

Effect of three different inoculum levels of root knot nematode *Meloidogyne* spp. in infection criteria and some growth traits of cucumber (*Cucumis sativus* L.)

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Received:	Abstract
Aug. 31, 2022	The study was conducted to find out the effect of three different
1108.01, 2022	levels of root-knot nematode inoculum on cucumber plants. An
	increase in infection criteria was observed when levels of contam-
	ination increased, as the highest number of root-knots, number of
Accepted:	females, egg masses, root-knot index and soft root weight on cu-
	cumber plants when contaminated with 2000 eggs were recorded.
Sept.10, 2022	/ pot, as it reached 26.33 knots. 2 g/root, 23.00 female and juvenile,
	2 g/root, 19.00 egg mass, 2 g/root, 3 root knot index and 11.33
	g/plant of soft root weight, respectively, while a gradual decrease
	in growth characteristics was observed. With an increase in the
Published:	levels of contamination in the lengths of the shoot and root, the
	vegetative fresh weight and the number of branches of the plant,
Sept. 20, 2022	the lowest values were recorded in the treatment of contamination
	with 2000 eggs/pot, which reached 25.33 cm, 15.00 cm, 16.33 g,
	and 5.66 branches/plant for each of them, respectively compared
	with in the treatment of control.
	Keywords: Meloidogyne spp., Different inoculums, cucumber.

Introduction

Cucumis sativus L. is one of the plants of the cucurbit family. It is a vegetable crop commonly grown in Iraq, in native to semi-tropical areas with moderate humidity. The importance of the crop lies in developing countries and due to the high demand for its consumption, it is grown all year round, Iraq's production of the cucumber crop in 2020 amounted to more than 242.6 thousand tons [1].

Cucumber plants using as index to many important plant diseases due to the sensitivity of the cucumber plant, including those affecting the root system, foremost of which are diseases caused by soil organisms, Soil-Borne pathogens, which are among the most dangerous pathogens, including fungi, led by *Pythium*, *Fusarium* and *Rhizoctonia*, in addition to plant-pathogenic nematodes, which are the most dangerous to the plant. Root knot worms, especially the common species of the genus *Meloidogyne*, which is one of the most important and widespread species in the world, as it affects all types of cultivated crops and in various agricultural lands [2]. Many studies have been conducted to find the relationship between nematode numbers and the amount of damage they cause to plant growth and productivity by



conducting infection at different levels (few, medium and high) of them and comparing with infection-free soil in the greenhouse, while in the field, infection is carried out with different levels of nematodes after Treating the soil to get rid of nematodes in it or conducting an experiment with different levels of nematodes in the originally contaminated field soil. [3,4]. found that there is an inverse relationship between the levels of contamination with nematode pollen and plant growth and productivity. When using high levels of nematodes The results of a study of four different levels of nematode inoculum for *M. incognita* (100, 1000, 5000 and 10000) second instars on four cultivars of bananas in Saray, Grand Nani, Maghribi and Williams There was a significant increase in the final densities of nematode numbers with the increase in contamination levels, while the reproductive factor was inversely proportional to the contamination levels and the percentage of low growth increased With increasing levels of contamination [5].

Another scientist [6] concluded that the different levels of root-knot nematode *M.incognita* (500, 1000, and 2000 second-stage juveniles. kg soil-1) negatively affected the growth characteristics of eggplant plants, as they recorded a decrease in root length and total vegetative and shoot weight, an increase in the fresh weight of the root system and the number of nodes on the root. While in a previous study [7] the effect of different levels of *M.paranaensis* inoculum (0, 500, 1,500, 3000, 5000 and 8000 eggs/plant) on three coffee cultivars were found to increase in the number of eggs and juveniles of the second instar was recorded with Increased levels of pollution. Therefore, This study was conducted to study different levels of plant pathogenic nematode pollen to identify its damage to cucumber plants in some growth traits.

Materials and Methods Prepare the soil and seeds

The experiment was carried out in one of the greenhouses, of the College of Agriculture, University of Karbala, during the agricultural season 2020-2021. The soil mixture was sterilized in a ratio of 1 soil: 2 with moss in an Autoclave at a temperature of 121°C and a pressure of 15 pounds/inch 2 for 60 minutes [8]. The sterilized soil was transferred to the greenhouse and distributed in plastic containers of a capacity of 2 kg. 4 seeds of cucumber plant DERVISH (225) F1 were planted in potting soil and irrigated with tap water.

Configuring the source of inoculum

Egg masses were collected from the roots of cucumber plants for the previous season from one of the greenhouses adjacent to the experiment site, and the highly infected plants that showed symptoms of nematode infection were selected [9]. The roots containing the knots were transported in nylon bags to the laboratory, then washed with water to get rid of the suspended lumps of clay and cut into small pieces 1-2 cm, and examined under the microscope to ensure the presence of eggs, 3 different



levels of egg mass were used in contamination Sterilized potting soil with the control treatment represented by healthy plants not contaminated with nematodes at the rate of (4) replications for each treatment after two weeks from the date of planting, after making holes in the potting soil with a depth of 3 cm at a distance of 2-3 cm around the stems of the growing plants and covered with moist soil.

The experiment included the following treatments:

- 1- Control (Cucumber plants without polluting)
- 2- Cucumber plants are contaminated with a rate of 500 ± 10 eggs/pot
- 3- Cucumber plants are contaminated with a rate of $1000 \pm 10 \text{ eggs/pot}$
- 4- Cucumber plants are contaminated with a rate of 2000 ± 10 eggs/pot

The plants were left for 60 days from the start of contamination to ensure the reproduction of nematodes to study the infection criteria and their impact on plant growth, taking into account watering when needed. Then the plants were uprooted, and the following was calculated:

1: Pathological characteristics:

Number of nodules 2 g/root, number of females and juveniles. 2 g/root, number of egg masses laid. 2g/root, number of eggs within one egg mass and the five-degree root knot index 1 = no nodes on the roots, 2 = nodes on the root 25 %, 3 = root nodes 50%, 4 = root nodes 75%, 5 = root nodes 100% [10].

2: Growth characteristics of the plant

Vegetative length in cm/plant, root length in cm/plant, fresh weight of vegetative group and fresh weight of root system in gm/plant, number of branches/plant According to [11].

Statistical Analysis

A Complete Randomized Design (CRD) was applied in the experiment, and the averages were tested using Duncan's Multiple Range at a probability level of 0.05 using the SAS program for statistical analysis [12].

Results and Discussion

Effect of different levels of root knot nematode inoculum on pathogenicity characteristics

Results in Table (1) showed that the highest number of knots was recorded when polluting seedlings with 2000 eggs/pot, as the number of knots, females, egg masses and root-knot index reached 26.33 knots. 2 g/root and 3 root knot index respectively for the mentioned traits followed by the contaminated seedlings treatment with 1000 and 500 eggs/pot, while the control treatment (unpolluted seedlings) recorded 0.00 except for the root knot index which recorded 1.00 with significant differences with the rest of the treatments.



Table (1): Effect of different levels of nematode inoculum on some infection criteria.

Treatments	Number of nodules 2 g/root	number of females and juveniles. 2 g/root	number of egg mass 2g/root	Knot in- dex	Number of egg inside egg mass
Control	0.00 c	0.00 c	0.00 c	1.00 c	0.00 b
plants con-					
taminated	16.00	14.00	11.00	2.00	275.00
with 500	b	b	b	b	a
egg/pot					
plants con-					
taminated	19.33	17.00	12.00	2.00	260.00
with 1000	b	b	b	b	a
egg/pot					
plants con-					
taminated	26.33	23.00	19.00	3.00	260.00
with 2000	a	a	a	a	a
egg/pot					

^{*} Similar letters within the same column are not significantly different from each other according to Duncan's test at the 0.05 probability level.

The number of eggs within one egg mass in all treatments results did not significant differences except for the comparison treatment (uncontaminated seedlings), which differed significantly from the rest of the treatments and recorded 0.0. The results of Table (1) are attributed to the fact that with the increase in the levels of contamination, the number of nematodes entering the root increased, and thus the nematodes were able to penetrate the roots and their entry was increased with the increase in the level of contamination. To provide it with food to complete its life cycle and development, and thus the mature females were able to lay egg masses on the root system of the plant. The increase in nematodes entry with increased levels of contamination led to an increase in the number of females and juveniles on the root system and an increase in the number of egg masses laid, thus increasing the number of nodes on the root total and the formation of nodes came As a natural reaction to the plant as a result of the entry of nematodes and feeding on its cells, where root-knot nematodes work to increase the number of hyperplasia cells surrounding the nematode head as a result of the accumulation of cytokinins and increase the size of

^{*}Each value in the table represents an average of three replicates.



hypertrophy cells as a result of the accumulation of oxins forming giant cells that appear in the form of knots on the root system [2]. When the level of pollution increased, a logarithmic increase was observed between the two levels contamination and nematode densities on plants, and this is in agreement with previous studies by [13,14].

Effect of different levels of root knot nematode inoculum on some growth characteristics of cucumber plants:

Table (2) recorded the effect of root knot nematode on some growth traits of cucumber, it appears, the significant effect of root knot nematode contamination levels on growth traits through a gradual decrease in vegetative and root lengths and the fresh weight of the vegetative group of plants in conjunction with an increase in the level of contamination, recording the highest values in the treatment The comparison (healthy plants not contaminated with nematodes), which reached 45 cm, 21 cm and 25 g, then decreased to 25.33 cm, 15.00 cm and 16.33 g when contaminating plants with 2000 eggs/pot, respectively, for the above-mentioned traits and differed significantly, as well as recorded differences Significantly between the comparison treatment and each of the two contaminating treatments were 500 eggs/pot and 1000 eggs/pot, respectively, in the aforementioned traits.

Table (2): Effect of different levels of root-knot nematode inoculum on growth characteristics

Treatments	Length of plant shoot (cm)	Length of plant root (cm)	weight of plant shoot (g)	weight of plant root (g)	No. of branche s/plant
Control	45.00 a	21.00 a	25.00 a	9.00 c	7 a
plants contami- nated with 500 egg/pot	38.66 b	18.66 b	23.00 b	10.00 b	6 ab
plants contami- nated with 1000 egg/pot	33.66 c	17.50 b	20.00 b	10.33 b	6 ab
plants contami- nated with 2000 egg/pot	25.33 c	15.00 c	16.33 c	11.33 a	5.66 ab

^{*} Similar letters within the same column are not significantly different from each other according to Duncan's test at the 0.05 probability level.

^{*}Each value in the table represents an average of three replicates.



Results also indicated that there is no significant difference among different treatment in branches of single plant, the root knot nematode had little effect on the number of branches, and the study period may not have been sufficient to show its effect on the number of branches.

The reason for the decrease in the values of the length of the vegetative and root system and the fresh weight of the vegetative total of the plant may be attributed to the formation or formation of giant cells around the vascular cylinder of the root that the nematode uses as its feeding cells. Water and nutrients from the soil lead to a decrease in the amount of water and nutrients that reach the vegetative total and this was reflected in the values of the length of the vegetative and root system and the fresh weight of the vegetative total of the plant. This result was identical to the results of previous studies [14,15,16,17].

The decrease in the concentration of nitrogen and protein and the amount of chlorophyll in the leaves with the increase in pollution levels leads to damage to the root system, and thus the efficiency of the root to absorb water and nutrients needed by the plant, including N, Mg, Fe, Cu, B and Zn, is an inevitable result caused by the effect of a complex nematode. Roots on the root system Many researches indicated the deficiency of these nutrients when infected with root-knot nematodes due to the lack of absorption by the plant [13,18,19].

Water is an important element in influencing plant growth, and as we mentioned earlier, nematodes affect the efficiency of the root in absorbing water, and this in turn leads to reducing the swelling of the guard cells in the leaves as a result of water tension, thus closing the stomata and reducing the amount of CO₂ entering the cells, which is very necessary in building chlorophyll as well. On the consumption of nutrients and energy needed for plant growth by nematodes [20].

References

- 1) Agricultural Statistics Directorate, (2020). Vegetable production report. Central Statistical Organization. Ministry of Planning, Iraq.
- **2)** Abu-Gharbieh, W.I., Ahmed, S.A., Zuhair, A.S., and Ahmed, A.D. (2010). Plant Nematodes in Arab Countries, Part One. First Edition, Darwael Publishing, Amman ,Jordan.586pp.
- **3**) Kinloch, R.A. (1982). The relationship between soil populations of *Meloidogyne incognita* and yield reduction of soybean in the coastal plain. J. Nematol. 14: 162-167.



- **4)** Al-Saadi, H.A.M. (1985). A Study of The Root Knot Nematode Disease Caused By *Meloidogyne* spp. on Eggplant in Iraq. Master Thesis, College of Agriculture, Baghdad University, Iraq.
- **5**) Kheir, A.M., Amin, A.W., Hendy, H.H. and Mostafa, M.S. (2004). Effect of Different Inoculum Levels of *Meloidogyne* on Nematode Reproduction and Host Respone of Four Banana Cultivars Under Greenhouse Condition. Arab Journal Plant Protection 22:97-102.
- 6) Hussain, M.A., Iram, M. T., Muhammed, N.A. and Muhammad, Z.K. (2015). Effect of Inoculum Density of Root Knot Nematode *Meloidogyne incognita* on Damage Potential in Eggplant. Research Article 13(1):33-36.
- 7) Andreazi, E., Gustavo, H.S., Ricardo, T.F., Tumoru, S., Ines, C.B., Anderessa, C.Z., Luciana, H.S., Filipe, G.C. and Fernando, C.C. (2015). Behavior of IPR 100 and Apoata IAC 2258 Coffee Cultivars Under Different Infestation Level of *M.paranaensis* Inoculum. Astralian Journal of Crop Science 9(11):1069-1074.
- **8**) Panhwar, Q.A., Radziah, O., Sariah, M. and Razi Ismail, M.(2009). Solubilization of Different phosphorus forms by phosphate Solubilizing Bacteria Isolated from Aerobic Rice. International Journal of Agriculture and Biology, 11(6):667-673.
- **9)** Kampfer, P. (2006). The family Streptomycetaceae, partI: taxonomy The Prokaryotes, 538-604.
- **10**) Dube, B. and Smart, G. C. J.(1987). Biological control of *Meloidogyne* incognita by Paecilomyces lilacinus and pasturia penetrans. J. Nematol., 9: 222-227.
- **11**) Al-Obadi, A.K.I. (2006). Resistace Mechanism of Some Pear Varieties To The Infection By Pear Lace Bug *Stephanitis pyri* (F.) (Tingidae: Hemiptera). Ph.D. Thesis College of Agriculture and Forestry, University of Mousl. Iraq.
- **12**) S.A.S. (2012). Statistical analysis system, users guide. Statistical. Version 9,1 nd SAS. Instituted Inc.Cary, N.C. USA.
- **13**) Abbasi, and Hisamuddin (2014). Effect of different inoculum levels of *Meloidogyne incognita* on growth and Biochemical Parameters of *Vinga radiate*. Asian Journal of Nematology 3(1):15-20.
- **14**) Jashanshahi, Afshar F., Sasanelli, N., Hosseininejad, S.A. and Tanha, Z. (2014). Effect of the root knot nematodes *Meloidogyne incognita* and *M.javanica* on Olive plants growth in glass house conditions. Helminthologia 51(1):46-52.
- **15**) 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 Journal of Biological Science 23: 288-292.
- **16**) Kankam, F. and Adomako, J. (2014). Influence of inoculum levels of root knot nematode (*Meloidogyne* spp.) on tomato (*Solanum lycopersicum* L.) Asian Journal of Agriculture and Food Science 2(Issue 2): 171-178.
- **17**) 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.



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- **18**) Giallaud, M.C., Dubreuil, G., Quentin, M., Perfus-Barbeoch, L. and Lecomte, P. (2008). Root Knot nematode Manipulate Plant Cell Functions During A Compatible Interaction. Journal of Plant Physiology, 165: 104-113.
- **19**) Korayem, A.M., Mohammed, M.M. and Abou-Hussein, S.D. (2013). Damage thershold of root knot nematode *Meloidogyne arenaria* on peanut in relation to date of planting and irrigation system. Canadian Journal of Plant Pathology 1:117:127.
- **20**) Al-Hakim, A.M.A. (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 Mousl. Iraq.