Evaluation the efficiency of some controlling methods on olive seedlings infected with root-knot nematodes

Meloidogyne spp.

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Abstract. The study was aimed to evaluate different controlling methods and agents including the nematocide Rugby, the bio-agents \textit{Trichoderma.harzianum} and \textit{Trichoderma.atroviride}, compost of poultry manure, cows and sheep dung. These agents were compared for their effect on nematode populations and plant growth parameters of olive infected plant. The results revealed that Rugby was the most effective treatment that decreased all measured criteria of nematode infection including gall number / 2 g root, Female number /2 g root, number of egg masses / 2 g root and number of males and juveniles/ 100g soil which recorded 0.33/ 2g root, 0.33/ 2g root, 0.33/ 2g root, 0.00/ 100g soil respectively. The highest values of some plant growth parameters including plant height , root length, number of branches/plant and leaf area/plant were gained from treated with the poultry manure treatment that resulted in 32.66cm, 34.00cm2, 22.88g, 7,00 branch/plant and 5.37cm2 respectively. Among all treatments compost of poultry manure, cow dung and sheep dung had increased biochemical indicators of plant health such as percentage of nitrogen content (1.93, 1.93, 1.89)% portion content (12.07, 12.07, 11.8)% and chlorophyll a, b and total chlorophyll content (0.604, 0.503, 0.488) , (0.302, 0.258, 0.244) , (0.906, 0.671, 0.732) mg/g fresh weight respectively.

1. Introduction
Olive is an evergreen wooden tree which belongs to the Oleacea family [1]. It is widely planting in the Mediterranean region where more than 1000 varieties are known there. There are more than 40 varieties of olive in Iraq [2].The economic importance of olive trees is in the possibility of obtaining olives oil using its wood in the profession of carpentry and can also be planted as a beautification of gardens. Olives trees are attacked by many pathogens such as nematodes which mostly affect the roots \textit{Meloidogyne} is one of the most important species affecting trees and seedlings of olive with a multiplicity of infection by different types of \textit{Meloidogyne} spp nematodes causing root knot [3]. There are many methods of control against root-knot nematodes .chemical control is the most successful and widely used method worldwide because its effective, fast results and can be easily applied. However, there are many consequences on public health. As a result the dependence on the chemical control methods was reduced more and more and alternative methods have been found such as the use of biological control [4]. Alternative methods pesticides that have proven successful are used by
adding degraded animal waste to the soil. It helps to improve the vegetative growth characteristics of plant [5]. It also helps increase the absorption of nutrients. Animal fertilizers also reduce the evaporation rate with availability material fermenting microorganisms in soil [5]. Statistics in Iraq indicate that the number of olive fruitful trees 459198 with production rate 18 Kg / tree [6]. According to the same statistics there is a decline in olive production recently in some provinces especially Baghdad, Karbala, Babylon and Diyala. The lack of nematode studies on olive trees in Iraq and the presence of many disease in the Zafaraniya farm (one of the largest farm that produce olive seedlings for farmers and greenhouses) we aimed to evaluate the effectiveness of some control factors on olive seedlings inoculated with root knot nematodes Meloidogyne spp.

2. Materials and Methods
Three types of animal waste were used cow dung, sheep dung and poultry manure which were preserved and fermented at high temperatures in July, August and September 2016. The flipping process was used with moisturizing whenever moisture was needed, then covered with polyethylene after each moisturing process. These wastes were used at a rate of 15g per pot. In this experiment the bio-control Trichoderma was used in both T.harzianum and T.atroviride at the rate of one dish per pack and the nematicide Rugby produced by American company FMC obtained from local markets. The pesticide was used depending on the concentration recommended by the company. Olive seedlings and sapling of Ashrasy cultivar were obtained from the Indian Horticulture Station / Karbala prepared and planted in thermally sterile soil. Control coefficients were distributed in experimental units as follows:

- negative control (1) non-treated olive seedlings.
- negative control (2) olive seedlings infected with root-knot nematodes at rate of 3000 ± 10 egg per each pot.
- Olive seedlings infected with root-knot nematodes at rate of 3000 ± 10 egg / pot+ poultry manure.
- Olive seedlings infected with root-knot nematodes at rate of 3000 ± 10 egg / pot+ sheep dung.
- Olive seedlings infected with root-knot nematodes at rate of 3000 ± 10 egg / pot+ cow dung.
- Olive seedlings infected with root-knot nematodes at rate of 3000 ± 10 egg / pot+ Nematicide Rugby.
- Olive seedlings infected with root-knot nematodes at rate of 3000 ± 10 egg / pot+ The bioagent T. harzianum.
- Olive seedlings infected with root-knot nematodes at rate of 3000 ± 10 egg / pot+ The bioagent T. atroviride

Plants were placed for 5 months since inoculation taking into account watering as needed. Plants were then carefully uprooted to estimate the following traits:

2.1. infection parameters
The numbers of root knot/ 2g root, numbers of females and juveniles/2g root, numbers of males and juveniles/100g soil, numbers of egg masses/2g root.

2.2. plant growth parameters
The shoot and root length cm² / plant, fresh weight of the root, shoot g/plant, number of branches / plant, leaf area cm² / plant.

Leaf area was measured by taking plants leaves from all treatments and a hole were printed on a white A4 sheet which has already taken its weight and area individually to replicate each transaction by three replicates then cut the corresponding part of the calculate the weight and then calculated the surface area of the leaf by the following equation:
The surface area of the leaf = the area of large white sheet(cm²) × the weight of the plotted plant leaf(g) / weight of the large white sheet(g). According to [7].
2.3. Chemical properties
Nitrogen% : nitrogen ratio is estimated by Kjeldahl method using stem distillation device Micro-Kjeldahel. The nitrogen ratio was then calculated in the following equation.

\[ \text{N\%} = \frac{V_1 \times N_1 \times V_2 \times 14 \times 100}{A \times B \times 1000} \]

V1: Volume of acid Hcl cm\(^3\) of a burette
N1: Standard Hcl used.
14: Atomic weight equivalent.
100: Conversion to percentage.
V2: The size of the digested sample diluted in the sense of total abstract size.
A: The size of the extract used 5cm\(^3\) in the distillation device.
B: The weight of dry plant sample used in the digestion process (0.2 g)
1000: Converting mlg to g.

a- Determination of leaf content of total protein% :
The ratio of total protein to nitrogen was estimated according to [8] by applying the following equation:

\[ \text{Percentage of total protein} = \text{percentage of total nitrogen} \times 6.25 \]

b- Determination of leaf content (mlg\times g-1 weight)
Determination of leaf content of chlorophyll a and b in olive leaf based on [9].
The light density absorbance of the filter was measured by measuring the light spectrum (spectrophotometer) at the wave lengths of 645 and 663 nm. Concentration of chlorophyll a, b was determined in plant leaves calculated on mlg/g soft vegetable texture:

\[ \text{Chlorophyll A} = \frac{[12.7(D 633 − 2.69(D 645))]\times V}{1000 \times W} \]
\[ \text{Chlorophyll B} = \frac{[22.9(D 645)−4.68(D 663))]\times V}{1000 \times W} \]
\[ \text{Total Chloropyll} = \frac{[20.2(D 645) + 8.02(D 663)]}{V} \]
V: The final size of the filter after completion of the centrifugation process.
D: Reading the chlorophyll photo voltaic density.
W: Soft weight (g), the unit of measurement of chlorophyll is mg per gram soft tissue.

2.4. Statistical analysis
Complete Randomized Design (CRD) was used and averages were tested using Duncan multi range and at P< 0.05 probability level using the SAS program.

3. Results and Discussion
3.1. the effect of control factors on infection criteria
Results of table(1) showed that treatment of seedlings with Rugby pesticide has significant effects by recording the lowest value of infection criteria in all studied traits (gall number/2g root, Female number/2 g root, number of egg masses/2 g root & number of males and juveniles/100g soil) that it reached 0.33/2 g root , 0.33/2g root , 0.33/2g root and 0.00/100 g soil respectively and did not differ significantly from the control treatment (non-infected seedlings) but showed significant differences with all tested parameters.
Table 1: Effect of different control factors on *Meloidogyne* nematode infected olive trees

| Treatment                                      | No. of gall / 2g root | No. of female and juveniles / 2g root | No. of Egg mass / 2g root | No. of males and juveniles /100g soil |
|------------------------------------------------|-----------------------|--------------------------------------|---------------------------|--------------------------------------|
| Seedling non infestation with nematode (control 1) | 0.0 d                 | 0.0 d                                | 0.0 d                     | 0.0 e                                |
| Seedling infestation with nematode (control 2)   | 42.60 a               | 77.00 a                              | 44.00 a                   | 43.00 a                              |
| Seedling infestation + rugby                     | 0.33 d                | 0.33 d                                | 0.33 d                    | 0.00 e                                |
| Seedling infestation + cow dung                  | 14.00 bc              | 24.00 bc                              | 22.00 bc                  | 23.30 cd                              |
| Seedling infestation + sheep dung                | 16.00 bc              | 26.00 bc                              | 23.30 bc                  | 27.00 bc                              |
| Seedling infestation + poultry manure            | 10.60 c               | 18.60 c                               | 17.60 c                   | 18.00 d                               |
| Seedling infestation + *T.atroviride*             | 20.00 b               | 37.30 b                               | 33.60 ab                  | 32.60 b                               |
| Seedling infestation + *T.harzianum*             | 17.30 bc              | 29.00 bc                              | 19.33 bc                  | 31.00 b                               |

* Similar letters on the same columns does not significantly different according to the Duncan test at a probility of P< 0.05.

Followed by the treatment of seedlings inoculated with root knot nematodes and treated with poultry waste a decrease was observed in the values of the traits studied above 10.6/ 2g root , 18.6/ 2g root , 17.6/ 2g root , 18.0/ 100g soil respectively which did not differ significantly compared to seedling treated with cow dung. While significant differences appeared sheep's dung and *T.harzianum* added and the number of males and juveniles only reached 27.0 , 31.0 / 100 g soil respectively. It was noted that there were significant differences between the treatment of seedlings with poultry waste and the treatment of seedlings with biocontrol agent *T.atroviride* of the above studied qualities while the control (1) treatment recorded the highest value of infection criteria reached up to 42.6/2g root , 77.0/2g root , 43.0/100g soil respectively and differed significantly from all experimental factors except for the number of egg masses did not recorded significant differences between them and the treatment of biocontrol agent *T.atroviride* recorded. It is known that the pesticide Rugby is one of the organic phosphorous pesticide affects the first and second juvenile stage inside the egg as the permeability of the egg shell before hatching as well as that the pesticide enters the egg through the hole caused by the second juveniles stage by the spear in the egg shell while exiting the outer perimeter. The pesticide also affects the second juveniles stage and males in the soil and on the parasites nematodes stages that existent inside the root as a nematicide . This pesticide works contact and enters into nematodes throw the external holes of the body or perhaps by the cuticle. The mechanism of killing of nematicide occurs by inhibiting the work of never enzymes such cholinesterase and acetylcholine esterase enzymes . The asterase enzyme is one of the vital enzymes and any defect at its work may cause to death since it works on decomposition the acetyl colin item after portaging the nerve orders at the synaptic area and that is a avital part of performing that mission but this difflicting inhibites by the organic phosphorous pesticide as a result of inhabiting the enzymes working and perhaps because of changing the enzymes chemical structure [10; 11; 12] . This in turn leads to the death of the nematode as a result of the accumulation of acetylcholine and not decomposition and leads to weakness in the nervous activity of paralysis and then reduce the movement of the nematode and body wrinkling and thus death after the end of food stocks [10; 11; 12].
The effect of degraded animal residues on the reduction of an infection with root knot nematodes was reflected on the number of gall shown on the root leading to the creation of an unsuitable environment that caused the death of large number of juvenile liberated from eggs after being poisoned by the release of ammonia gas in the area around the root and decline of the number of juvenile penetrating the root [15;16].

Animal manure also provides plants with essential nutrient that make it more potent and resistant to the nematode [17] also degraded animal residues act to alter soil pH within an in appropriate range of nematode activity and reproduction also improve soil composition and control nematode. Improve soil composition lead to increase soil porosity, improve ventilation and permeability, increase soil water retention increase the maintain soil mass clusters that inhabit movement of juvenile. Also provide the soil with organic matter improve ion exchangeability improve soil pH and provide soil with Humus , Vitamins, Hormone and plant enzymes that are not provided by chemical fertilizers [18 ; 19 ; 20]. The reason for the superiority of poultry waste compared to sheep and cow dung is due to its high nitrogen content which directly affects the reduction of nematodes by releasing the ammonia gas produced by the microbial activity which dissolves the substance more organic than in cows and sheep dung, nitrogen compounds released by organic waste also reduce the number of nematodes [21; 22; 23;24] Bio-fungus may be the reason of reduction the incidence of root knot nematodes to the mechanism of the fungus *Trichoderma* which is parasitic directly on eggs surrounds them also for its capability of penetration the egg shell by the enzymes they release and thus break the egg shell and begin to grow inside until it reaches the juveniles leading to inhibition of hatching process as well as the direct parasitize the fungus *Trichoderma* on the second stage juvenile and penetration of the body wall by the hypha of the fungus [25]. Since the second stage juvenile cuticle is mainly composed of protein the fungus secrete an enzyme that dissolves the proteolytic protein which is working on decomposition of nematode body wall. Which finally lead to its death, as well as the direct affecting of *Trichoderma* it is also has undirected affection on the nematode through its work to stimulate defensive responses of plants at the level of tissue systems and to build phytoalexins in parts of the plant away from the site of injury and build related defence proteins such as chitinase, peroxidase, polyphenoloxidase, ammonia and phenylalamine [13]. In addition to the accumulation of callus phenols and lignin after the infection sites that act as barriers to prevent the spread of infection. The fungus *Trichoderma* is a non-pathogenic fungus that settles the root region and contributes to prevent many pathogenic fungi in different ways and methods which include parasitic antithesis and competition [13; 26; 27].

### 3.2. The effect of biocontrol agents on some plant growth parameters

The result of Table 2 indicating the effect of the control factors used on the growth characteristics of the olive seedlings indicate a significant superiority for the treatment of the root-knot nematodes contaminated seedlings to which the poultry manure were added. The highest values of plant growth parameters including plant height, root length, shoot fresh weight, number of branches/plant and leaf area. Plant were gained from plants treated with the poultry manure treatment resulted in 32.66cm, 34.00cm, 22.88g., 7.00 branch/plant and 5.37cm respectively. Followed by root-knot nematode compost cows dung treated seedling reached in plant growth to 32.66 cm, 31.66 cm root length, 20.34g shoot fresh weight, 5.33 branch/plant, 4.11 cm.

This is a significant difference compred to seedlings contaminated with root-knot nematodes with poultry manure but did not differ significantly from the treatment of seedling infected with root knot nematodes with sheep dung residues to above characteristics except for fresh weight of vegetative total. Followed by the treatment of seedlings infected with root-knot nematodes and the both fungus species *T.harzianum*, *T.atroviride* there were no significant differences between them. The values for the above growth traits reached 29 cm, 30cm, 15.35g., 5.66 branches/plant and 3.51cm respectively while their values at the treatment with the fungus *T.atroviride* reached 28.33 cm, 30.00cm, 16.31g., 5.66 branches/ plant and 3.77 cm respectively.
This was followed by the treatment control (1) with the values of the above mentioned growth traits reaching 29.66 cm, 27.66 cm, 14.03 g, 4.33 branch/plant and 3.10 cm respectively. A significant difference was observed in the length of the root total and leaf area compared with rest of the treatments. As for the vegetative total, the control (1) treatment did not differ from the treatment of root knot nematode contaminated seedlings with the addition of cows and sheep dungs and fungus *T. harzianum*.

The best treatment was the addition of disintegrated animal residues in contrast to the results when calculating the infection criteria to the superiority of the pesticide on the rest of the treatments. Plant nutrients only have chemicals that kill nematodes and the soil remains poor in the nutrients the plant needs to support its growth as a result of the destruction of most nutrients during autoclave. The animal residues ranked first in the improvement of the growth characteristics of the plant due to their direct impact on reducing the number of nematodes. It is also increase the resistance of the plant to the infection and have a role in improving the properties of physical and chemical soil and to provide the plant with the necessary nutrients. Current research indicated the containment of nitrogen and phosphorus and potassium, In poultry manure as compared to sheep and cow manure, which reached 3.03 for nitrogen, 2.63 for phosphorus and 1.4 for potassium [28; 23] This explains the superiority of the characteristics of growth when treated with poultry manure on sheep and cows.

**Table 2. Effect of different control factors on plant parameters of infected olive trees**

| Treatment                  | Plant height cm | Root length cm | Shoot fresh weight g. | Root fresh weight g. | Number of branch / plant | Leaf area cm²/plant |
|---------------------------|-----------------|----------------|-----------------------|----------------------|--------------------------|-------------------|
| Control 1                 | 29.66 b         | 27.66 e        | 14.03 e               | 10.55 e              | 4.33 d                   | 3.10 d            |
| Control 2                 | 25.33 e         | 23.83 f        | 11.36 f               | 13.23 a              | 3.33 e                   | 2.69 e            |
| Pathogen + rugby          | 28.00 d         | 29.66 d        | 15.13 de              | 10.66 de             | 4.00 d                   | 3.43 c            |
| Pathogen + cow dung       | 30.66 b         | 31.66 b        | 20.34 b               | 10.90 bcd            | 5.33 bc                  | 4.11 b            |
| Pathogen + sheep dung     | 30.33b          | 31.00 bc       | 17.76 c               | 11.00 bc             | 5.00 c                   | 4.05 b            |
| Pathogen + poultry manure | 32.66 a         | 34.00 a        | 22.88 a               | 10.80 bcd            | 7.00 a                   | 5.37 a            |
| Pathogen + *T. atroviride*| 28.33 d         | 30.00 cd       | 16.31 cd              | 11.00 bc             | 5.66 b                   | 3.77 bc           |
| Pathogen + *T. harzianum* | 29.99 cd        | 30.00 cd       | 15.35 de              | 11.17 b              | 5.66 b                   | 3.51 c            |

* Similar letters on the same columns does not significantly different according to the Duncan test at a probility of P< 0.05.

Seedlings treated with Trichoderma showed improving in growth properties of the plant to the effect of the fungus directly on nematode that infect roots and indirectly by stimulating the defense responses of the plant. In addition, Trichoderma increases the efficiency of plant nitrogen, increases the melting of phosphorus and aids in the decomposition of ions Zn, Cu, Fe and Mn and the micro nutrients needed by the plant in its growth and make it ready in the soil, which facilitates the absorption of the plant by the roots feeder. The fungus stimulates the production of proteins rich in proline, which leads to the accumulation of silica in the walls of cells, that earns strength and works as a barrier.
between the pathogen and healthy tissues as well as production of growth regulators and vitamins. In addition, it stimulates and encourages the growth and development of plants also stimulates the growth of the total root and increase the weight of the fungus also produces antibiotics. That lead to an increase in the efficiency of photosynthesis and thus increase the solubility (major metabolic products) that tend to build new tissues [3].

3.3. The effect of control factors used in the study on olive seedlings chemical properties

The highest nitrogen ratio was recorded in the control of poultry waste, cow dungs and sheep dungs, which differed substantially with the rest of the experimental factors, but did not differ significantly with each other as it reached (1.93, 1.93, 1.89%) respectively. The control (2) treatment recorded the lowest percentage of nitrogen with significant differences with all the parameters of the experiment as it reached 1.34%. The results also showed that the treatment of the control of poultry, cow and sheep wastes was the highest protein percentage (12.07, 12.07, 11.8%) respectively, and did not differ significantly between them but differed with other treatments except for the treatment of sheep dungs with biological fungi *Trichoderma* that not show any significant differences. While the lowest proportion of protein was recorded in the comparison treatment (seedlings infected with nematode) and differed significantly with all the parameters of the experiment as it reached 8.38%.

As for the amount of chlorophyll a and b and total chlorophyll indicate a significant superiority of treatment of poultry manure on other experimental factors, except for the treatment of cow and sheep waste, which did not differ significantly. The values reached 0.604 and 0.302 and 0.907 respectively, while the lowest amount of chlorophyll a and b and total chlorophyll recorded in the control treatment (contaminated seedlings) which was significantly different with all the experimental parameters, with 0.057, 0.028 and 0.085 respectively. This decrease in nitrogen, protein and chlorophyll may be due to the effects of root-knot nematodes while feeding on the root system due to the efficiency of the root on water absorption and the necessary nutrients, which reflected negatively on the various vital activities of the plant and thus an effect on the amount of nitrogen and protein and chlorophyll produced in comparison with other treatments used used compared to pesticide which had a clear role in minimizing the impact of nematodes and thus enabling the plant to produce a higher amount of nitrogen, protein and chlorophyll. As for the treatment of seedlings with animal wastes and fungi *Trichoderma* may be due to the fact that these factors in addition to their direct impact on the nematodes and reduces its number, which led to natural absorption of water and nutrients in roots. It provides the plant with necessary compounds and increase the strength of absorption of the plant and this is reflected in the values of nitrogen, protein, chlorophyll a, b and total chlorophyll [29].

**Table.3** Effect of control factors used in the study on olive seedlings chemical characteristics.

| Treatment                  | Nitrogen % | Protein % | Chlorophyll a (mg . g⁻¹ fresh weight) | Chlorophyll b (mg . g⁻¹ fresh weight) | total chlorophyll (mg . g⁻¹ fresh weight) |
|----------------------------|------------|-----------|---------------------------------------|---------------------------------------|------------------------------------------|
| Control1                   | 1.510 c    | 9.450 c   | 0.267d                                | 0.127d                                | 0.394d                                   |
| Control 2                  | 1.340d     | 8.380d    | 0.057e                                | 0.028e                                | 0.085e                                   |
| Pathogen + rugby           | 1.510 c    | 9.440 c   | 0.412 bcd                             | 0.206 bcd                             | 0.618 bcd                                |
| Pathogen + cow dung        | 1.930 a    | 12.070a   | 0.503 ab                              | 0.258 ab                              | 0.761 ab                                 |
| Pathogen + sheep dung      | 1.890 a    | 11.810 ab | 0.488 abc                             | 0.244 abc                             | 0.732 abc                                |
| Pathogen + poultry manure  | 1.930 a    | 12.070a   | 0.604a                                | 0.302a                                | 0.906a                                   |
| Pathogen + *T.atroviride*  | 1.760 b    | 11.020b   | 0.342 bcd                             | 0.171 cd                              | 0.513 cd                                 |
| Pathogen + *T.harzianum*   | 1.600 c    | 10.020c   | 0.330 cd                              | 0.164 cd                              | 0.494 cd                                 |
* Similar letters on the same columns does not significantly different according to the Duncan test at a probility of P< 0.05.

4. Conclusions
The present assessment indicates that all control agents used from decomposed animal waste and fungi *Trichoderma* examined showed robust antagonistic activity against root-knot nematodes. Consequently, they could be used as alternative methods of nematicide application for management of the diseases caused by root-knot nematodes in plants.

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References
[1] Katib Y M 2000 *Classification of seed plants* (Mosul, Iraq: Dar Al-Kitab Printing Press).
[2] Al-Douri A H A and Al-Rawi A S 2000 *Fruit production* (Mosul, Iraq: Dar Al-Kitab Printing Press).
[3] Stephan Z A 1988 Newly reported hosts of root-knot nematode in Iraq.Int. *Nematol.Net Work. Newsl. 5(3) 36-43
[4] Khan Z et al 2007 Observation of the suppression of root-knot nematode (*Meloidogyne arenaria*) on tomato by incorporation of cyanobacteria power (Oscillatoria chlorine) into potting filed soil. *Bioresour. Technol. 98* 69-73
[5] Abu Garbia W I and Al-Hassany N R 2010 *Nematode control using agricultural measures, plant nematodes in Arab countries* (Aman, Jordan: Dar Wael Publishing).
[6] Central statistical organization 2015 *Summer Fruit Production Report Central Statistical Organization* (Bagdad,Iraq: Ministry of Agriculture).
[7] Abadi A I 2006 *Mechanism of resistance of some varieties of pears to the infection of the insects bugs stencils Steganitis pyri (F) (Tingida: Hemiptera)* (Mosul, Iraq: PhD thesis, University of Mosul).
[8] Ibrahim A A and Ibrahim I K 2000 Evaluation of non – chemical treatment in the control of *Meloidogyne incognita* on common bean *Pakistani J. Nematol.* 18 51-57.
[9] Mackinney G 1941 Absorption of light by chlorophyll solution.*J. Biol. Chem.* 140 315 -322.
[10] Hauge N R 1979 A technique to assess the efficacy of nonvolatile nematicides against the potato cyst nematode *Globodera rostochiensis*. *Ann. Appl. Biol.* 93 205-211.
[11] Wright D J 1981 Nematicides : mode of action and new approaches to chemical control. in: *Plant Parasitic Nematodes*. (B.N. Zuckerman and R.A. Rohde, eds). P. 421-449. Academic Press, New York.
[12] Shab'an A et al 1993 Pesticides Dar Al Kutub for Printing and Publishing, University of Mosul, 520 pages.
[13] Al-Hakem A M 2009 Histological and Biological study on citrus Nematode *Tylenchulus semipenetrans* Cobb, 1913. With some methods of it’s control. Ph.D. Thesis , College of Agriculture and Forestry , University of Mosul.
[14] Soltani T et al 2013 Chemical Control of Root Knot Nematode (*Meloidogyne javanica*) on Olive in the Greenhouse Conditions . *Journal of Plant Pathology and Microbiology* 4(6) 1- 4.
[15] Radwan M A et al 2004 Management of *Meloidogyne incognita* root – knot nematode by interaction of *Bacillus thuringiensis* with other organic amendments or carbofuran . *Pak.J.Nematol.* 22 135 – 142.
[16] Abd-Elgawad M M M et al 2006 Efficacy of selected bio - control agents on *Meloidogyne incognita* on eggplant . *Nematol. Medit.* 34 105 - 109.
[17] Khalil Ashraf Al-Saeed 2012 Integrated control of nematode diseases affecting vegetable and fruit crops. Agriculture Research Center. Plant Pathology Research Center./ https://hamzehammada.wordpress.com

[18] Maina Y T et al 2012 The Use of Organic Manure in The Management of Plant Parasitic Nematode in Nigeria. Journal of Environmental Issue and Agriculture in Developing Countries 4 54-64.

[19] Renco M 2013 Organic amendments of soil as useful tools of plant parasitic nematodes control. Helminthologia. 50(1) 3-14

[20] Al-Jubouri F K D 2013 Molecular and immunological diagnosis of the virus. PhD thesis, Faculty of Agriculture and Forestry, University of Mosul.

[21] Lazarovits G et al 2001 Organic Amendments as a Disease Control Strategy for Soil Borne Disease of High Value Agricultural Crops. Australasian Plant Pathology 30 111.

[22] Kerkeni A et al 2011 Suppression of the root knot nematode Meloidogyne incognita on tomato by composted animal manures. The African Journal of Plant Science and Biotechnology 5(1) 60-62.

[23] Uko A E et al 2013 Effect of poultry manure and plant spacing on the growth and yield of waterleaf (Talinum fructicosum (L.) Juss). Journal of Agronomy 12(3) 146-152.

[24] Ruhollah N et al 2016 The Effect of composted municipal waste, sheep manure and urea nitrogen on the growth and chemical composition of Tow Rapeseed Cultivars. Journal of Plant Nutrition. 39(9) 1328-1335.

[25] Sharon E et al 2001 Biological Control of the Root Knot Nematode Meloidogyne javanica by Trichoderma harzianum. Phytopathology 91(7) 687-693.

[26] Abdul-rasool A M and Ibrahim B Y 2011 Effect of treatment with Trichoderma spp on the level of Peroxidase, Polyphenoloxidase and Chitinase in Cowpea plant Vigna unguiculata effected by root-knot nematodes Meloidogyne javanica. Mesopotamia Journal of Agriculture 39(4) 257-265.

[27] Sharf R and Hisamuddin 2016 Potential for Biological Control of Nematode by Trichoderma spp. and Its Effect on Growth and Yield of Phaseolus vulgaris. World Journal of Pharmaceutical Research 5(6) 1044-1064.

[28] Reddy T Y and Reddi G H 1995 Principles of agronomy. 2nd Edition, Kalayani Publisher, New Delhi, India, 223 pages.

[29] Al-Hazmi A S and Javeed M T 2016 Effect of Different Inoculum Densities of Trichoderma harzianum and Trichoderma viride Against Meloidogyne javanica on Tomato. Saudi J. Biol.Sci. 23 288-292.