \texttt{+} oxamyl$

T₇=Conocarpus water leave extract 75%+Punica water extract 75%+Citrullus water extract+oxamyl

T₈= Recommended dose of Oxamyl 0.3 ml/plant

T₉=Nematode alone

Table 3: Nematode parameters of *M. incognita* infecting eggplant cv. black long as influenced by three water extracts *Citrullus*, *Punica* and *Conocarpus* integrated with oxamyl under greenhouse conditions $(25^{\circ}C \pm 3^{\circ}C)$.

The current investigation focused on the potential use three water extracts of Citrullus, Punica and Conocarpus (dried leaf) as alternative method control for the root-knot nematode, M. incognita under in vivo and in vitro conditions. Conocarpus water dried-leaf extract at the highest conc. (75%) ranked first in increase percentage of nematode paralysis, which amounted to 24.5, 28.5, 39.75 and 92.75%, for the three consecutive days and total nematode paralysis rate under laboratory conditions. However, the quadrant treatment of Conocarpus water leave extract 75%+Punica water extract 75% +Citrullus water extract+oxamyl, recorded the lowest values for the same plant measurements that amounted 73.3, 86.7and 83.3%, respectively as well as surpassed other tested materials and recorded highest values for reduction percentage of the final nematode's population (92.8%) galls (88.1%) and egg masses numbers (94.4%) respectively. These results are in conformity by Radwan, et al. that recorded the effectiveness of bio-products adjacent to M. incognita by using nematicides such as oxamyl to the soil. In the current study, all experienced plant materials showed hopeful results and gave significant decrease in all tested nematode criteria i.e. root galling, juveniles in soil, developmental stages in root, egg masses and eggs/egg mass. Al-Shatti et al. revealed that the ornamental plant, Conocarpus lancifolius, (Combretaceae) do not encourage growth of other plants

and not attacked by common herbivores [14]. C. lancifolius appears to be wholly devoid of plant pathogens. These notes signify that C. lancifolius may have mechanisms either resistance or tolerance that are effective when it is attacked by herbivores or pathogens. Such plant might contain biochemical reactions that produce compounds that are toxic to agricultural pests. These inhibitory materials may be induced as allelochemicals or secondary metabolites of such plants. The analysis of C. lancifolius leaves extract showed the occurrence of many inhibitory compounds i.e. phenolics; tannins; flavonoids; steroids and terpenoids. The technique of action of such compounds may be direct or indirect. Direct action includes effects on plant growth and metabolism and indirect effects are in purview of modification of soil property and nutrition as well as changes in useful and harmful soil microbial populations [15]. Some microorganisms and dissimilar chemical processes in the soil environment might inactivate secondary metabolites or break them into novel toxic allelochemicals [16]. Tannins are known to be microbial inhibitors asphenolics were growth substrates [17]. Some phenolics in forests soils were used by fungi and cellulose hydrolyzers whereas growth of heterotrophic bacteria was inhibited [18]. Therefore, allelo-chemicals do not only influence changes in plant communities but also microbial populations in the soil. However, oxamyl as nematicide was the greater in reducing M. *incognita* pollution at the suggested dose and rising plant strength. Normally, oxamyl, showed strong actions than tested extracts in respect to reduction of all tested nematode criteria such as galls, juveniles, developmental stages, egg masses numbers. These data are in accord with those obtained formerly by Hadad and Al- Hashmi, and Khalil et al., It was accepted that carbamate nematicides acted by the inhibition of acetylcholinesterase (ACHE) at cholinergic synapses in the nematode nervous system Wright; Opperman and Chang. Thus, reducing their movement, host attack, feeding and accordingly the rate of development and reproduction Kheir et al.

Conclusion

In conclusion, tested extracts, applied as safer and eco-friendly alternatives to synthetic nematicides, gave encouraged and suitable results in approximately applications. Therefore, field experiments on a great scale are necessary for the management of the goal nematodes. Moreover, phytochemicals separation and classification of secondary metabolites compounds are desirable for additional studies at laboratory and greenhouse conditions to appreciate their method of action and nematicidal behavior and to verify their efficacy at field conditions. Applications of screened botanicals and bio-products at integrated manage program might make available effectively manage of phytonematodes nematodes infecting economic plants.

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