Plant Archives Vol. 19 No. 2, 2019 pp. 4321-4330 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

ECO-FRIENDLY MANAGEMENT OF ROOT KNOT NEMATODE *MELOIDOGYNE* SPP. ON CUCUMBER CROP UNDER PLASTIC HOUSE CONDITIONS

Qais Kadhim Zewain* and Shaker Aliwe Ali

Department of Plant Protection, Faculty of Agriculture, University of Tikrit, Iraq
Corresponding Author: Qais K. Zewain. email address: qzewin@tu.edu.iq,

Summary

To evaluate the role of three plant extracts namely: *Azadirachta indica*, *Riciuns communis* L., *Thuja standishii* and bio-control fungus *Paecilomyces lilacinus* individually and collectively on compatibility basis, in reducing the symptoms of *Meloidogyne* spp and improving the yield, vegetative and root system characteristics of *Cucumis sativus* L. cucumber; a field trial was conducted inside a greenhouse in Kirkuk Governorate/Iraq during autumn season of 2017. The results showed the superiority of combined treatment between neem extract and biocontrol fungus *P. lilacinus* in giving the best averages for most of studied traits: root knot index and numerical density of nematodes per 100 g soil, number of egg sacks, length of plant (cm), wet and dry vegetative weight (g/plant), yield (g/plant), root length (cm), wet and dry root weight (g/plant), peroxidase and chlorophyll content (mg/g weight wet), with averages of 1.44%, 375.11, 2.11, 316.02, 48.233, 2495.3, 35, 5.41, 0.71, 4.823 and 13.067 respectively except the trait of plant length, where the combined treatment of castor plant extract and *P. lilacinus* gave a higher average of 181.333 cm.

Key words: cucumber, *Meloidogyne spp, Paecilomyces lilacinus, Azadirachta indica, Riciuns communis.*

Introduction

Cucumber crop is infected by many important agricultural pests included root knot nematode *Meloidogyne spp* and this genus is considered as a most dangerous genus among all plant parasitic nematodes (Abu Garibiah, 2010), economic losses caused by plant parasitic nematode estimated by $100 billion per year, 50% of which is caused by root knot nematode alone, causing losses estimated by 5% of total global agricultural production (Hussey and Janssen, 2002), (Abbas *et al.*, 2009).

Bio-Control method using bio-control fungi is introduced on the wide commercial scope of application at the beginning of 1990s to control root knot nematode and its interaction with pathogenic soil fungi, where the use of some nematodes or fungal formulations was found to have no negative effect on the vitality and efficacy of these bio-fungi so, it was gradually replaced the chemical control method (Antoine, 2014). It was also found that some fungi like *Paecilomyces lilacinus, Trichoderma harzianum* are among the most influential biological factors in nematode activity so it has attracted scientist's interest in isolating and diagnosing other fungi as biological control agents that have proven effective characteristics in preventing crops from infecting by many nematode species (Meyer, 1990).

In Iraq, research on using bio-control technique has recorded a significant progress in controlling of many fungal and nematode diseases in recent years, since it relied primarily on the efficient use of natural resources to activate the activity of useful organisms against harmful pathogens in the root zone and adjacent soil, many researches and studies have been carried out dealing with the role of biological control alone or along with chemical and natural control agents in combating pathological complexities between nematodes and fungi in addition to availability of many bio-control formulations for this purpose in the Iraqi market as a result of these researches (Istifan *et al.*, 2002).

Using of plant extracts from (Fruits, leaves, roots) is one of the most successful and frequently used method for controlling many plant pathogens. Dewan *et al.,* (1983)

used some plant extracts to evaluate its impact on juveniles mortality and egg hatching of root knot nematode *M. exigua* that infects coffee plants. Soil applications of neem cake at 100 g/plant at planting time and subsequently, at 3-month intervals, reduced the population density *of Pratylenchus goodeyi* and *Meloidogyne* spp. on par with a chemical nematicide named; Furadan 5G (carbofuran) applied at 40 g/plant at planting time and then at 6-month intervals to banana plants cultivated 6-l-capacity pots with controlled levels of nematode infestations. Eight months after planting date, banana plants treated with neem cake, had 4 to 95 times less parasitic nematodes than the untreated control. However, only powdered neem cake applied to un pared banana plants kept the nematode population below the economic threshold (Musabyimana and Saxena, 1999).

It is also reported that neem Fertilizer *Azadirachta indicia* is used to control 16 species of nematodes, of which infects the roots on Honey Pumpkin Plant and 400 species of insect pests (Schmutterer and Singh, 1995).

Mostafa (2000) reported that use of castor leaf powder with the fungus *Arthrobotrys oligospora* and chemical nematicide Oxamyl on *Meloidogyne* spp has achieved significant results in nematode control and reduced the number of root knots and increased vegetative growth and dry weight of tomato plants.

The impact of two bio-control fungi *T. harzianum* and *P. lilacinus* with castor seed oil were evaluated against *M. javanica* on tomato crop in the plastic house, it been indicated that using of castor oil alone with nematode had led to an increase in growth variables as the length of roots reached (14.40 cm) and the stem length (11.90 cm) compared with nematode infected control treatment which gave (11.81 and 6.85 cm) respectively. It had also reduced values of infection variables as the number of root knot reached (43.66), the number of egg sacks for whole root system (54.76), the number of juveniles in 100 g of soil (1000.01) and the number of eggs per sack (130.87), compared with nematode infected control treatment which recorded (80.76, 69.76, 10910.65 and 150.76) for these variables, respectively (Abed *et al.*, 2011).

The current study aims to develop an integrated management program to manage root knot nematode on cucumber crop relying on eco-friendly materials through using of bio-control fungi *Paecilomyces lilacinus*, natural neem fertilizer (neem cake), castor and tannin extracts individually as well as collectively on compatibility basis under plastic house conditions.

Materials and methods

1. Preparation of nematode inoculum: A continuous source of *Meloidogyne* spp inoculum was obtained from plant roots of cucumber and eggplant from infected fields in Kirkuk governorate showing the symptoms of root knots. Nematode inoculum was extracted according to (Hussey and Barker, 1973) and (Javed *et al.*, 2007). 15-day old cucumber seedlings are inoculated with egg masses. Plants have been placed in the plastic house and irrigated as needed. From time to time, new cucumber seeds were planted and new seedlings were polluted in the same way, thus obtaining a permanent source of the nematode inoculum.

2. Collection of trial materials: Leaves of Castor plant *Riciuns communis* L. and fruits of Tannin plants *Thuja standishii* were collected from areas in Kirkuk Governorate while natural neem Natural (Neem Cake) *Azadirachta indica* was ready insured from Indian manufacturer named Scientific Fertilizer Company. The isolate of bio-control fungus *Paecilomyces lilacinus* was obtained from the biology laboratory belongs to Ministry of Science and Technology-Baghdad.

3. Plant material Preparation and extraction: The fresh plant samples were thoroughly washed and individually furnished in the shade for several hours and then cut into small pieces, brushed on a piece of cloth under lab temperature, with continuous flipping to prevent rot then pulverized by a mill (National mark) and screened by a sieve of 50 mesh and the powder of each plant was preserved in a paper bag tagged with a sheet indicating all necessary sample collecting data.

For extraction purpose, (10) g of each sample powder was taken and dissolved in 100 ml of distilled and sterilized water (10% w/v) at room temperature (22±2°C) and left for 24 hours with occasional shaking. The aqueous extract was filtered by several layers of gauze to dispose the plant parts, followed by centrifugation at 10000 rpm for 10 minutes to dispose of leftover impurities. Extracts were aseptically preserved in dark glass bottles placed in the refrigerator for further use (Hassoun *et al.*, 2007), (Atwan *et al.*, 2005).

4. Compatibility test: Poisoned Food method was applied in this experiment to estimate the compatibility of used plant extracts with bio-control fungus *Paecilomyces lilacinus*. Each 125 ml of sterilized PDA media was amended by 10% concentration of aqueous extract of used plants. The mycelial growths of *Paecilomyces lilacinus* were evaluated in 850 mm petri dishes with sterilized solid PDA medium amended by 10% aqueous extract of each plant, then the center of each petri dish was inoculated with 5 mm diameter disc of fungal colony

taken from the tip of pure culture (5 days old) by cork borer. All inoculated petri dishes were incubated at 28°C for 7 days post inoculation. Radial mycelial growth was measured at the time on which petri dishes of control treatment got filled with the fungal mycelial growth. Three replicates were maintained for each treatment while PDA medium without plant extract was maintained as a control treatment in three replicates as well. The antifungal activity for each plant extract was calculated as an inhibition percentage of mycelial growth by applying the following formula:

$$\% \text{ inhibition} = (dc-dt)/dc \times 100$$

Where "dc" is the average increase of mycelial growth in control treatment and "dt" the average increase of mycelial growth in extracts treatments. (Jasso de Rodriguez *et al.*, 2004), (Bouskani, 2008).

5. Calculation of aqueous dilution of bio-control fungus: *Paecilomyces lilacinus* fungus was developed on millet seeds, 600 grams of millet seeds and were distributed on three glass jugs, seeds were water soaked and sterilized in the autoclave for 30 min. at 121°C under pressure of 1.5 bar, After that parts of PDA medium containing the mycelial growth *Paecilomyces lilacinus* were aseptically placed and incorporated into millet seeds and placed in the incubator after got it sealed with pieces of medical cotton at a temperature of 25°C with an occasional shaking to ensure homogenous distribution of the fungus according to (Alwan *et al.*, 2012), then (1gm) of the seeds containing the bio-control fungus *Paecilomyces lilacinus*, was took and placed in a test tube containing (9ml) distilled water which considered as a first concentration and then (1ml) took from this concentration and placed in another test tube containing (9ml) of distilled water and this is considered as a second concentration and so on until getting the desired dilution of 10^{-5}.

6. Greenhouse Experiment: The field experiment was carried out in the plastic house located in the nursery belongs to Kirkuk Directorate of Agriculture where it soil was subjected to soil solarization for 8 weeks during hottest summer months in Iraq July and August 2017 in order to obtain sterile soil in an economical and environmentally safe way (Abu Gharibiah, 2000). Roni F1 cultivar was used in this trial produced by National Seeds Company with purity 95% and germination 99%. cultivation lines were determined (1m) between one line and another, and cucumber seedlings were planted 40 cm between the plant and another within the same line. All cultural operations were maintained according to Iraqi Ministry of Agriculture' recommendations regard cultivation of cucumber crop in Iraq. Ten treatments were included in this experiment as follow: natural neem fertilizer, bio-control fungus *Paecilomyces lilacinus*, castor leaf powder, Tannins fruit powder, natural neem fertilizer + *Paecilomyces lilacinus*, castor leaf powder + *Paecilomyces lilacinus*, Tannins fruits powder + *Paecilomyces Lilacinus*, Standard chemical nematicide Oncal 5% G, Nematode inoculated control and Nematode non-inoculated control. Each treatment included ten plants and three replicates were maintained for each treatment. One meter was left as a free distance between each two treatments to avoid any contamination. The randomized complete block design (RCBD) was applied for this trial.

7. Nematode inoculum preparation and inoculation: Nematode eggs were extracted using a sieve of 200 mesh and 500 mesh then collected in a glass baker and counted the egg number in 1 ml of egg suspension under dissection stereo-microscope and then estimated the total number of eggs. Cucumber seedlings after 10 days of its transplanting into the greenhouse were inoculated by pouring 10 ml of water containing 3000 eggs to the soil by a glass pipette at depth of 2cm through making three pits with equal distances 3cm around each tested plant.

8. Treatments application: Evaluated treatments were applied by homogenous mixing of 10g of treatment material with plant surrounded soil while neem natural fertilizer was added by 40 g/plant in same way according to its producer instruction. Bio-control fungus *Paecilomyces lilacinus* was applied by rate of 3 g/plant into longitudinal slit around the plant while its interaction treatments with plant materials were added by same rate and methodology considering applying plant materials first then bio-control agent.

Experiment observations were taken 60 days after transplanting date, plants were up-rooted and root galls and following plant characteristics were determined.

A. Root galls: root galls index is used according to (Bridge and Page, 1980). as follows:

0 = No knots, 1 = Few small knots difficult to find, 2 = small knots only but clearly visible, 3 = Some larger knots visible, main roots clean, 4 = larger knots predominate but main roots clean, 5 = 50% of the roots effected, knotting on some main roots, 6 = knots spread over 60% of the root, 7 = knots spread over 70% of the root, 8 = knots spread over 80% of the root, 9 = knots spread over 91% of the root, 10 = knots spread over 100% of the root.

The changes in the values of the studied characteristics were estimated as a percentage by applying of following formula reported by (Eksteen *et al.*, 2001):

$$\% \text{ of reduction} = \frac{\text{Average of treatment - Average of check treatment}}{\text{Average of check treatment}} \times 100$$

B. Length of plant vegetative and root system: Length of plant stem and root were measured using the metric tape after separating the vegetative system from the root system on crown area.

C. Wet weight of plant vegetative and root system: Wet weight of plant vegetative and root systems for all treatments were measured at the end of experiment. plants were uprooted and its root system was cleaned by water from all of the dust stuck then vegetative system was separated from root system and wet weight was took for each of them.

D. Dry weight of plant vegetative and root system: Each of vegetative and root systems of plants for each treatment were kept in a perforated paper bags and placed under solar drying for two weeks and weighed for the purpose of obtaining dry weight where the plant parts were confirmed dry after weight stability for two consecutive readings.

E. Yield weight per plant: Three yield harvests were taken and weighed for each treatment in its three replicates and then total weight was divided on the number of included plants to get the yield weight/plant.

F. Root knot index: Root infection index for each treatment was calculated according to (Bridge and Page, 1980).

G. Nematode numerical density in the soil: Numerical density for nematode juveniles were calculated in 100g of soil by according to (Coyne *et al.*, 2007).

H. Counting of nematode egg masses: Plant roots were carefully washed by water to remove all soil stuck materials and egg masses were counted under stereomicroscope.

I. Estimation of Peroxidase concentration: Concentration of peroxidase enzyme as an indicator for induced systemic resistance was estimated in plant roots for each treatment following (Hammerschmidt *et al.*, 1982).

J. Chlorophyll content: Chlorophyll content was measured in randomly chosen 5 plants from each treatment using Chlorophyll content meter.

9. Statistical analysis: Data of the present investigation were subjected to the analysis of variance test (ANOVA) as (RCBD) and treatment means were compared by Duncan's Multiple Range Test. It was necessary to transform the data for nematode populations (loge) before analysis. Probability level was (P<0:05). (Al-Rawi and Abdulaziz, 2000).

Results and discussion

Table 1 shows means comparison for root knot index, numerical density of nematode/100g soil and number of egg masses in a multi-range Dunkin test, which are clearly indicating the significant impact of applied plant extracts and bio-control fungus in reducing the disease symptoms caused the nematode on cucumber plant roots. The superior effect in reducing the percent of root knot index and numerical density of nematode in 100g of soil was achieved by standard nematicide Oncal 5% G 1.11%, 371.11 followed by integrated treatment of neem fertilizer + *P. lilacinus* 1.44%, 375.11 respectively with no significant difference between them, then followed by integrated treatment of castor oil + *P. lilacinus* and neem fertilizer with means of 1.667, 1.778 for root knot index and 475, 450.33/100g soil for nematode density respectively with no significant difference among the means of three mentioned treatments. For the number of egg masses, there are no significant differences between standard nematicide Oncal 5% G treatment which was achieved the superior mean and the rest of individuals and integrated treatments means ranged between 2.111 and 3.333 egg mass/plant for neem fertilizer + *P. lilacinus* and *P. lilacinus* alone treatments respectively. The effective role of using bio-control fungus with plant materials as natural neem fertilizer has given the highest reduction in

Table 1: Means of Treatments effect in pathological characteristics of root knot nematode.

Egg mass/plant	Nematode numerical density/100 g soil	Root knots index	Traits / Treatments
bc 3.33	bc 757.33	bc 2.33	*P. lilacinus*
bc 2.55	de 450.33	d 1.77	Natural neem fertilizer
bc 2.88	d 525.11	cd 2	Castor
b 4.22	b 780.11	b 2.66	Tannin
bc 2.11	e 375.11	de 1.44	Neem fertilizer + *P. lilacinus*
	de 475	d 1.66	Castor+ *P. lilacinus*
b 3.77	c 659.44	bc 2.33	Tannin+ *P. lilacinus*
bc 1.77	e 371.11	b 2.55	Oncal 5% G
a 26.55	a 1646.11	e 1.11	Nematode inoculated control
c 0	f 0	a 5.22	Non-inoculated control

Each value represents an average of three values. * The averages that share the same letter are not significantly different according to Duncan's multiple test at the probability level of 0.05.

Table 2: Means of Treatments effect in plant vegetative characteristics and yield.

Yield/plant (g)	Vegetative system weight (g/plant)		Plant height (cm)	Traits / Treatments
	Dry weight	Wet weight		
abc 2245.3	d 34.00	d 266.07	ef 165.11	*P. lilacinus*
abc 2205.3	c 41.02	bcd 282.38	de 167.44	Natural neem fertilizer
bc 2047.0	d 36.02	cd 276.11	cd 169.66	Castor
c 1981.0	d 33.12	d 268.11	f 163.33	Tannin
a 2495.3	a 48.23	a 316.02	c 171.33	Neem fertilizer + *P. lilacinus*
abc 2187.7	bc 44.21	abc 302.29	a 181.33	Castor+ *P. lilacinus*
bc 2041.7	d 33.07	bcd 278.09	cd 169.22	Tannin+ *P. lilacinus*
bc 2055.3	ab 45.21	ab 308	b 177.55	Oncal 5% G
d 468.3	e 22.30	e 128.63	g 111.55	Nematode inoculated control
ab 2361.7	ab 47.61	abc 305.33	ab 180	Non-inoculated control

Each value represents an average of three values. * The averages that share the same letter are not significantly different according to Duncan's multiple test at the probability level of 0.05.

pathological evaluated traits due to nematode inhibition achieved by *P. lilacinus* as a result of availability of fungus food requirements and mechanisms already owned by *P. lilacinus* including secretion of many antibiotics that have an inhibition effect for nematode growth as compounds (isonitriles, steroids, polyketides and Peptaibols) (Sallam *et al*., 2009), or induced a plant systemic resistance through increasing the efficacy of Peroxidase and chitinase enzymes in treated plant after 48-72 hours (Harman, 2006) or competing nematode on nutrients and place and thereby reduce the chance for pathogen developing. The efficacy of neem fertilizer against insects and other agricultural pests in general comes from the possibility of its working as an antifeedant, repellent, anti-ovi-position, nematicide, insecticide, microbial growth inhibitor and also as a disabled substance for growth of target organisms. Its distinctive efficacy against nematode may belong to its potential to reduce nematode female capability of egg position, inhibiting egg hatching, restricting nematodes movement in the soil, lowering the numerical density of nematode, inhibition the penetration of plant's roots by the second-phase juveniles and thereby as a result significantly reducing the number of nematode root knot. The action mechanism of neem fertilizer also includes its working as an antifeedant for second phase of nematode juveniles. These juveniles when they are hungry and wants to feed on plant roots, the presence of active substances of Azadirechtin, Salannin and Meliantriol in the soil surrounding the roots as a result of soil application with neem fertilizer leads to stop the torsional movement for Juvenile gastrointestinal tract and subsequently feeling like the desire to vomit which lead finally, to refrain from feeding. The second mechanism of action for this fertilizer is preventing nematode female to place their eggs on plant roots treated with neem fertilizer or even neem oil. It also acts as a growth regulator which is similar in this behavior to juvenile hormone application technique currently applied in the field of insect pest management (Technical Bulletin of neem cake, 2017). The results also pointed to the active role of bio-control fungus *Paecilomyces lilacinus* in reducing the number of root knots and nematode numerical density due to the high capability of these high parasitism fungi on nematode eggs, by penetrating the fungal hyphae for nematode egg crust (Istifan *et al*., 2002). Many researchers were pointed out that the effect of these fungi filtrates on the nematode juveniles is due to the range of antibiotics such as giotoxin, viridin and hadeciden contained, which inhibit the movement of juveniles and subsequently disable its ability to penetrate the roots and forming nematode knots on them (Khan *et al*., 2001), (Khan *et al*., 2002), (Sukumer *et al*., 2005). These results were agreed with similar studies stated that using of bio-control fungus *Paecilomyces lilacinus* was effectively reduced the number of nematode root knots (Hinawi *et al*., 2014).

Table 2 shows efficacy means of evaluated treatments in plant characteristics of vegetative system growth and yield, which indicates that all plant extracts and bio-control fungus *P. lilacinus* and its integrated treatments have improved the growth quality of vegetative system of cucumber plants infected with root knot nematode *Meloidogyne* spp. Among them, plant height character, where integrated treatment of castor and bio-control fungus *P. lilacinus* was significantly superior among all other treatments, including the standard pesticide Oncol 5% G in achieving the highest plant of 181.33 cm, followed by non-inoculated control 180 cm with no significant difference between them. Inoculated control treatment recorded the lowest plant height by mean of 111.556 cm.

For wet vegetative weight, the treatment of natural neem + *P. lilacinus* was recorded the highest average of 316.02 g/plant with no significant difference with each of standard nematicide of Oncol 5% G, non-inoculated control and Castor + *P. lilacinus*, by averages of 308, 305.33, 302.29 g/plant respectively with significantly differed with the rest of treatments according to Duncan's multiple test. As for dry weight of vegetative system, neem Fertilizer + *P. lilacinus* was achieved the highest

rate of dry vegetative weight by average of 48.233 g/plant, superior to both treatments of Oncol 5% G and non-inoculated control with no significant difference while it was differed significantly with the rest of studied treatments. Same treatment of natural neem Fertilizer + *P. lilacinus* was also superior in recording the highest yield average of single plant, which reached 2495.3 g/plant with significant differences with most of evaluated treatments, except non-inoculated treatment, *P. lilacinus*, natural neem Fertilizer and castor + *P. lilacinus* with averages of 2361.7, 2245.3, 2205.3 and 2187.7 g/plant respectively.

The positive impact of plant extracts, bio-control fungus *P. lilacinus* and their integrated treatments is due to the nature and content of these substances and specially the active substance that have a significant role in influencing the pathogen by secretion of many growth regulators and enzymes that analyzed nematode's walls and cells and thus increase in plant growth and yield, many studies have referred to the active role of microorganisms in resisting the pathogen and improving the characteristics of plant growth (Silva *et al*., 2008). It was found that microorganisms has an important role in production of iron-siderophores compounds with low molecular weights which are produced under the conditions of iron deficiency in the soil and have high ability to link with ferrous ion to form complexes where iron is separated from siderophore and the later is released to associate again with iron and so on, by this process the availability of iron element is increased for plants and subsequently improve its growth characteristics and productivity (Najib *et al*., 2007). Superiority of neem and Castor extracts with bio-control factor *P. lilacinus* may also be attributed to their synergistic effect in reducing the biological tension of nematode infection. Bio-control fungi *P. lilacinus* produces some of growth regulators that participating in improve some of the plant growth characteristics and increase the efficiency of absorption of micro-nutrients Mn, Fe, Cu, Zn and K by plants due to its high readiness by effect the fungus, thus increasing the growth of the root system and its activity thereby promoting vegetative growth (Al-Shaibani, 2005). In addition, fungus secretions provide a protection to the roots from attack by pathological causes (Whipps, 1997). It is also found that use each of abamectin and *Paecilomyces lilacinus* and Oxamyl had a significant impact against *M. Incognita* on tomato and improved plant growth characteristics and highly increased its wet and dry weights (Khan *et al*., 2012). On the other hand, the impact of plant extracts may attribute to their content of some substances that stimulate plant growth such as hormones and vitamins (Al-Nuaimi, 1999), also it may contain toxic substances for nematode, alert natural enemies to these substances or increase the level of plant resistance as well as improve the soil fertility and thus plants become more tolerant to nematode pathological impact (Vawdrey and Stirling, 1997).

Table 3 shows the means of root system traits out of which we found that integrated treatment of neem fertilizer + bio-control fungus *P. Lilacinus* was characterized by the best means of root length, wet and dry root weight of 35 cm and 5.41 g/plant and 0.71 g/plant respectively, which were significantly superior to all other treatments, including the standard nematicide treatment except non-inoculated control treatment in dry root weight 0.645 g/plant with no significant difference between them according to the result of means test by Dunkin's multi-range method while some treatments recorded lower means for wet and dry root weight compared with inoculated control treatment, this variation may attributed to root system deformation as a result of nematode infection and nematode galls formed on the roots. The reasons for increasing root length and wet and dry weight of root system of cucumber plants may belong to the role of applied treatments in boosting the growth characteristics of root system. From the previous studies, (Istifan *et al*., 2006) reported that adding cauliflower *Brassica oleracea* leaf powder by 1-2 g/kg a week before transplanting date of eggplant seedlings provided almost complete protection of 97% against root knot

Table 3: Means of Treatments effect in root system characteristics.

Root dry weight g/plant	Root wet weight g/plant	Root length	Traits / Treatments
cd 0.54	de 3.51	de 24.33	*P. lilacinus*
bc 0.58	bcd 3.92	c 29	Natural neem fertilizer
de 0.48	cde 3.60	d 25.22	Castor
e 0.42	e 3.25	e 23.11	Tannin
a 0.71	a 5.41	a 35	Neem fertilizer + *P. lilacinus*
bc 0.58	cd 3.81	bc 30.11	Castor+ *P. lilacinus*
cd 0.53	cde 3.6	d 26.22	Tannin+ *P. lilacinus*
bc 0.59	bcd 4	c 29.22	Oncal 5% G
cd 0.50	bc 4.03	f 18	Nematode inoculated control
ab 0.64	b 4.32	b 31.66	Non-inoculated control

Each value represents an average of three values. * The averages that share the same letter are not significantly different according to Duncan's multiple test at the probability level of 0.05.

nematode *Meloidogyne* spp roots after 60 days of inoculation with nematode pathogen and also led to significant improvement of vegetative and root growth and its dry weight compared with inoculated control treatment. This result is agreed with (Amee and Ahmed, 2010) findings that nematode inoculated control treatment of cucumber crop achieved the minimum weight average of the root system, attributing that to consumption the required nutrients and energy for natural plant growth by the nematode, in addition to reducing the efficiency of nematode infected roots in absorption of water and nutrients and its translocation to plant vegetative system, because it is less branched and contains less root hairs compared with healthy roots due to the deformation of the vascular system in the area of infection, which also resulted in the stunting of plants, as well as increased breathing speed in the tissues affected by the root knots that led to an imbalance between the catabolism and anabolism in the plant which reduced its length and followed by manufacturing decrease of food and energy needed for root growth and its consumption by nematode (Choudhury, 1980). The toxic residues and enzymes introduced by nematode in the roots were negatively impacted on plant growth as it caused a disruption in its vital metabolism and subsequently negatively reflected on its length and dry weight. (Malekeberhan *et al*., 1985). Dawoud (2015) reported the superiority of garlic treatment in increasing the wet and dry weight of eggplant root system respectively 191.3, 73.3g compared to other evaluated treatments. Increasing of dry and wet weights of plants applied with plant materials and bio-control fungus *P. lilacinus* came as a result of its potential in combating root knot nematode and reducing the number of its resulted root knots. Biological control of pathogens that occur in plant root zone is usually carried out by secretion of anti-parasitic compounds by involved bio-control agents, the importance of these compounds appears in depriving of root knot nematode from some essential and important compounds. Therefore, bio-control fungus and plant materials are important for provision of plant nutrients, food competition, enhancement of plant acquired systemic resistance and biological availability of important minerals (Rosenblueth and Romero, 2006), (Sessitsch *et al*., 2002). Hinawi *et al*., (2014) also pointed to similar results on the positive role of bio-control fungi of *T. harzianum* and *P. lilacinus* in improvement of root system characteristics of tomato plants compared with control treatment with significant difference among their means (Hinawi *et al*., 2014).

Table 4 shows averages of evaluated treatments impact in peroxidase enzyme level and chlorophyll content. Among them, superiority of the natural neem fertilizer + *P. lilacinus* in increasing the level of peroxidase enzyme in the roots could be noticed as it scored an average of 4.823 unit/g wet weight, achieving the second place after standard nematode Oncol 5% G with no significant difference between them and significantly differed with all other individual and integrated treatments of plant extracts and bio-control fungus *P. lilacinus*.

Plants can obtain systemic and localized resistance mediated by many biological agents, such as non-pathogenic fungi, which live in plant rhizosphere, this type of resistance is called induced systemic resistance, including the induction of plant defensive responses at the level of histological systems and organs and phytoalexin building in plant parts away from infection site and building of defense-related proteins (PR proteins). These proteins that are induced by an elicitor as a result of the infection and pre-synthesized proteins in the plant considered as defensive means, including enzymes like; Polyphenol oxidase, peroxidase, Chitinase and Phenylalanine ammonia-lyase (Van loon *et al*., 1998). In addition to accumulation of callus, phenols and lignin behind the sites of infection, which act as barriers to prevent spreading of infection therefore; the induction of systemic resistance in plants is considered as one of biological control methods for soil endemic pathogens and can contribute to reduce the use of chemical pesticides in plant protection (Yedidia *et al*., 2000).

Table 4: Means of Treatments effect in level of peroxidase enzyme and chlorophyll content.

Chlorophyll content Spad	Level of peroxidase enzyme unit/g wet weight	Traits / Treatments
a 13.09	bc 3.81	*P. lilacinus*
cd 10.24	b 4.2	Natural neem fertilizer
d 9.41	de 2.04	Cast
d 9.25	de 2.08	Tannin
a 13.06	a 4.82	Neem fertilizer + *P. lilacinus*
bcd 10.73	c 3.51	Castor+ *P. lilacinus*
bcd 10.85	bc 3.92	Tannin+ *P. lilacinus*
ab 12.43	a 5.11	Oncal 5% G
e 6.87	e 1.80	Nematode inoculated control
abc 11.68	d 2.5	Non-inoculated control

Each value represents an average of three values. * The averages that share the same letter are not significantly different according to Duncan's multiple test at the probability level of 0.05.

From the previous studies in this field. Woo *et al.,* (1999) inoculated cucumber plants with spores of *Trichoderma harzianum* by spraying the vegetative system or soil application and found that this treatment led to reduce infection severity with *Botrytis cinerea* and increase cellulose content of cell walls and raised the level of peroxidase and chitinase enzymes in leaves and root tissues. The effectiveness of enzymes peroxidase and chitinase β-1, 3-glucanase and cellulase have increased in the plants treated with Trichoderma spp compared to non-treated plants (El-Katatny *et al*., 2000), (Yedidia *et al*., 2000). Treating cotton seeds by *Trichoderma virens* led to increase roots content from peroxidase and terpenoid compared to non-treated seeds (Howell *et al*., 2000). It has been found that efficacy of Peroxidase and polyphenol oxidase is directly linked with host induced resistance against *Pythium* sp. and *Rhizoctonia* sp. (Karthikeyan *et al*., 2006). For the characteristic of chlorophyll content in the leaves, the treatment of bio-control fungus *P. lilacinus* and integrated treatment of neem fertilizer + *P. lilacinus* is achieved highest means of chlorophyll content 13.09 and 13.067 mg/g wet weight respectively, thus significantly superior to all other treatments except standard chemical nematicide and non-inoculated control treatments that followed them. The superiority of neem fertilizer + *P. lilacinus* in chlorophyll content trait may be due to the increase in plant size, noting that this treatment has achieved high averages of plant height and its wet weight. Al-Nuaimi (1999) indicated that effect of plant extracts is due to its content of some substances that stimulate growth in the plant, such as vitamins and hormones. The increase in total chlorophyll content can also be attributed to the abundance and readiness of nutrients. The increase in plant leaves as a result of plant nutrient spraying contains nutrients (nitrogen, phosphorus and potassium) due to the important role of these elements in plant growth as nitrogen element enters into synthesis process of proteins and enzymes found in the plant, it also participates in the synthesis of porphyrins aggregates involved in the synthesis of chlorophyll and cytokromes essential for photosynthesis and respiration processes. Phosphor element also activates P-6-Glucose P-1-Glucose saccharides that enter the course of important processes in the formation of cellular membranes and then increase the proportion of chlorophyll in the leaves (Muhammad, 1985).

References

Abbas, S., S. Dawar, M. Tariq and M.J. Zaki (2009). Nematicidal activity of spices against *Meloidogyne javanica* (Treup) *Chitwood. Pak. J. Bot*., **41(5):** 2625-2632.

Abed, A.F., S.A. Salman and A.H. Amer (2011). Integrated control of root knot nematode *Meloidogyne javanica* on tomato plants under plastic house conditions. *Al-Furat Journal for Agricultural Sciences*., **3(3):** 66-73.

Abu-Gharibiah, W.I. (2000). Practical applications of solar sterilization of agricultural soils. Meeting of consultants for integrated production and prevention management of protected agriculture in the Arabian Peninsula. Dubai. U.A.E. The Arabian Peninsula programme. ICADA.

Abu-Ghraibiah, W.I. (2010). Plant nematodes in Arabic Countries. The University of Jordan - House Wael For publication. 1242.

Al-Nuaimi, Saadallah Najm (1999). Fertilizers and soil fertility. Second revised edition. University of Mosul. 381.

Al-Shaibani, J.A.K. (2005). The effect of adding organic matter (compost), bio-pesticide (*Trichoderma harzianum*) and the bacteria (*Chroococcum azotobacter*) in the growth and yield of tomato plants. P.hd. Thesis. Baghdad University. College of Agriculture.

Al-Rawi, K.M. and K.A. Abdulaziz (2000). Design and analysis of agricultural experiments. University of Mosul-Ministry of Higher Education and scientific research - Republic of Iraq.

Alwan, D.S., A.S. Abdelkarim and A.A. Najm (2012). Evaluation of efficacy of bio-control fungus *Trichoderma Harzianum* and *Trichoderma viride* in protecting the seeds of black seed *Nigella sativa* L. and its seedlings from the infection of field pathogens *Fusarium Lateritium*, *Fusarium Solani* and *Rhizoctonia* sp. and its effect on some growth characteristics. *Diyala Journal of Agricultural Sciences*., **2(4):** 105-115.

Amee, S.N. and A.I. Ahmed (2010). The numerical density of root knot nematode on cucumber plant and some methods of its control. *The Journal of Al-raffedain Agriculture*.

Antoine, B.G. (2014). Root knot nematode. *Iraqi Agricultural Journal*., **(1):** 56-59.

Atwan, Z.W., S. Fatima and N.J. Ferdous (2005). Testing the bio-effectiveness of safflower extract against germs and fungi. *Basra Research Journal Sciences.,* **31(3):** 39-47.

Bouskani, J.H. (2008). The effect of extracts of some wild plants in control of Ascochyta blight and Fusarium wilt diseases on chickpea crop in Sulaimaniyah governorate. P.hd. Thesis. Baghdad University. College of Agriculture. Plant protection Dept.

Bridge, J. and S.L.J. Page (1980). Estimation of root-knot nematode infestation levels on roots using a rating chart. *Topical Pest Management.*, **26:** 296-298.

Choudhury, B.C. (1980). Effect of different inoculum levels of *Meloidogyne incognita* on tomato varinties. *Bangladesh J. Agric. Res.,* **5:** 13-16.

Coyne, D.L., J.M. Nicol and B. Claudius-Cole (2007). Practical plant nematology: a field and laboratory guide. SP-IPM Secretariat, *International Institute of Tropical Agriculture (IITA)*, *Cotonou, Benin*., 1-38.

Dawoud, H.S. (2015). Evaluation of the efficacy of some plant extracts and bio-control fungus *Trichodema harzianum* in control of white rot disease on eggplant caused by *Sclerotinia sclerotiorum*. M.Sc. Thesis. College of Agriculture. Tikrit University.

Dewan, M.M., A.H. EL-Behadli, S.L. Alwan and A.M. Hamarach (1983). Root of unfermented animal manures in spreading some important crop pathogens in Iraq. *Zanco*., **8(1):** 21-31.

El-Katatny, M.H., W. Somitsch, K.H. Robra, M.S. El-Katatny and G.M. Gübitz (2000). Production of chitinase and â-1, 3-glycanase by *Trichoderma harzianum* for control of the phytopathogenic fungus *Sclerotium rolfsii*. *Food Technol. Biotechnol*., **38:** 173-180.

Eksteen, D., J.C. Pretorius, T.D. Nieuwoudl and P.C. Zietsman (2001). Mycelial growth inhibition of plant pathogenic fungi by extracts of south African plant species. *Ann, Appl. Biol*., **139:** 243-249.

Hammerschmidt, R., E. Nuckles and J. Kuc (1982). Association enhanced systemic resistance cucumber to *Colletotriccbum lagenarium*. *Physiol. Plant pathol*., **20:** 71-82.

Harman, G.E. (2006). Overview of mechanisms and uses of *Trichoderma* spp. *Phytopathology*., **96:** 190-194.

Hassoun, I.K., A.A. Ahd and A.O. Abed (2009). Assessment of the efficacy of bio-control fungus *Trichoderma harzianum* and the powders of some plants in controlling the fungus of *Rhizoctonia solani* the causing agent of stem canker disease black scurf disease on potatoe under the conditions of lath house conditions. *Babylon University Journal of Pure and Applied sciences*., **17(1):** 212-225.

Hinawi, M.J., S.H. Ali, N.H. Zaid and M.A. Hadi (2014). Evaluation of the efficacy of soil solarization and fungi of *Trichoderma viride*, *Trichodema harzianum*, *Glomus Mosseae*, *Paecilommyces lilacinus* and furfural nematicide against root knot nematode *Meloidogyne javanica* on tomato plant. *Journal of Biotechnology Research Center*., **8(2):** 73-79.

Howell, C.R., L.E. Hanson, R.D. Stipanovic, L.S. Puckhaber and M.H. Wheeler (2000). Induction of terpenoid synthesis in cotton roots and control of *Rhizoctonia solani* by seed treatment with *Trichoderma virens*. *Phytopathology.,* **90:** 248-252.

Hussey, R.S. and K.R. Barker (1973). A comparison of methods of collecting inocula of *Meloidogyne spp*., including a new technique. *Plant Disease Reporter*., **57:** 1025-1028.

Hussey, R.S. and G.J.W. Janssen (2002). Root-knot nematodes *Meloidogyne* species. In Plant resistance to parasitic nematodes, J.L. Starr, R. Cook & J. Bridge, (Eds), 43-70, CAB Publishing.

Istifan, Z. A., S.H. Mohammed and K.H. Ibrahim (2002). Efficacy of Fennamos nematicide and bio-control fungi *Trichoderma harzianum* Rifani and *Paecilomyces lilacinus* (Thom) Samson and some organic soil additives in controlling disease complex of root knot nematode and wilt diseases on eggplant. *Arab Journal of Plant Protection*., **20(1):** 1-5.

Istifan, Z.A., K.R. Omar., B.D. Hadeel and H.T. Kawther (2006). Efficiency of cauliflower powder in controlling of root knot nematode *Meloidogyne javanica* on eggplant and cucumber. *Iraqi Agriculture Journal.,* **1(2):** 60-67.

Jasso de Rodriguez, D., D. Hermández-Castillo, R. Rodriguez-Garia and J.L. Angulo Sánchez (2004). Antifugal activity *in vitro* of *Aloe vera* pulp and liquid fraction against plant pathogenic fungi. *Indust. crops & products.,* **21:** 81-87.

Javed, N., S.R. Gowen, M. Inam-ul-Haq and S.A. Anwar (2007). Protective and curative effect of neem (*Azadirachta indica*) formulations on the development of root-knot nematode *Meloidogyne javanica* in roots of tomato plants. *Crop Protection.,* **26:** 530-534.

Karthikeyan, M., K. Radhika, S. Mathiyagan, R. Bhaskaran, R. Samiyappan and R. Velazhan (2006). Induction of phenolic and defense-related enzyme in coconut (*Cocos nucifera* L.) roots treated with *biocontrol agent. Baz. J.*

Khan, H.U, W. Ahnad, R. Ahmad and M.A. Khan (2001). Evaluation of culture filtrates of different fungi on the larval mortality of *Meloidogyne incognita. Pakistan Journal of phytopathology.,* **12:** 46-49.

Khan, H.U., W. Ahmad, R. Ahmad, S.M. Khan and M.A. Khan (2002). Evaluation of the combined effects of *Paecililomycis lilacinus and Trichderma harzainum* against root-knot disease of tomato. *Biological Research.*, **3:** 139-142.

Khan, M.R., F.A. Mohiddin, M.N. Ejaz and M.M. Khan (2012). Management of root-knot disease in eggplant through the application of bio control fungi and dry neem leaves. *Turk. J. Biol*., **36:** 161-169.

Malekeberhan, H., R.C. Brooke, J.M. Wester and J.M. Dauria (1985). Response of *Phaseolus vulgaris* to a single generation of *Meloidogyne* of *Phaseolus vulgaris* to a single generation of *Meloidogyne incognita*. *Nematropica*., **31:** 190-220.

Meyer, S.L.F. (1990). Evaluation of potential biocontrol agents for soybean cyst nematode. *Mycological Society of America Newsletter.*, **41:** 29.

Mostafa, F.A. (2000). Integrated control of root-knot nematode *Meloidogyne spp* infecting sunflower and tomato. *Pakistan Journal of Biological Sciences.,* **4(1):** 44-46.

Muhammad, A.K. (1985). Plant Physiology, parts I and II, the University Press Directorate, University of Mosul, 1059.

Musabyimana, T. and R.C. Saxena (1999). Efficacy of Neem Seed Derivatives against Nematodes Affecting Banana *Phytoparasitica.,* **27(1):** 43-49.

Najib, L.M., Q.A. Mohamed and J.M. Khalaf (2007). Isolation and diagnosis of soil bacteria produced for siderophore iron compounds and the effect of sodium chloride in inhibition of some types of pathological bacteria. *Anbar Journal of Agricultural Sciences.,* **5:** 305-311.

Rosenblueth, M. and E.M. Romero (2006). Bacterial Endophytes and Their Interactions with Hosts. Molecular Plant-*Microbe Inter actions*., **19(8):** 827-837.

Sallam, N., A.A. Abd-Elrazik, M. Hassan and E. Koch (2009). Powder formulations of *Bacillus subtilis*, *Trichoderma* spp. and *Coniothyrium minitans* for biocontrol of onion white rot. *Phytopathol. & Plant Prot*., **42(2):** 142-147.

Schmutterer, H. and R.P. Singh (1995). List of insect pests susceptible to neem products. *In:* Schmutterer, H. (Ed.) The Neem Tree*, Azadirachta indica* A. Juss. and Other Meliaceous Plants: Sources of Unique Natural Products for Integrated Management, Medicine, Industry and Other Purposes. VCH, Weinheim, *Germany*., 326-365.

Sessitsch, A., J.G. Howieson, X. Perret, H. Antun and E.M. Romero (2002). Advances in Rhizobium research. *Crit. Plant Sci*., **21:** 323-378.

Silva, L.H.C.P., J.R. Campos, A.C. Sousa, (2008). Chractererization of *Streptomyces* with tential to promote plant growth and *biocontrol V*., **65:** 55.

Sukumer, J., S.D. Padma and U.D. Bongale (2005). Biological control of mulberry root-knot nematode *Meloidogyne incognita* by *Trichoderma harzianum. Internal Journal Entomology India*., **8:** 175-179.

Technical Bulletin of Neem cake Scientific Fertilizer Company (P) Ltd. (2017). Coimbatore, India.

Van Loon, L.C., P. Bakker and C.M.J. Pieterse (1998). Systemic resistance induced by rhizosphere bacteria. *Annu. Phytopathol*., **36:** 453-483.

Vawdrey, L.L. and G.R. Stirling (1997). Control of root-knot nematode (*Meloidogyne javanica*) on tomato with molasses and other organic amendments. *Australasian Plant Plathology.,* **26:** 179-187.

Whipps, J.M. (1997). Development in the biological control of soil borne plant pathogens., **26:** 1-134.

Woo, S. E., B. Donzelli and F. Scala (1999). Disruption of ech 42 (endo chitinase encoding) gene affect biocontrol activity in *Trichoderma harzianum* PI. Mol. *Plant Microbe. Interact*., **12:** 419-429.

Yedidia, I., N. Benhamou, Y. Kapulnik and I. Chet (2000). Induction and accumulation of PR proteins activity during early stages of root colonization by the mycoparasite *Trichoderma harzianum* strain T-203. *Plant Physiol. Biochem.,* **38:** 863-873.