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Efficacy of *Nerium oleander* Leaves extract on controlling *Meloidogyne incognita in-Vitro* and *in-Vivo*

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ABSTRACT

Experiments were carried out to evaluate the nematicidal effect of Oleander *(Nerium oleander)* extracts on controlling root-knot nematode *Meloidogyne incognita in-Vitro* and *in-Vivo*. Five different organic solvents i.e., Petroleum Ether, Chloroform, Acetone, Ethyl Alcohol and Methyl Alcohol were used. Plant extracts screened for nematicidal activity against egg hatching and mortality of second-stage juveniles of *M. incognita* under laboratory conditions and on nematode infecting tomato plants under greenhouse conditions. Results showed significantly effect of all tested oleander extracts on egg hatching and larvae mortality under laboratory conditions. A great reduction in number of galls; eggmasses; females/root system of tomato plants and number of second stage juveniles (J2S)/250 g soil was recorded compared to the treated plants with nematode alone. Tomato plant growth parameters and oxidative enzymes were markedly enhanced compared to the non-treated control.

Key words: Biopesticides, Plant extracts, *Meloidogyne incognita*, ,Oleander *(Nerium oleander)* ,Tomato plants.

INTRODUCTION

Root knot nematode, M. incognita had proved itself as an important limiting factor for cultivation and productivity of vegetable crops throughout the worldwide. Meloidogyne spp. cause annual losses of about USD$ 100 billion worldwide (Brand et al., 2010). It considered a limiting factor for Egyptian vegetables, which do not only cause low yield but also discourage most farmers from cultivating the crop especially in the newly reclaimed land (Ibrahim et al., 2010; Bakr et al., 2011). It is affecting the quantity and quality of marketable vegetable yields (Kingland, 2001). Infected plants suffer vascular damages which disturb water and mineral uptake. Intensity galled roots of infected tomato plants are unable to uptake enough water and nutrients for normal plant growth. Yield losses over 30% in eggplant, tomato and melon (Sikora and Fernández ,2005). The extent of damage is influenced by the cultivar, nematode species, level of soil infestation and environment. Zero yields of tomato plants may occur when grown in sandy soils infested with high nematodes population, especially in the summer season.

Chemical synthetic nematicides were highly toxic to both human and the environment (Abawi and Widmer, 2000). Thus, the development of alternative eco-friendly control strategies and long-term integrative approaches using biodegradable pesticides is urgently needed in order to replace chemical nematicides (Martin, 2003). Thus; control strategies are now directed towards the use of natural products. Worldwide awareness and considerable efforts have been done at screening ornamental plants in order to develop new safety botanical nematicides as alternatives to the existing nematicides. Plant extracts have the advantages of environmental safety (Chitwood, 2002 and Adegbite, 2003). less toxic to mammals, more selective in action, easily and cheaply produced by farmers, safer to farmers, consumers and the environment, easily degraded and non-persistent under field conditions, and non expected residues on the products or in the environment. Organic amendments are not only safe to use but also have the capacity to improve soil structure and fertility. Plant extract using in an environmentally conscious world also holds promise for

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their acceptability and use by resource-poor farmers.

The present research was undertaken to evaluate N. oleander leave extracts to control root-knot nematode M. incognita on tomato plants.

MATERIALS AND METHODS

Collection and preparation plant material samples

Oleander (*N. oleander*) leaves was collected from the farm of Faculty of Agriculture, Menoufia University, Egypt. Its botanical identity was further confirmed and authenticated at the Agricultural Botany Department, Faculty of Agriculture, Menoufia University, Egypt. The collected leaves were thoroughly washed under running tap water, distilled water, and finally air-dried. The dried materials were ground into powder form using a Micro-hammer-mill (KINEMATICA AG, PX-MFC).Then stored in air-dried containers until required for use.

Plant extracts preparation

Extracts prepared using the sequencing extraction methods by using five different organic solvents i.e., Petroleum Ether, Chloroform, Acetone, Ethyl Alcohol and Methyl Alcohol as follow: 100 g powder sample were soaked for 84 hours in 2 litre conical flask containing 1 litre of petroleum ether at 100 rpm under laboratory conditions. Extraction was done by using filter paper (Watmann No.1) and received the extract solution in a mortar and leave it to allow the solvent to totally evaporate. The collected crude was weighed and redissolved in water till 100 ml to prepare a final concentration of 1%. The treated powder was left to dry on tissue paper under room temperature. Then re-extracted with chloroform for another 48 hours and follow the same procedure to prepare a final concentration of 1% as mentioned before. The same steps were done using the same treated powder with serial different solvents i.e. acetone, ethyl alcohol and methyl alcohol to prepare the same concentration from each solvent.

Effect of plant extracts on egg hatching of M. incognita

All different plant extracts were screened against egg hatching of M. incognita. Eggs were extracted from infected tomato roots by using NaOCl technique as described by Hussey and Barker (1973).The effects of plant extracts on egg hatching was measured by putting 0.9 ml of each plant extract into each well of sterile 72-well microtiter plates then each well received 0.1 ml water containing 1000 eggs of *M. incognita* (Munif, 2001). Sterilized water was used as control. Three replicates were used for each particular treatment. After 24 hr eggs were washed by tap water and put in water then calculate the percentage of eggs hatching under light microscope by calculating the percentage of eggs hatching after 24, 48 and 96 hours.

Effect of plant extracts on larvae mortality of M. incognita

Plant extracts were also screened for its effectiveness on larvae mortality of M. incognita. Eggs were extracted from infected tomato roots as described before. Eggs were allowed to hatch and fresh hatched second-stage juveniles (J2) were collected. The effects of plant extracts on larvae mortality was measured by putting 0.9 ml of each plant extract into each well of sterile 72-well microtiter plates then each well received 0.1 ml water containing 1000 fresh hatched second-stage juveniles of M. incognita (Munif,2001). Sterilized water was used as control. Each particular treatment was replicated three times. After 24 hours, activity of juveniles was examined under the microscope. Inactive juveniles appear to be rigid and elongated with head and tail sometimes slightly bent in total. Juveniles were collected over 20 µm sieve and washed with tap water to remove the residual plant extracts and transfer in well of microtiter plates with water and counted after 24 hours .Juveniles still inactive classified as dead.

Effect of plant extracts on J2 penetration rate and control of M. incognita infecting tomato

The effect of plant extracts on the juveniles penetration of M. incognita was evaluated by preparing the extracts as described before. Four weeks old tomato seedlings (Cv. GS) were transplanted into plastic pots 15 cm in diam. containing sterilized mixed sand / clay soil (2/1 v/v). At the same time of transplanting each plant inoculated with 2000 freshly second stage juveniles of M. incognita around the young hairy roots. Plant extracts were pipetted at 2.5, 5 and 10 ml around the seedlings roots at the same time of transplanting. Plants received 5 ml distilled water served as a control. Each treatment was replicated 6 times. Plants were watered and weekly fertilized with a nutrient solution as described by Epestein (1972). After 15 days from nematode inoculation 3 replicates were uprooted and their roots were carefully washed under running tap water. Penetration rate was determined after staining the roots with Sodium hypochlorite–acid fuchsin by transfers the roots into a beaker containing boiling acid fuchsin for 30 seconds as described by Byrd et al., (1983). Excess stain was removed by rinsing in running tap water. Root materials then placed in 20-30 ml glycerin acidified with a few drops of 5N HCL, heated to boiling and cooled. The numbers of penetrated juveniles were counted under a stereomicroscope by pressed the root segment between microscopic slides.

Remaining plants were irrigated with the different plant extracts again after 15 days of nematode inoculation with the same rates as mentioned before. Two months after nematode inoculation plants were uprooted and their roots were carefully washed under running tap water. Numbers of galls, egg masses, females, developmental stages/root system and number of J2S /250g soil as well as the plant growth parameters i.e. shoot and root fresh and dry weights were determined and recorded. Egg-masses were stained prior to counting by dipping the infected roots in 0.015% Phloxine-B solution for 20 minutes as described by Daykin and Hussey (1985). Females were collected 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 (Mahdy,2002). The roots were then washed through 500 and 250 µm sieves to separate the females and developmental stages from the root debris and counted under a stereomicroscope.

Determination of antioxidant enzymes activity

Peroxidase and phenoloxidase activities were determined in fresh leaf samples by using spectrophotometer (CT-2200 Spectrophotometer–Medline, Scientific limited). Peroxidase activity was expressed as changes in absorbance per minute per gram fresh weight (Reuveni et al., 1992). The increase in absorbance density at 470 nm was recorded. Activity of Phenoloxidase was expressed as the change in the absorbance of the mixture at 495nm (Matta and Dimond, 1963).

Determination of root permeability

The Membrane leakage (%) was estimated following the method of Leopold et al., (1981) using the equation:

$$\text{Membrane leakage (ML \%)} = \frac{T_1}{T_2} \times 100$$

As T1= Initial Absorbance of Bathing Medium T2= Final Absorbance of bathing Medium

Statistical analysis

Data statistically analysis using Duncan's Multiple Range test (P=0.05) using costat 6.3 version program.

RESULTS

Effect of plant extracts on egg hatching of M. incognita

Data in Table (1) showed the effect of oleander plant extracts on percentage of egg hatching of M. incognita. The extracts were significantly effective in inhibiting egg hatch throughout the period of observation. Egg hatch was low with all tested extracts than the control. Ethanol extract was significantly more effective than other extracts with a cumulative egg hatch of 94.66, 87.66 and 80.66 % after 24, 48 and 96 hr of exposure. There was a gradual

decrease in egg hatching with increase in extract concentration. Petroleum Ether was the second effective solvent in inhibition of egg hatching by 92.66, 87.66 and 80.33 %after 24, 48 and 96 hr of exposure compared with untreated control

Table (1): Effect of oleander leaves extract on egg hatching of *M. incognita*

Treatment		Mean number of un-hatched eggs after		
		24 hours	**48 hours**	**96 hours**
Petroleum Ether	1%	75.00 cde	67.666 cd	61.00 cde
	2%	83.333 bcd	76.333 abcd	70.666 abcd
	3%	92.666 ab	87.666 a	80.333 b
Chloroform	1%	72.333 de	64.666 d	54.00 ef
	2%	79.00 cd	71.00 bcd	65.666 bcde
	3%	91.666 ab	85.666 a	77.333 a
Acetone	1%	67.666 e	63.00 d	48.333 f
	2%	78.333 cd	75.00 abcd	60.00 de
	3%	83.666 bcd	82.00 ab	72.00 abc
Ethyl Alcohol	1%	72.333 de	70.00 bcd	57.666 ef
	2%	84.00 bc	79.666 abc	73.00 ab
	3%	94.666 a	87.666 a	80.666 a
Methyl Alcohol	1%	75.333 cde	71.666 bcd	58.00 ef
	2%	80.666 cd	76.00 abcd	65.333 bcde
	3%	92.333 ab	87.666 a	77.666 a
Control		36.00 f	26.666 e	15.00 g
LSD (0.05)		**6.969**	**8.483**	**7.987**

Effect of plant extracts on larvae mortality of *M. incognita*

Results of the present work revealed that some plant extracts were highly toxic against nematodes in a laboratory exposure. Data presented in Fig. (1) shows the effect of plant extracts with different organic solvents on the larvae mortality of M. incognita. Oleander petroleum ether extract at the high concentration (3%) was the most effective in larvae mortality by 88% followed by chloroform extract by 83% compared to the untreated control which recorded 7 % of larvae mortality

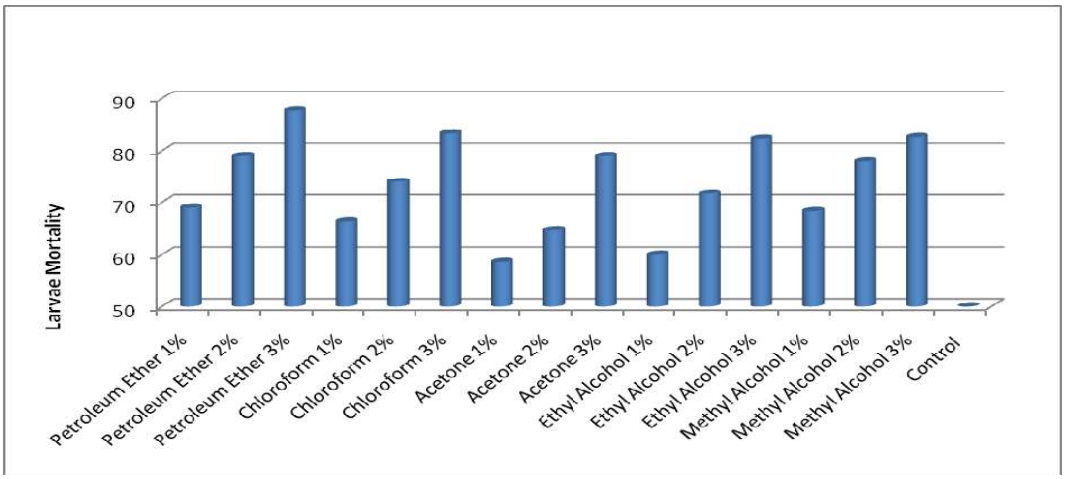
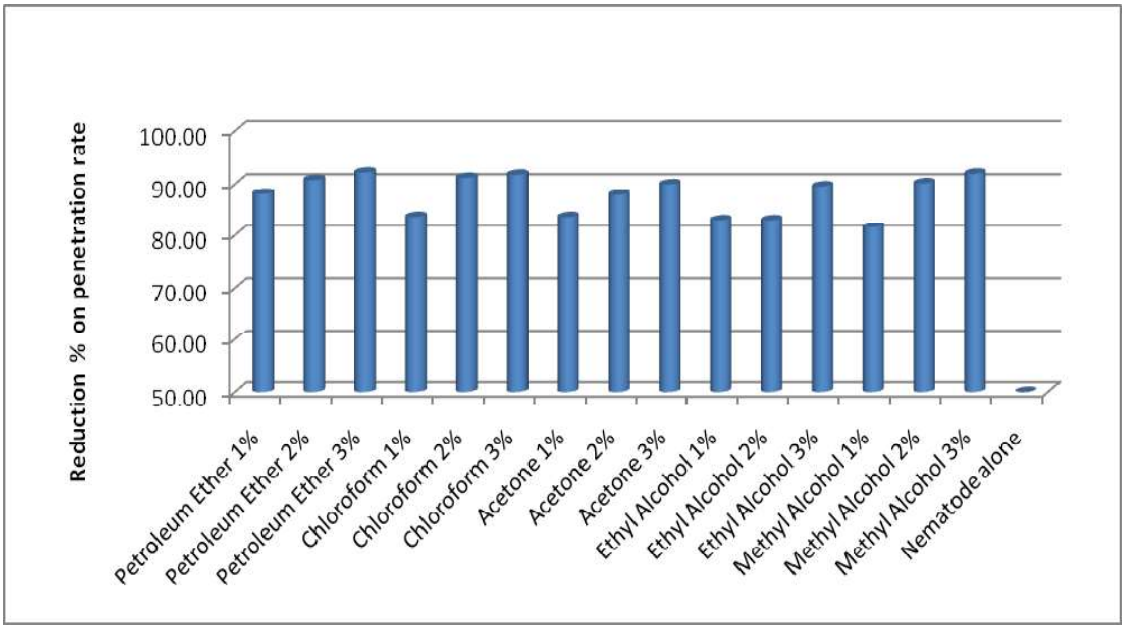


Fig (1): Effect of oleander leaves extract on larvae mortality of *M. incognita*

Effect of plant extracts on penetration rate of second stage of M. incognita

Data illustrated in Fig.(2) showed the effect of plant extracts on the penetration rate of second stage juveniles of *M. incognita* into tomato roots after 15 days of nematodes inoculation. The penetration rate was different between the examined extracts on all treatments compared with plants treated with nematode alone. Results also indicated that all plant extracts reduced the penetration rate of second stage juveniles of *M. incognita* after 15 days from nematodes inoculation compared with nematode alone treatment. Petroleum ether extract treatment of oleander at 3% showed the highest reduction of penetrated larvae into tomato roots followed by methyl alcohol at 3 % while, the lowest one was noticed with methyl alcohol at 1%.



Fig(2): Effect of plant extracts on penetration rate of second stage of M. incognita to tomato roots

Effect of plant extracts on *M. incognita* parameters

Results presented in Table (2) showed the effect of oleander plant extracts on nematodes parameters i.e. galls, egg masses, females and developed stages /root system of tomato plants infected with M. incognita. Results clear that all nematode parameters on tomato plants were affected by using oleander different extracts under greenhouse conditions. The reduction in number of J2S /250 g soil varies from treated and non-treated pots. The 100 % reduction in number of J2S /250g soil was recorded in the treated pots with all tested solvents at the high concentration (3%) compared to control. The least reduction was notice on plants treated with methyl alcohol at 1%. Examination of tomato root system revealed that the number of galls /root system was markedly affected by all applied oleander extracts treatments. All the application reduced the number of galls/ root system up to 100 % compared with untreated control. The least reduction on number of galls/root system was observed with chloroform extract at 1%. Observation of root system revealed that the number of egg masses/root system

was affected by treatments with oleander extracts compared with untreated control tomato plants. The same trend was recorded as with the number of galls /root system. Developmental stages/root system were also affected by the different oleander extracts. The reduction in number of developmental stages reach to 100 % with the high concentration of four solvent of the five used.

Table (2): Effect of oleander on *M. incognita* control

Treatment	J2/ 250gm soil	Mean number of nematode parameters/root system of tomato plants			
		Galls	Egg masses	Females	Developed stages
Petroleum Ether 1%	4.333	2.66	1.666	5.00	3.666
2%	2.333	1.33	1.00	2.333	1.666
3%	00.00	0.0	00.00	00.00	00.00
Chloroform 1%	3.00	9.0	8.00	8.00	3.00
2%	1.333	4.33	3.333	4.00	1.666
3%	00.00	0.0	00.00	00.00	00.00
Acetone 1%	4.666	7.33	7.00	3.333	4.00
2%	3.00	2.33	2.666	2.333	1.666
3%	00.00	3.0	3.333	1.333	0.666
Ethyl Alcohol 1%	5.00	4.0	4.666	1.666	3.666
2%	1.666	1.66	2.00	0.666	1.00
3%	00.00	0.0	00.00	00.00	00.00
Methyl Alcohol 1%	7.00	6.33	7.00	6.333	3.333
2%	1.666	2.33	2.333	1.666	2.00
3%	00.00	0.0	00.00	00.00	00.00
Nematode Only	29.666	56.66	45.666	25.00	15.333
LSD (0.05)	**2.640**	**4.919**	**5.215**	**4.354**	**2.778**

Effect of oleander leaves extract on fresh shoot and root weights and dry shoot weight of tomato plants infected with M .incognita. Results in Table (3) show the effect of plant extracts on plant growth parameters of tomato plants. Results also indicate that tomato plant growth parameters were affected by the different tested extracts under greenhouse conditions. Results of plants treated with oleander plant extract revealed that the highest fresh weight of root was observed in tomato plants due to chloroform at 3% application, followed by the methyl alcohol at 3% treatment, whereas petroleum ether at 1% treatment achieved the lowest effective one when they compared to the untreated control.

Data indicated that the highest tomato shoot weight was recorded on plants treated with chloroform at 3%, followed by methyl alcohol at 3% treatment, whereas methyl alcohol at 1% treatment had the lowest effective one compared to control. Shoot dry weight showed the highest value in plants treated with chloroform at 3% followed by the acetone at 3% treatment, whereas petroleum ether at 1% treatment had the lowest effective one compared to control

Table (3): Effect of oleander leaves extract on fresh shoot and root weights and dry shoot weight of tomato plants infected with *M .incognita*

Treatment	Vegetative plant growth parameters		
	Root weight (g)	Shoot weight (g)	Shoot dry weight (g)
Petroleum Ether 1%	1.566	5.90	0.466
2%	2.533	7.366	0.83
3%	1.90	5.933	0.913
Chloroform 1%	2.166	6.166	0.526
2%	0.933	5.833	0.59
3%	4.00	11.533	1.773
Acetone 1%	2.133	8.40	0.963
2%	2.466	6.233	1.23
3%	3.112	8.90	1.54
Ethyl Alcohol 1%	2.266	8.20	0.67
2%	2.066	6.266	1.023
3%	3.166	6.466	0.82
Methyl Alcohol 1%	1.966	5.533	0.536
2%	2.766	5.666	0.64
3%	3.266	9.433	1.126
Nematode alone	1.40	5.533	0.506
Control	1.511	5.761	0.578
LSD (0.05)	**1.910**	**3.0100**	**0.539**

LSD =Least significance difference at 5%

Effect of plant extracts on phenoloxidase and peroxidase activity on tomato plants infected with M .incognita

Results in Table (4) showed the effect of plant extracts on phenoloxidase and peroxidase activity on tomato plants. It was noticed that, all tested treatments enhanced the activity of phenoloxidase enzymes when compared with the untreated plants. Results of plants treated with oleander leaves extract revealed that the highest significant activity of phenoloxidase enzyme was observed in tomato plants due to petroleum ether at 3% application, followed by the chloroform at 3% treatment, whereas ethyl alcohol at 1% treatment had the lowest effective one when they compared with the untreated control as illustrated in Fig.(3).

Data showed that all the tested treatments enhanced the activity of peroxidase enzymes when compared to the untreated plants. Results revealed that the highest significant activity of peroxidase enzyme was observed in tomato plants due to acetone at 3% application, followed by the chloroform at 3% treatment, whereas ethyl alcohol at 1% treatment the lowest

effective one compared to the untreated control as illustrated in Fig.(4).

Data presented in Fig.(5) showed the effect of plant extracts on the Membrane leakage (%) in tomato plant roots treated with plant extracts.

All the tested treatments clear that plant extracts reduced membrane leakage of tomato root cells compared to the untreated plants. Results obtained from plants treated with oleander leaves extracts revealed that the highest reduction in membrane leakage was observed in tomato plants treated with petroleum ether at 3% application, followed by methyl alcohol at 3% treatment, whereas acetone at 1% treatment was the lowest effective one when they compared to the untreated contro

Table(4): Effect of oleander leaves extract on the antioxidant enzymes ,Phenoloxidase and peroxidase and root permeability in tomato infected with *M. incognita*

Treatment	Phenoloxidase (O.D.g-1 dr. wt. after 45 min).	Peroxidase (O.D.g-1 dr. wt. after 2 min)	Membrane permeability
Petroleum Ether 1%	0.192	0.139	42.424
2%	0.201	0.344	21.153
3%	0.303	0.465	18.518
Chloroform 1%	0.200	0.211	47.272
2%	0.230	0.317	32.142
3%	0.258	0.677	25.00
Acetone 1%	0.233	0.266	51.666
2%	0.239	0.450	38.823
3%	0.224	0.775	26.470
Ethyl Alcohol 1%	0.151	0.134	50.00
2%	0.216	0.195	48.484
3%	0.207	0.330	37.50
Methyl Alcohol 1%	0.178	0.194	40.322
2%	0.226	0.262	32.692
3%	0.211	0.370	19.047
Nematode alone	0.110	0.081	69.533
Control	0.149	0.131	19.565
LSD (0.05)	**0.00167**	**0.00530**	**0.0657**

LSD =Least significance difference

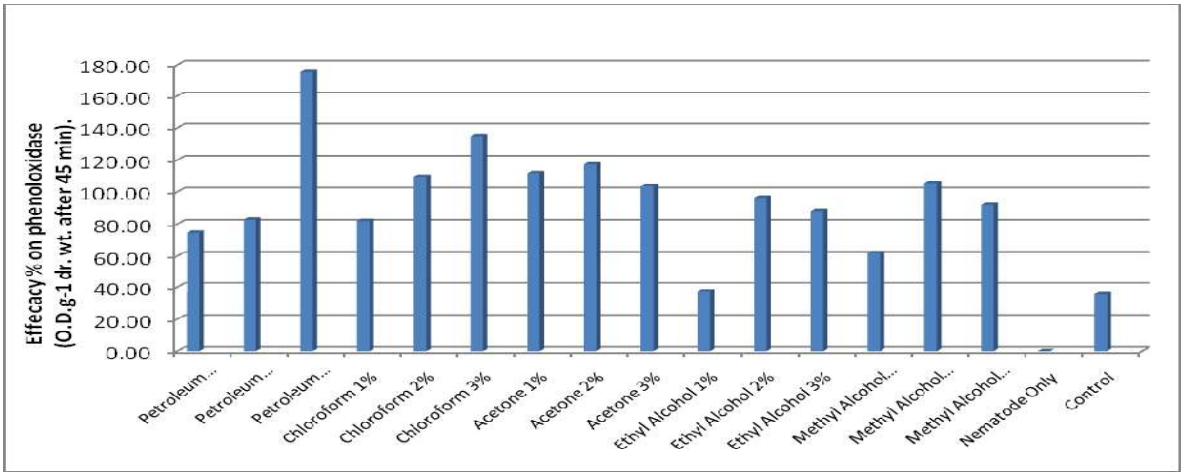


Fig.(3): Effect of oleander leaves extract on efficacy % on Phenoloxidase (O.D.g^{-1} dr. wt.)

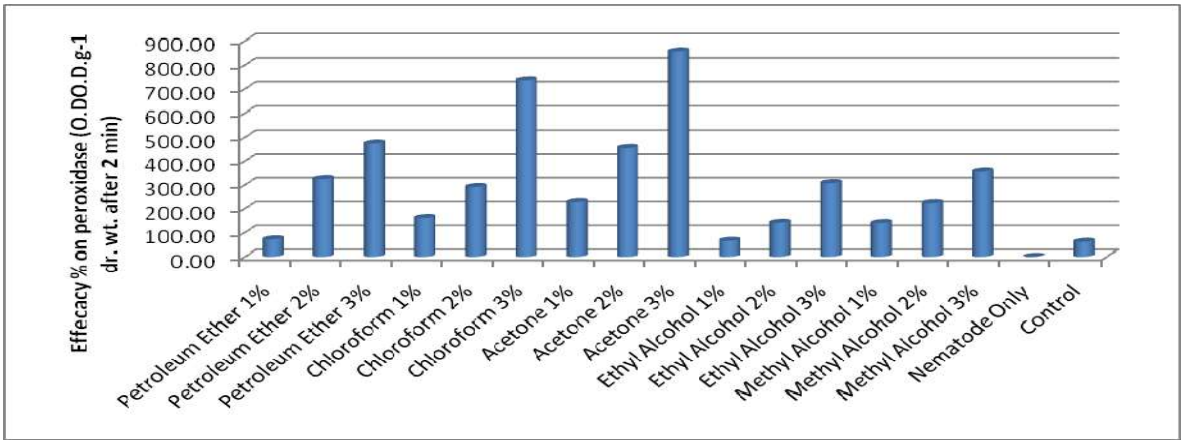


Fig. (4): Effect of oleander leaves extract on efficacy % on Peroxidase (O.D.g^{-1} dr. wt.)

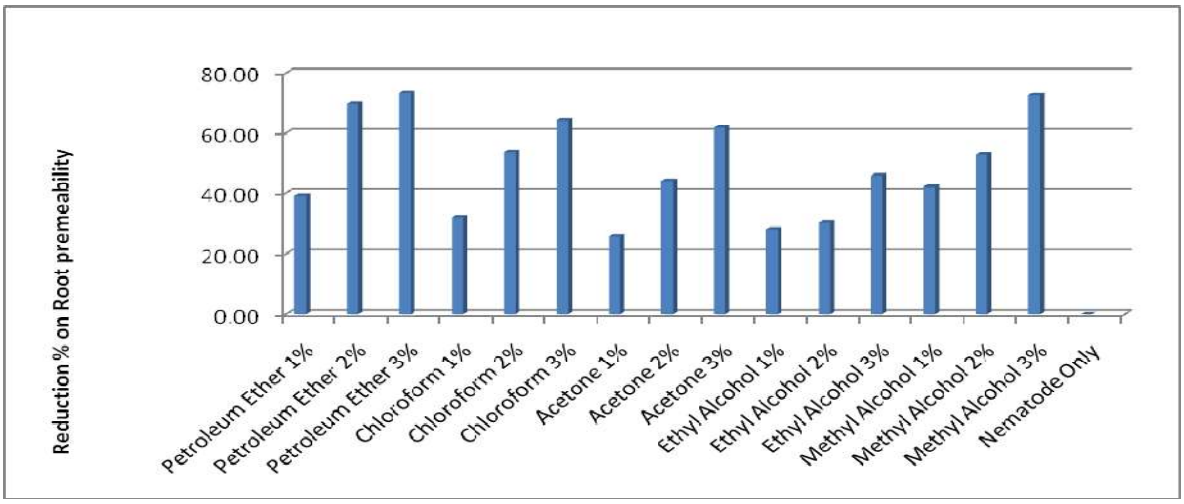


Fig. (5): Effect of oleander leaves extract on reduction % in root membrane permeability

DISCUSSION

The plant extracts of certain plants are known to have nematicidal or nematostatic properties against several plant-parasitic nematodes by their efficacy in inhibiting the egg hatching and larvae mortality in vitro investigation. Phytochemical analyses reveal that the three plants contain different constituents which are useful for medicinal purposes. The infra-red spectroscopy of the oleander extract showed the presence of different functional groups such as alkanes, amides, amines, aromatics aliphatic amines, alkyl halides.

Results of the current study revealed that the plant extracts of oleander were highly toxic against nematodes in a laboratory exposure. Meloidogyne incognita egg hatching was markedly affected under laboratory conditions. These data support previous reports of nematicidal activity by some of these plants against plant parasitic nematodes who reported that the plant extracts of certain plants are known to have nematicidal or nematostatic properties against several plant-parasitic nematodes by their efficacy in inhibiting the egg hatching in vitro investigation. The nematicidal effect of the tested extracts may possibly be attributed to higher contents of certain oxygenated compounds which are characterized by their lipophilic properties that enable them to dissolve the cytoplasmic membranes of nematode cells and their functional groups interfering with enzyme protein structure (Knoblock et al., 1989). The different in extracts activity tested at all concentrations could be due to the presence of different dissolved phytochemicals at the different extracts according to the different ability to dissolve in the different organic solvants. Egg hatchability may be attributed to the permeability of M. incognita egg shells to the toxic materials contained in the plant extracts used, consequently, killing the developing juveniles. Current results in agreement with those by Zasada et al., (2002) who reported that the water extract of N. oleander reduced M. javanica egg hatching.

The obtained results obtained revealed that tested plant extracts were highly toxic against root-knot nematode M. incognita larvae mortality in a laboratory exposure. The differences in the toxicity of different extracts could be attributed to the presence of the active compounds in the plant material that may be influenced by several factors such as age of plant, method of extraction and type of extracting solvent (Qasem and Abu-Blan, 1996; Nicolls, 1969).

Oleander petroleum ether extract at the high concentration 3% was the most effective in larvae mortality could be attributed to the predominant presence of acidic compounds and their derivatives and amino acids and their derivatives. Where similar results were reported by Zasada et al., (2002) and Elbadri et al., (2008).

The effective role of leaf extracts may be related to the role of leaves as centres of intermediary metabolism leading to biologically active secondary metabolites.

Results also clear that tested treatments reduced such nematodes parameters i.e., number of galls, egg-masses, females and developmental stages/ root system. Plant extracts promoted the plant growth parameters i.e. fresh root and shoot weight; and dry shoot weight compared to non-treated plants. The potential of using plant extracts in controlling plant parasitic nematodes has been shown by several researchers. The inhibitions of nematode development in-vitro and in-vivo strongly suggested the presence of nematicidal substances in the plant extracts and the possibility of using the plant species for control of any plant parasitic nematodes.

Plant extracts were able to decrease the number of galls/root system on tomato plants treated with the different extracts. These results were in agreement withthat reported by Tiyagi et al., (2012) who mentioned that N. indicum significantly reduced the number of root galls caused by M. incognita in treated plants and that of Zasada et al., (2002) who reported that N. oleander reduced the root galling caused by M. incognita on tomato plants. Plant growth parameters were enhanced by using the plant extracts of the tested plants

in addition to chlorophyll content and peroxidase and phenoloxidase activity. Obtained results were also in agreement with those of Tiyagi et al., (2012) who mentioned that N. indicum significantly improved the plant growth parameters i.e. plant weight and chlorophyll content.

The basis for physiological action of N. oleander cardenolides is similar to that of classic digitalis glycosides, i.e. inhibition of membrane Na+/K+ ATPase pump, resulting in deficit in conduction of electrical potential, (Cheeke, 1998).

Vrain (1980) confirmed that the toxicity of fatty acids on second stage juveniles of Meloidogyne hapla increased with increase in carbon number C3 to C11. The reduction of nematode population may be attributed to the production of nematicidal substances i.e. terthienyl, triterpenoid and other alkaloids by organic compounds.

In this study, different fatty acid esters were identified in the different plant extracts. A variety of fatty acid esters have been used to control nematodes in-vitro and in-vivo. A mixture of sodium lauryl sulphate and citric acid immobilised some nematodes, this mixture also reduced nematode growth significantly when applied at planting. Some fatty acids that can be in the epoxide, cyclopropane, methylated or hydroxylated forms have also been confirmed to be toxic to nematodes (Pinkerton & Kitner, 2006). The fatty acid esters contain long chain carbon numbers, and it has been established that toxicity increase with carbon chain length (Fabiyi et al., 2012).

In this investigation the highest effective treatment on activity of phenoloxidase enzyme was observed in tomato plants treated with petroleum ether at 3% and this may be revealed to its contents of acidic derivatives, amino acids and their derivatives, phenol derivatives, terpenes derivatives, aldehydes derivatives, alkaloid, ketones and vitamins, alcohol and amid groups. The present findings revealed that the highest significant activity of peroxidase enzyme was observed in tomato plants due to oleander acetone extract and this may be related to the content of this extract of acidic compounds, their derivatives, and amino acids and their derivatives, which play a good role in the enzyme activity. Results also showed that petroleum ether at 3% was the highest effective extract on reduction of membrane leakage in tomato plants roots and this may be due to its contents of acidic compounds and their derivatives, amino acids derivatives, alcohols, simple sugars, antibiotic and phenols, vitamins and amides. Based on the photochemical results of the present study, it could be said that the plant extracts contain chemical constituents of nematicidal significance. The presence of these chemical constituents in this plant is an indication that the plant, if properly screened using additional solvents, could yield a significance bionematicides. However, studies are to be continued to find out the active principal involved in them and to find out the effects of these leaves in controlling nematodes when they are incorporated into the soil as green manure, at the same time the limitation of availability in large quantities to use them as green manure will be there. For the wide practical application of such plant extracts as bio-nematicides against Meloidogyne spp. Infecting such crops. further research is therefore needed to:1) Isolate, purify and characterize these chemical constituents; 2) use single and combination of the compounds to know whether the nematicidal activity revealed to one or mixed compounds; and 3)develop formulation to improve their efficacy and stability.

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