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Utilization of Oleander Leaves and Fish Waste on Root-Knot Nematodes Control

R.A. Bakr*

Agricultural Botany Department, Faculty of Agriculture, Menoufia University, Egypt

**Corresponding author*

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A B S T R A C T

Root-knot nematode (RKN), *Meloidogyne incognita* considered as destructive pathogen to eggplant. Even though the chemical nematicides the effective option, more safety biological control methods are urgently required. Experiments were conducted to study the nematicidal potential of oleander *(Nerium oleander)* and fish waste (FW) in management of root-knot nematodes *Meloidogyne incognita*. Three concentrations of oleander dry Leaves(ODL),oleander fresh leaves(OFL) and fish waste(FW) were tested on egg hatching, larvae mortality and nematode management under greenhouse conditions. Results pointed out that all used treatment at the different concentration affected the egg hatching, larvae mortality and nematode management in pots experiment. The highest inhibition of egg hatching was achieved with ODL at 300ppm after 24, 72 and 7 days of treatments starting by 70.59, 76.47 and 79.12 respectively. Also the oleander dry Leaves (ODL) at 300ppm exhibited the highest mortality rates by 63.75, 84.75 and 90.75 % respectively after 24, 48 and 72 h of exposition to the tested treatments. A high reduction in nematode related parameters was observed in eggplant root. Oleander dry Leaves (ODL) at 2%was more effective in reducing the penetration rate of J2s into the eggplant roots after 15 days followed by OFL at 2%. Number of J2s/250 g soil and number of egg-masses; galls; females/root system was decreased markedly by using the different treatments compared with infected un-treated control. The highest significant reduction in all nematode parameters of *M. incognita* was presented in plants previously treated ODL at 2% while; the least reduction was recorded with FW at 0.5%. Eggplant growth parameters were increased by amended the soil with the different treatments. The highest values of plant high, root length and fresh weight were obtained in plants treated by FW2% followed by OFL 2%. Fresh and dry shoot weight were recorded the highest weight when pots soil treated by FW2% followed by ODL 2% comparing with untreated plants and infected untreated plants. Thus, further study is needed to maximize the efficacy of oleander leaves and fish waste in nematodes management.

Introduction

Organic matter (OM) percent in soil refer to biological, chemical and physical soil characters and this affect the soil agricultural production. Additives of organic amendments to soil increase the eco-friendly benefit of compatibility with organic production

programs and the sustainable agriculture especially in intensive cropping systems. Willer and Kilcher (2013) reported that 162 countries worldwide have 1.8 million organic farmers exceed 37 million hectares agricultural land. Vegetable grown in organic farm sometimes suffer from many plant pathogens such as, Fungi, nematodes, Bacteria and viruses. Root-knot nematode (RKN), *Meloidogyne incognita* is very destructive endoparasite with a wide host range from cultivated plants all over the world. It causes a several threats to the plants and considers some time the main limiting factor of some plants especially in the sand new reclaimed land (Bakr *et al.,* (2011). Vegetable grown in greenhouse recorded 50% of RKN infection and the estimated annual losses reached more than 400 million dollars (Huang *et al.,* 2014). With the high population of RKN in the intensive cropping agricultural system the most effective control methods was chemical nematicides. Unfortunately the chemical nematicides were environmentally hazardous, high cost and toxic for non target microorganisms. Therefore, the searching for eco-friendly, nonchemical and sustainable management strategies is an urgently need for controlling RKNs (Huang *et al.,* 2016) and using for plant nutrition. Thus, researcher worldwide trying to use and development modern trends of alternative control strategies for long-term and sustainable integrative new approaches by using biodegradable nematicides. Also the alternatives should be easy handle, cheap, safer and suitable for poor farmers, less toxic to mammals, selective in action, no persistent and degradable (Bakr *et al.,* 2015). Organic amendments from plant materials and organisms is an environmentally safety alternatives to the present nematicides. The biopestcides from botanical origin can be obtained from plants materials such as fresh or dried roots, leaves, barks, rhizomes flowers, bulbs, cloves, seeds or fruits. The Dried plant materials are preferred because it reduces water concentration and resulting in high yield of active ingredient (Chougule and Andoji, 2016). Oleander (*Nerium oleander L*.) family *Apocynaceae* is a cultivated throughout wide area in the Mediterranean region. Gas chromatography of oleander leave ethanol, methanol, acetone, chloroform and petroleum ether revealed that the extract containing alkaloids, phenols, terpenes, flavonoids, steroids, tannins essential oils and different chemical component (Ali *et al.,* 2010; Bakr, 2014). Leaves of *N. oleander* showed high antimicrobial activity towards a wide spectrum of bacteria and fungi (Hussain and Gorsi, 2004). Many natural compounds with potency against mammalian parasites could be effective in plant parasitic nematodes control strategy (Chitwood, 2002). Previous researcher confirmed the efficacy of oleander in RKN larvae mortality and nematodes control (Rahmani *et al.,* 2016; Salim 2016a). Several commercially fertilizers comes from fishmeal are permitted for use in farms tracking organic agriculture legislation (EC Regulation 2092/91, 1991). According to the chemical analysis FW is rich content of nutrients (Illera *et al.,* 2010). Fish waste (FW) come from fish market, home use and industrial company and representing about 30-45% of row materials used (Illera-Vives *et al.,* 2015).

Fish waste was rich cheap organic materials and can be used in agricultural proposes (Radziemska *et al.,* 2016). Fish meal or dried fish waste has been used for plant pathogens management (Akhtar and Mahmoud, 1994; Abbasi *et al.,* 2009; Abbasi 2011, 2013). Slight information is available about using of soil amendment with fish waste for control and suppression of different plant parasitic nematode (Roldi *et al.,* 2013). Therefore the present study was conducted to evaluate the efficacy of oleander leaves and fish waste in egg hatching, larvae mortality and the

management of RNK in eggplant as a safety alternative control methods.

Materials and Methods

Preparation of fish waste and oleander

Fish residuals collected from fish shops and houses and dried using oven at 60 °C for 3 days then the dried materials grounded to a fine powder using Microhammer-mill (KINEMATICA AG, PXMFC).Then stored in a sealed plastic bag until use. Oleander (*Nerium oleander*) fresh leaves were used directly but dried leaves powder prepares according to Bakr *et al*., (2015).

Extraction of *M. incognita* eggs

Heavily galled eggplant roots infested with *M. incognita* were used for eggs extraction. Procedure was done according to the sodium hypochlorite (NaOCl) technique by Hussey and Barker, (1973). The Gently washed eggplant roots was cut and macerated in blender (Monlinex) at maximum speed two times for 10 seconds. Produced root suspension transfer to plastic jar containing fresh prepared 0.5% sodium hypochlorite solution and shaken for 3 min. Then suspension passes during serial sieves to separate root tissues and wash several times by tap water to remove the NaOCl residuals to avoid the harmful effect on extracted eggs. Eggs up the 20µm sieve were collected in a glass flask containing tap water. Number of eggs / ml was counting using stereomicroscope (Bel photonics, Biological Microscope, Bio 1-B) at 100x.

Preparation of *M. incognita* second stage juveniles

Extracted eggs as described before were put on a filter paper with distilled water in a Baermann funnel. Two days later fresh second stage juveniles were collected and placed in a flask, then counted microscopically at and number of J2s/ml recorded as an average of three replicates.

Preparation of Extracts

Extracts prepare by soaking 100 g sample of fish and oleander powder and fresh oleander leaves in 2 litre flask containing 1 litre of water at rotary shaker at 100 rpm for 84 hours at room temperature. After that, extract was filtered by filter paper (Watmann No.1) and received in a glass beaker. After water totally evaporates the crude was weighed and suspended in distilled water and Triton X-100 (0.01%) to prepare 1% concentration as a stock.

Effect on Egg Hatching

For evaluating the effect of fish waste and oleander extracts on egg hatching of *M. incognita*. Two ml of egg suspension containing approximately 2000 eggs/ml put in 3cm dim Petri dish and the stock extracts used to give a final extract concentration of 100, 200 and 300 ppm. Control dishes received eggs and distilled water only. Each treatment repeated three times. Then dishes have been kept in the laboratory in the dark at 25°C. Number of hatched J2s was recorded after 24 and 72 hr. The egg hatch inhibition percentage calculated using the following equation.

$$\text{Egg Hatching Inhibition \%} = \frac{\mathbf{C} - \mathbf{T}}{\mathbf{C}} \times 100$$

as C= hatched J2s in control, T= hatched J2s in treatments

Then eggs sieved by 20 µm sieve and rinsed in distilled water for 4 days in the previous same conditions and the inhibition percentage of egg hatching calculated as mentioned before.

Effect on larvae Mortality

Two ml of distilled water containing 2000 fresh hatched *M. incognita* J2s were placed in 3cm dim glass petri dish and extracts were add from the prepared stock to give the final concentration of 100, 200 and 300 ppm. Distilled water was used in the control treatment and three replications for each treatment were setup. Dishes then kept at 25°C in the laboratory in the dark. After 24 and 48 hr Nematode activity was calculated under the microscope. Inactive juveniles almost are rigid and elongated and head and tail sometimes slightly bent in total. For check the recovery of juveniles the J2s were washed by distilled water over 20 µm sieve and re-incubated in distilled water for another 24 hr (Bakr, 2014) and examined, Juveniles considered dead when it still inactive.

Effect on *M. incognita* control and plant growth under greenhouse conditions

The nematicidal potential of oleander fresh leaves and its powder and fish residuals on root-knot nematodes control was determined on eggplant (*Solanum melongena* L.). Plastic pots 30 cm in diameter filled with autoclaved mixed sand / clay soil (1/1). Treatment included oleander fresh and dry leaves powder and its powder and fish residuals at 0.5, 1 and 2% of soil weight and well mixed with the pots soil and watered. One week later Thirty days old white eggplant seedlings (Cv. Balady) were transplanted in the pot centre. Two days later nematode inoculums add at 2000 eggs of *M. incognita* in a small hull behind the youngest hairy roots. Plants inoculated by nematode only served as a positive control and Plants untreated considered as negative control. Six replicates were done for each treatment. Plants watered and needed agricultural processing done. Penetration rate of juveniles calculated after 15 days from nematode inoculation. Three plants uprooted from the pots then roots gently washed by tap water. Roots were stained using Sodium hypochlorite-acid fuchsin technique according to Byrd *et al.*, (1983).Staining process done by put the roots in boiling acid fuchsin for 30 seconds then removed and rinsing in tap running water. Root transfer to 20-30 ml glycerin acidified with a few drops of 5N HCL then heated to boiling and cooled. Penetration rate calculated by counting the penetrated juveniles to the roots by squeezed the root segments between microscopic slides and counting using the stereomicroscope.

Sixty days from nematode inoculation, plants have been plucked from the pots and roots were separated from plant shoots and washed softly with water to remove the adhesive soil particles. Plant growth parameters i.e. plant high, root length, shoot and root fresh weight and shoot dry weights were writing down. Then roots were used for determination of nematode related parameters. The galls number /root system were counted directly by naked eye. Egg assess have been counted after placed the roots in Phloxine-B stain solution at 0.015% / 20 minutes as described by Daykin and Hussey (1985).Then roots washed with water and Egg-masses were stained with pink colour. For females counting the roots were then transfer to a beaker containing water and left in room temperature for 4 days until be soft. For extraction the females and developmental stages roots washed through 250 and 500 µm sieves. Females number/root system were counted using stereomicroscope. Soil samples from pots were collected for determine the number of J2 / 250gm soil using Baermann trays technique.

Statistical analysis

Analysis of variance (ANOVA) was done for the laboratory and pot experiment by

Duncan's Multiple Range test at P=0.05. The significant differences among the treatments means were calculated according to Least Significant Difference (LSD) using costat 6.3 version program software.

Results and Discussion

Obtaining results presented the nematicidal activity of oleander and fish waste on egg hatching and larvae mortality and nematode control. Results illustrated in table 1 showed the ovicidal activities effect of different concentration of dry and fresh oleander leaves and fish waste on egg hatching percentage of *M. incognita*. Observation of egg hatching after 24 h revealed that the oleander dry leaves powder at 300ppm was significantly ($p<0.05$) effective in reducing the egg hatching as it recorded 7.5 % followed by OFL at 300ppm by 8.25% compared with 25.25 % in control treatment. After 72 h from starting eggs treatments examination of egg hatching showed differences between the treatments. The least egg hatching was recorded 9% with ODL300ppm followed by 10% in OFL at 300ppmtreatment while the fish waste at 1% recorded 15.75% of egg hatching compared with 38.25% in control treatment. The accumulative effect of oleander dry leaves powder after 7 days was significantly difference comparing with the other treatments and control. The least accumulative egg hatching15.50 % recorded after 7 days with 300ppm ODL followed by16.75% in OFL at 300ppm while the highest egg hatching value was 23.50% in FW at 300ppm treatment compared with 74.25% in control treatment. In general, the most effective ovicidal activities treatment in inhibit egg hatching was ODL at 300ppm in all intervals examined time as it recorded 70.59,76.47 and 79.12% of egg hatching inhibition after 24,72 and 7 days after treatment compared with control treatment. The least egg hatching inhibition percentage was recorded with FW at 100ppm by 43.14, 58.82 and 68.35% compared with untreated control. From the previous results, there was a piecemeal increase between egg hatching and increasing the concentration of different treatment inside each treatment. Results in table 2 presented the larvicidal potentials activity of used treatments on Juvenile mortality of *M. incognita* under laboratory conditions. These results revealed that all treatments were toxic to nematode juveniles and mortality percentage increased with increasing of exposure time within the same treatment. The most significantly ($p<0.05$) effective treatment in causing highest percentage of juvenile mortality after 24h of exposure to treatment was ODL300ppm by 63.75% followed by OFL300ppm by 61% while least mortality 42.50% recorded by FW100ppm when comparing with 3% juveniles mortality in control treatment. The highest larvicidal potentials after 48h of exposure also recorded with ODL at 300ppm followed by OFL at 300ppm by 84.75 and 81 % respectively compared with 5% in control treatment. Fish waste at 100ppm showed the lowest efficacy in juvenile's mortality after 48h by 58.25%. Juvenile's mortality after washing the larvae with water after 48h of exposure to treatment then transfer to distilled water and incubation for 24 hours illustrated that the treatment had a nematicidal effect against larvae mortality. After 72h from starting treatments more than 90% of juvenile's mortality recorded with ODL at 300ppm treatment and 87.25 % with OFL at 300ppm while it was 62.75% with FW at 100ppm compared with 6.75% in distilled water (control) treatment.

Effect on *M. incognita* control and plant growth of eggplant under greenhouse conditions

Results clear that using of oleander leaves and fish waste was effective in controlling

infestation of nematodes into roots of eggplant. The effects of oleander leaves and fish waste on nematode control under greenhouse experiment were differing between treatments as illustrated in table 3. Observation of root system of eggplant after 15 days refers to a great reduction in penetration rate of *M. incognita* J2S. The least penetration rate of *M. incognita* J2s was observed in plants treated with ODL 2%(16) followed by OFL 2% (20)while the highest penetration of J2s was recorded in plants treated with FW 0.5% (32) compared with plants infected by nematode alone (149). All of the treatments significantly ($p<0.05$) decreased the number of J2/250gm soil in pots soil but not all of the treatments significantly between each other. The most effective treatments were ODL2% followed by OFL2% and FW2% but there is no significant difference ($p<0.05$) between the treatments. Application of ODL 2% concentration exhibited the highest potency in reducing the number of galls /root system (41.50) followed by OFL2% (45.50), while the lowest effective treatment in reducing the number of galls /root system recorded with FW 0.5% (67) compared with 263 galls in infected untreated plants. Calculating of egg masses number /root system revealed that there is no significance differences ($p<0.05$) between ODL2% and OFL2% even they recorded the least number of egg masses/root system (45 and 49) respectively. Females number/root system were significantly ($p<0.05$) affected by the treatments compared with untreated infected control. The lower number of females/root system was reported in plants treated with ODL2% by 48.25 while the next effective treatment in reducing number of females was OFL2% by 54.25 followed by 57.50 in FW 2% with no significant differences($p<0.05$).

Results from current study confirmed that treatments stimulated significantly ($p<0.05$) the plant growth parameters of eggplant as illustrated in table 4. No recorded phytotoxicity to eggplant due to application of the different treatments. Obtained data showed that the all treatments significantly ($p<0.05$) enhancing the plants height compared with control plants and infected untreated plants. Adding the FW2% to the soil recorded the highest plant height (84.75cm) followed by OFL 2% by 82.50cm compared with 54.25 and 71.50cm in plants infected with nematode only and untreated control plants. Plant root length was also affected markedly as the results showed significant differences between the treatments. The same trend recorded with the same treatments as in plant height. The fresh root weights of eggplant were increased significantly ($p<0.05$) after adding the treatments. Fresh and dry shoot weights were also enhancing by the used treatments. Plants treated with FW3% showed the highest improvement of shoot fresh and dry weight by 59.24 and 3.34 respectively. The other treatments also enhanced the fresh and dry shoot weight significantly comparing to nematode alone treatment but no significant comparing with untreated control. The lowest improvement of shoot fresh and dry weight noticed in plants treated with FW 0.5% compared to untreated control.

The current study confirms the using of eco-friendly bio-nematicides as organic matter and botanicals to replace chemical nematicides with hazardous effects to humane and environments to control *M. incognita*. The results revealed that oleander leave extract and fish waste were effective in reducing the egg hatching of *M. Incognita* under laboratory conditions with different values. The obtained results are in agreement with results by previous researcher confirmed the ovicidal effect of oleander in root-knot nematode egg hatching. Zasada *et al.*,(2002) pointed out that *N. oleander* water extract

reduced *M. javanica* egg hatching. Bakr *et al*, (2015) revealed that oleander leaves extracts using Acetone, Petroleum Ether, Ethyl Alcohol, Chloroform and Methyl Alcohol significantly inhibited the egg hatching *M. incognita* throughout the period of observation. Phytochemical analyses of oleander leave extracts explain the ovicidal effect of oleander leaves by the GC analysis which confirmed the presence of amino acids and their derivatives, alkaloid, phenol derivatives, terpenes derivatives, ketones and aldehydes derivatives (Bakr, 2014). Cardenolides from *N. oleander* physiologically inhibit the membrane Na+/K+ ATPase pump which resulting in deficit the electrical conduction (Cheeke, 1998).The differences in efficacy between treatments my affect the obtained results and this may be due to dissolved phytochemical amount and chemical constituents. The ovicidal effect may be attributed to the transfer of toxic plant extract chemical component by permeability from the egg shell then kill the juveniles inside the eggs. Also, the high concentration of certain oxygenated compounds with lipophilic properties and good ability to dissolve the cytoplasmic membranes of nematode cells may be elucidate the nematicidal effect (Knoblock *et al.,* 1989). Results concerned to juvenile mortality showed that the treatments were effective in increasing the *M.incognita* juvenile's mortality and the mortality rates of nematode juveniles increased when the exposure time treatments increased. This result is agreed with those by different researchers. Elbadri *et al.,* (2008), reported that *N. oleander* leaf Hexane or Methanol gives 29, 31.5 and 60.3 mortality rates of *M. incognita* juveniles after 24, 48 and 72 h of exposure under laboratory. Moosavi (2012) indicated that *N. oleander* leaves extracts present higher mortality in *M. javanica* juveniles. Salim *et al.,* (2016b) mentioned that 5% and 10% concentration of *N. oleander* extracts gives mortality rate of up to 78 and 95% on *Meloidogyne* sp. Juveniles. The nematicidal potency of oleander leaves also was recorded against different phytonematodes such as reported by Ahmed (2015) against *Ditylenchus Dipsaci, Aphelenchoides fragariae* and *Pratelenchus penetrans* using water extract at 70°C and 22°C (room temperature) and *Tylenchulus semipenetrans* (Elzawahry *et al.,* 2014). The current study showed that oleander leave water extracts was highly toxic to *M. incognita* under in-vitro examination. Using of leaf extracts was more effective and this could be due to the leaves job as the main centres of photosynthesis and metabolism which manufacture active secondary metabolites.

The current study confirmed the potential of using oleander leaves and fish waste in controlling root-knot nematode *M. incognita*. All the used treatments markedly decreased all the related nematode parameters on eggplants such as, penetration rate, number of egg masses, galls, females /root system and number of J2s /250gm pots soil. These data agree with previous reports by nematlogistis using oleander and fish waste against root-knot nematodes. Combination of oleander leaves and chicken litter treatment reduce the root galling by 97.73% in eggplant infected with *M. incognita* (Osman *et al.,* 2016). Also Rahmani *et al.,*(2016) demonstrated that aqueous extract of oleander represent a great reduction on number of galls and *M. javanica* larvae population in soil. Results illustrated that oleander dry leave is more effective that fresh leaves in reducing nematode related parameters in eggplant and this is totally in agreement with results reported by Youssef and Lashein (2013) who stated that dry leave of oleander (5g/kg soil) is more effective in decreased the number of galls, egg masses, females and developmental stages of *M. incognita.* In a previous study by Jayakumar *et al.,* (2004) they found that adding 10 g/pot

of fish meal was effective against *M. incognita* infecting tomato plants under glasshouse conditions. The nematicidal mode of action of organic amendments could be attributed to the production of secondary substances from the decomposition which presents nematotoxic ability (Mahmood and Saxena 1992). The Variation in the toxicity of different used treatments could be due to active components and the concentration affected by several factors starting from source plant, solvent and extraction methods (Qasem and Abu-Blan, 1996). And the potency in the soil influenced by several factors during decomposition process such as, disruption of the organic amendment tissue into soil, the sufficient soil moisture and soil temperature during tissue decomposition (Ploeg and Stapleton, 2001, Morra and Kirkegaard, 2002, Lopez-Perez *et al.*, 2005). The nematicidal effect may be attributed to the interaction between the phenolic compounds and bio-membrane of microorganism (Veldhuizen *et al.*, 2006). Also the presence of terpens which can penetrating into the organismis cells and interacting with critical interacellular sites which causing the death of the cell (Cristani *et al.*,2007). The efficacy of organic matter on disease suppression could be attributed to many different mechanism but the soil and the rate of organic amendment will play important role (Abbasi, 2011).The nematicidal effect of fish waste maybe attributed to nutrient content which enhance the plant health and growth which help in reduced the plant infection with nematode and pathogens.

Table.1 Effect of oleander dry and fresh leaves and fish waste on egg hatching of *M. incognita*

Treatments	Time					
	24h		72h		7 Days*	
	Hatching %	Inhibition %	Hatching %	Inhibition %	Hatching %	Inhibition %
FW 100 ppm	14.50	43.14	15.75	58.82	23.50	68.35
FW 200 ppm	12.00	52.94	14.75	61.44	21.00	71.72
FW 300 ppm	8.50	66.67	12.00	68.63	18.00	75.76
ODL 100 ppm	12.75	50.00	15.00	60.78	22.00	70.37
ODL 200 ppm	11.50	54.90	14.25	62.75	20.50	72.39
ODL 300 ppm	7.50	70.59	9.00	76.47	15.50	79.12
OFL 100 ppm	13.00	49.02	15.50	59.48	22.50	69.70
OFL 200 ppm	11.75	53.92	14.50	62.09	20.75	72.05
OFL 300 ppm	8.25	67.65	10.00	73.86	16.75	77.44
Control	25.50	0.00	38.25	0.00	74.25	0.00
LSD 0.05	2.19		2.22		3.25	

* After transferred the eggs from the treatments to distilled water for 4 days

Table.2 Effect of oleander dry and fresh leaves and fish waste on juvenile's mortality of *M. incognita*

Treatments	% Mortality		
	24h	48h	72h*
FW 100 ppm	42.50	58.25	62.75
FW 200 ppm	50.75	69.00	76.25
FW 300 ppm	56.50	80.50	83.25
ODL 100 ppm	45.75	65.50	70.00
ODL 200 ppm	53.25	76.75	81.50
ODL 300 ppm	63.75	84.75	90.75
OFL 100 ppm	45.00	65.00	66.75
OFL 200 ppm	52.50	71.75	76.75
OFL 300 ppm	61.00	81.00	87.25
Control	3.00	5.00	6.75
LSD 0.05	2.583	3.447	3.324

* After transferred the larvae from the treatments to distilled water and incubation for 24 hours

Table.3 Effect of oleander dry and fresh leaves and fish waste on number of J2s, galls ,egg masses and females in eggplant infected with *M. incognita*

Treatments	Penetration rate after 15 days	Effect on nematode parameters			
		J2s / 250gm soil	Galls	Egg masses	Females
FW 0.5%	32.25	176.25	67.00	69.75	74.25
FW 1%	26.00	163.75	57.00	62.50	68.00
FW 2%	22.25	152.50	48.25	53.00	57.50
ODL 0.5%	26.75	165.00	61.50	66.25	68.50
ODL 1%	24.00	155.00	51.25	55.75	62.25
ODL 2%	16.0	142.50	41.50	45.00	48.25
OFL 0.5%	28.75	166.25	65.25	68.25	70.50
OFL 1%	25.00	157.50	55.25	59.00	64.00
OFL 2%	20.00	148.75	45.50	49.00	54.25
Nematode Alone	149.25	394.25	263.00	279.00	302.25
LSD 0.05	5.014	10.462	5.667	3.910	5.497

Table.4 Effect of oleander dry, fresh leaves and fish waste on plant height, root length, fresh, dry shoot and root weight of eggplant infected with *M. incognita*

Treatments	Effect on plant growth parameters				
	Plant height (cm)	Root length (cm)	Fresh root weight (g)	Fresh shoot weight (g)	Dry shoot weight (g)
FW 0.5%	74.25	33.00	11.342	52.022	2.987
FW 1%	77.00	33.75	11.835	53.975	3.122
FW 2%	84.75	40.00	12.682	59.242	3.342
ODL 0.5%	76.5 0	33.50	11.537	52.98	3.112
ODL 1%	78.50	34.50	12.142	55.782	3.137
ODL 2%	80.75	35.75	13.817	57.902	3.187
OFL 0.5%	75.00	33.25	11.515	52.455	3.035
OFL 1%	77.25	34.25	12.022	54.182	3.135
OFL 2%	82.50	37.50	13.855	57.365	3.147
Nematode Alone	54.25	25.75	8.605	45.717	2.785
Control	71.50	30.25	10.355	51.927	2.950
LSD 0.05	2.121	1.761	0.561	1.647	0.128

Results of present investigations proved that using of the treatments not only control *M. incognita* but also increase fertility of soil which reflected on the growth related parameters of eggplant. The current evaluation indicated that application of organic material such as oleander leaves and fish waste was improved clearly the plant root length, plant high, plant shoot and root weight. During organic material decomposition of some nutrients release in the soil and this will help in enhancing the plant growth which help the plants in avoid or escape from nematode infection.

This explanation was suggested by different study using different organic materials. Using of fish effluent in Irrigation increased the production yield of cherry tomato in Brazil (Castro *et al.,* 2006). In a pot experiment Mazur and Radziemska (2014) found that treated soil by compost (79.3 % fish waste + 19.7 % bark + 1 % brown coal) increased markedly the above part and root yield of radish.Tiyagi *et al.,* (2015) observed that organic amendment improved the plan growth of tomato plants. Using of Compost containing 79.3% fish waste + 19.7% straw +11% lignite recorded the highest crop yield of maize comparing with control (Radziemska and Mazur, 2015). The water extract of oleander enhanced the growth parameters of tomato plants infected with *M. javanica* (Rahmani *et al.,* 2016). Application with fish waste increased the potato production yield (Illera-Vives *et al.,* 2017). Using of oleander leaves and bio-waste not only increased soil nutrient concentration but also change the soil texture and this will affect the soil moisture. The sufficient soil moisture could help plant water supply which will affect the plant growth.

In conclusion, the inhibition of nematode and enhancing plant growth obtained in this study is important for strongly support the possibility using of oleander leaves and fish waste as effective method in controlling root-knot nematodes instead of using chemical nematicides to avoid environmental pollution and direct toxicity to beneficial organism, pollinators and human. Adding the used treatments in the soil not only root-knot nematodes control but also increased the soil

fertility, improves soil physical properties to sustain the productivity and solve some disposal problems. Therefore, using of oleander leaves and fish waste for nematode control alone or can be developed to be as a part of integrated nematode management programs especially under organic production programmes.

Future prospective

Further studies are urgently needed to improve the biopesticides combination and formulation to increase their applicable efficacy and stability. Another point is to use the new trend in nanotechnology techniques to maximize the potency and safety for plant parasitic nematode management.

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