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# Plant-parasitic nematodes affecting vegetable crops in greenhouses

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# ABSTRACT

This work focuses on investigating plant-parasitic nematodes that affect greenhouse vegetables. The study took place in the Rostov region (Russian Federation) between May 2019 and May 2020 and involved 180 samples of soil and roots of 30 different vegetables in the families *Cucurbitaceae* (6), *Solanaceae* (8), *Umbelliferae* (8), *Lamiaceae* (4) and *Allioideae* (4) from 20 intensive farming locations. In this study, 11 nematode genera were detected. The most common genus was Meloidogyne, followed by Helicotylenchus, Pratylenchus, and Scutellonema. The highest Meloidogyne densities were detected in cucumbers, green peppers, carrots, eggplants, basil, and celery. Onions were not infected with Meloidogyne at all. Plant diseases caused by Pratylenchus, Scutellonema and Helicotylenchus were present in 29.7%, 51.5% and 81.6% of all crops examined, respectively. Xiphinema were found exclusively in carrots and celery, while Ditylenchus were only present in tomatoes and carrots (for each, the prevalence was 2.1%). The relative abundance of Meloidogyne, Helicotylenchus, and Pratylenchus was 58.3%, 10.4%, and 2.1%, respectively. As regards other genera, the relative abundance was less than 1%. The results show that soil properties are as important for the abundance, distribution and structure of the plant-parasitic nematode communities as the host plant. Findings may be helpful in improving the vegetable pest controls. © 2021 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access

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## 1. Introduction

Nematodes are microscopic worms that cause billions of dollars in crop losses annually, and all crops in the world are susceptible to at least one species of nematode parasites (Bozbuga et al., 2018). Nematodes are among the most important and abundant animals in the animal kingdom and are able to survive in any environment (Aleuy and Kutz, 2020; McSorley, 2003). Plant-parasitic nematodes are the major biotic stressor in crop cultivation (Treonis et al., 2018). Plant-parasitic nematodes are called plant-parasitic because nutrients they receive come directly from plants. They have a needle-like structure called a stylet that helps them to pierce the plant cell walls and reach the juicy contents on which they feed

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(Bernard et al., 2017). Correct identification of nematode species is essential for choosing the proper methods of control. The morphology-based taxonomy of nematodes is complex due to intraspecific variation, but the tools and techniques based on biochemical and molecular markers can successfully diagnose a wide range of nematode species (Carneiro et al., 2017). The economic consequences of crop losses caused by nematodes come in many variations and are associated with a decrease in the crop quality and yield. The assessment of such losses and periodic updates of these estimates may be of great use to set research priorities. In addition to that, it can serve as a benchmark for policy planners/funding agencies to support research in this field, as well as for public/private sector to make appropriate investments in management (Kumar et al., 2020).

Plant-parasitic nematodes are recognized as one of the greatest threats to crops worldwide. It has been found that nematodes, alone or in combination with other soil microorganisms, infect almost every part of the plant, including roots, stems, leaves, fruits and seeds. Although worldwide recognition of nematodes as important cause of plant diseases did not occur until the middle of the last century, nematodes were studied more than 150 years earlier. The most damaging plant-parasitic nematodes are

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considered to be the root-knot nematodes (RKNs), *Meloidogyne* sp. (Almohithef et al., 2018), which are responsible for losses in vegetable crops throughout the world and determine the common use of chemical pesticides (Sikandar et al., 2020). In Spain, RKNs can be found in all horticulture areas (Archidona-Yuste et al., 2018), causing yield losses in essential vegetable crops, such as cucumber (85%), tomato (59%), zucchini (40%), watermelon 36%, and lettuce (29%) (Gullino et al., 2019). RKNs can also be seen posing a threat to vegetable production in other European and transcontinental countries such as Turkey, Slovenia, Brazil, and Greece. In Turkey, for example, they can attack numerous higher plants, including monocotyledonous, dicotyledonous, herbaceous, and woody plants, all thanks to it wide host range (Aydınlı et al., 2013).

The most popular methods to control nematodes include soil fumigants and nematicides (Giannakou and Panopoulou, 2019). However, in order to preserve the beneficial soil organisms, it is necessary to use alternative ways of neutralizing parasitic nematodes. A sustainable production system uses environmentally friendly alternatives to maintain and improve the health of antagonist organisms. Soils with high antagonistic potential lead to the suppression of soil-borne pathogens. In suppressive soil, pathogens do not form, persist or establish, but cause little or no damage at all (Topalovic et al., 2020). Suppressive soils have already been described for many soil-borne pathogens, including plantparasitic nematodes (Romanenko et al., 2008). Soils that suppress the development of nematodes have been intensively studied over the past several years, but the mechanisms of suppression are not entirely understood. (Ghini and Morandi, 2006) summarized the biotic and abiotic factors associated with soil suppression. Among them; soil microbiota plays an important role (Topalovic et al., 2020) in maintaining biological productivity (Dutta et al., 2019).

Nematode infection in soil can lead to secondary infection with fungal and bacterial pathogens and even to the transmission of plant-infecting viruses, which negatively affect the yield. In the presence of plant-parasitic nematodes, the drop in the yield of vegetables can sometimes reach 29% for susceptible genotypes (Sabeh et al., 2019). Note that vegetable crops are usually among the most susceptible to nematodes. When colonizing the roots, nematodes alter the uptake of water and nutrients and inhibit the translocation of minerals. Such alterations can affect the shoot-to-root (S/R) ratio, leading to poor plant growth (Gullino et al., 2019).

The role of nematodes in limiting vegetable production largely depends on the farming system used, on the physical and/or chemical environment in the soil, and on the climate (Moura and Franzener, 2017; Treonis and Wall, 2005) reported that the strength of the relationship between nematode community and soil type varies with nematode species and that nematode reproduction was positively correlated with relative humidity and negatively correlated with air temperature. In addition to that; they found that soil texture is the most important factor explaining the presence of some nematode species and that environmental factors affect the ability of nematodes to parasitize and reproduce on their host.

The greenhouse cultivation of vegetables often involves utilizing the plastic mulches. This cultivation system is mainly used to cultivate tomato, eggplant, cabbage, pumpkin, squash, cucumber, watermelon, melon, and bell pepper. However, intensive cultivation of vegetables on plastic mulch can increase the risk of problems with soil pests and pathogens, including plant-parasitic nematodes (Perry, 2006). This is especially important in a second or third crop grown on the same plastic mulch because reusing the mulch promotes the accumulation of populations of these pathogens in the soil. In fact, crop damage is often increased when two vegetable crops are grown each year. Many root diseases, including those caused by plant-parasitic nematodes, thus become widespread in the soil and interfere with vegetable production.

Identifying new or potentially harmful nematode species is essential to agricultural success and helps to design and evaluate measures aimed at minimizing their distribution. As the world travel and transport of plants increases, the need to monitor the movement of destructive nematodes increases. Accurate identification of nematode species is a foundation of effective nematode control and successful plant quarantine (Dutta et al., 2019). Employment of preventive procedures allows for the avoidance of parasitic nematodes and cost-effective minimization of crop losses (Del Prado-Vera et al., 2018). The importation of exotic plants and the spread of local quarantine nematodes are prevented through the implementation of stringent measures such as import and domestic quarantine controls, certification schemes, pest risk analyses, and clean treatment procedures in diagnostic and research laboratories (Knoetze et al., 2017).

The common symptoms of nematode infection are the root growth reduction, increased wilting, mineral deficiency-like symptoms, decreased winter hardiness, and dieback in perennial plants. Although these symptoms are related to root impairment, some species of nematodes (*Anguina* spp.) can also affect other parts of the plant, leading to the production of galls on seeds, leaves, and stems (Kumar et al., 2020). By definition, the seed gall nematodes produce seed galls, whereas the stem gall nematodes cause swelling and distortion of stems, and the leaf nematodes cause leaf discoloration. However, the terrestrial symptoms of nematode infection are predominantly indirect and rather subtle; among them are decreased energy, stunted growth, reduced yield, and leaf chlorosis.

Given the variation in symptoms of nematode infection, the differences that exist between species, and the lack of a complete description of plant-parasitic nematodes, the present paper seeks to investigate the diversity and population density of plantparasitic nematodes that attack greenhouse vegetables in different agroecological zones. The findings of the study may serve as a framework for improving vegetable pest control methods to minimize crop losses.

# 1.1. Problem statement

Nematodes parasitizing on greenhouse vegetable crops is an acute problem, considering the possible economic losses they may cause. Among all the nematodes known to science, some species exhibit high parasitic activity, thereby posing serious danger. When it comes to distribution, however, even these species are dependent upon many factors, among which the most important ones are the host plant and the soil properties. This study supposes that soil moisture content can indirectly affect the distribution of parasitic nematodes among different species of cultivated plants, as well as the health of these plants. In this regard, it is important to identify the species diversity of nematodes in the studied soil and cultivated greenhouse vegetables in order to obtain useful information that may be helpful in improving the existing vegetable pest controls. Therefore, this study aims to investigate the diversity and population density of plant-parasitic nematodes that attack greenhouse vegetables in different agroecological zones. The **objectives** of the study are as follows:

- testing soil and root samples taken under and from the most common vegetables grown in a greenhouse for the presence of parasitic nematodes;
- (2) determining the taxonomic diversity of the plant-parasitic nematodes and their quantitative characteristics;
- (3) finding a relationship between nematodes and vegetable crops, as well as the most infected crops;

- (4) establishing a relationship between soils and prevailing nematodes;
- (5) identifying the most common plant-parasitic nematodes and their population densities for further research.

# 2. Methods and materials

# 2.1. Sampling

Soil and root samples were taken from 20 intensive vegetable farming areas between May 2019 and May 2020 in the Rostov Region (Fig. 1). The climatic conditions of this region did not change substantially during the study period, which means there is no dependence between nematode distribution and temperature swing.

These locations were selected based on their level of importance for vegetable production, the variability in vegetables being cultivated and their geographic distribution. The geographic coordinates of each location were determined using the Global Positioning System (GPS). A total of 180 soil samples and 180 root samples from 30 different vegetable crops were taken. In each location, there were 3 beds per crop and 5 plants per bed randomly selected. All plants were rooted out with a garden trowel (Usha et al., 2015). Samples were placed onto separate root and soil structures (at least 1 kg per structure) and stored in plastic bags until nematodes were removed.

#### 2.2. Extraction of nematode

Nematodes were extracted separately from roots and soil for each of the collected samples. The roots were gently washed to remove as much soil as possible and then cut into pieces of about 0.6 cm. Nematodes were removed from the 10-gram root sample using the modified Berman method for 48 h (Hernandez-Chavarria and Avendano, 2001). Nematodes were also extracted from 60 g soil per sample using the same extraction method. The



Fig. 1. Sites of research in the Rostov region (2019/2020).

The suspensions of nematodes were collected in beakers, allowed to settle for 2 h, and the supernatant was separated out. The final volume (about 2 ml per suspension) of the supernatant was poured into a 10 ml tube and mixed with hot (65 °C) 4% formalin. The tubes were kept in a refrigerator at 4 °C until nematodes were identified and their population density assessed. Endoparasitic nematodes were examined on a small amount of plant tissue using a stereoscopic microscope (15 × magnification) with transmitted or incident light. All assays were carried out in triplicate.

#### 2.3. Statistical analysis

Data on the population density of nematodes for ANOVA (Analysis of Variance) analysis were normalized by converting them to log10 (De Smith, 2018). The nematode density was assessed separately for each sample and the mean population density, defined as the sum of nematode populations per gram of roots and per gram of soil, divided in half, was identified. This was done to make the presentation of results easier, and it was possible to calculate the mean density because all nematodes that were detected were endoparasitic. Data were compared using the Multivariate Analysis of Variance (MANOVA) in Microsoft Excel and Statistica 10. Differences were considered significant at  $P \leq 0.05$  (according to Student's *t*-test). The relationship between the nematode population density and the physicochemical properties of the soil was assessed through correlation analysis using the SAS software for the Windows operating system.

# 3. Results

# 3.1. Estimation of nematode population density and qualitative analysis

The total number of samples collected per crop varied depending on the crop. Plant parasites of 11 genera have been encountered, namely: *Scutellonema*, *Helicotylenchus*, *Aphelenchoides*, *Hemicriconemoides*, *Ditylenchus*, *Meloidogyne*, *Rotylenchulus*, *Xiphinema*, *Quinisulcius*, *Pratylenchus* and *Tylenchulus*. *Meloidogyne* was the most densely populated genus with the average population density of 50 nematodes per gram of sample volume, followed by *Helicotylenchus* (5 nematodes per gram), *Pratylenchus* (4 nematodes per gram) and *Scutellonema* (2 nematodes per gram). As for other nematode species, there was less than 1 nematode per gram detected in the sample. As can be seen, the population density of the first 4 nematode species was higher (P  $\leq$  0.05). The highest population densities of *Meloidogyne* sp. were recorded in cucumbers, green peppers, carrots, eggplant, basil, and celery in all repetitions.

Investigation involved greenhouse vegetables in the families *Cucurbitaceae* (6), *Solanaceae* (8), *Umbelliferae* (8), *Lamiaceae* (4) and *Allioideae* (4). Among all vegetables examined, onion was the only vegetable that was one not infected with *Meloidogyne*. Yet, there were nematodes of other genera detected (Table 1), namely *Pratylenchus* (29.7% of the crops sampled), *Scutellonema* (51.5%) and *Helicotylenchus* (81.6%).

Nematodes of the genera *Ditylenchus, Xiphinema* and *Tylenchulus* were extracted from two cereals each and amounted to 8.1% of all crops sampled. All crops under study, barring parsley, onion and cucumber, were hosts to mixed populations of nematodes, with 9 species found on tomato and a hot peppers. Genera *Hemicriconemoides, Aphelenchoides, Ditylenchus, Quinisulcius, and Scutellonema* are uncommon for the Rostov region. During the

	Ditylenchus	8.1	±0.8	
Percentage of greenhouse vegetables infected with parasitic nematodes (detected in the period from May 2019 to May 2020).	Tylenchulus	8.1	±0.7	
	Scutellonema	51.5	±5.0	
	Aphelenchoides	62.7	±5.9	
	Rotylenculus	30.2	±3.2	
	Quinisulcius	30.2	±2.8	
	Meloidogyne	95.7	±10.1	
	Xiphinema	8.1	±0.7	
	Helicotylenchus	81.6	±8.5	
	Hemicriconemoides	30.2	±3.7	
	Pratylenchus	29.7	±2.3	
	Genus of the plant-parasitic nematodes Pratylenchus	Percentage of infected crops, %		

**Table** 

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experiment, it was found that the susceptibility of crops to these newly registered nematodes depends on the species of nematodes, and the infection level can range from 30.2% to 62.7%.

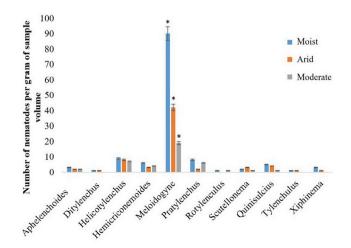
The number of samples (roots or soils) taken from the moist area, the arid area, and the moderate area was 88, 39 and 45, respectively (Fig. 2). It was found that wet soils are habitats to the greatest variety of plant-parasitic nematodes (11 nematode species). In arid and moderate zones, *Meloidogyne* had the highest population density (42 and 19 nematodes per gram of sample volume, respectively), followed by *Helicotylenchus* (8 and 7 nematodes per gram, respectively).

The population density of other nematode genera was significantly lower ( $P \le 0.05$ ). In the humid zone, *Meloidogyne* remained the most densely populated nematodes with 90 nematodes per gram of sample volume, whereas the population density of other genera was significantly lower (for example, the population density of *Helicotylenchus* was 9 nematodes per gram).

#### 3.2. Abundance and prevalence of plant-parasitic nematodes

According to the results of the assays, *Meloidogyne* was the most common genera encountered in the samples (80.9% of the 180 root and soil samples examined), whist the prevalence of the second common genus, *Helicotylenchus*, was approximately 69.8%. Note that other genera were present in less than 15% of the samples. Notably, *Xiphinema* (2.1%) were found on the roots of carrots and celery, and *Ditylenchus* (2.1%) were only present in tomatoes and carrots. The relative abundance of nematodes *Meloidogyne*, *Helicotylenchus*, and *Pratylenchus* was 58.3%, 10.4%, and 2.1%, respectively. For other genera, it is less than 1%. Although it was less common than *Helicotylenchus* and *Pratylenchus*, *Tylenchulus* caused the second highest level of infection (29.5%) after *Meloidogyne* (95.7%), higher than *Pratylenchus* and *Helicotylenchus* (19.8% and 16.5%, respectively).

The coefficients of correlation between the physicochemical properties of the soil and the population density of the 3 most common nematodes (in terms of population density, prevalence and relative abundance) are rather low (maximum value, r = 0.39). The organic matter to C/N ratio correlated neither with the population densities of individual nematode species, nor with the overall nematode density.



**Fig. 2.** Number of plant-parasitic nematodes per gram of the sample volume found in samples from different agroecological zones in the period from May 2019 to May 2020.\**the values are significant at*  $P \le 0.05$ .

# 4. Discussion

This study provides detailed information about the plantparasitic nematodes found in vegetable crops to expand the limited research in this field. (Seid et al., 2015) focused mainly on tomato; whereas (Reddy, 2008) focused on a broader range of crops; including eggplant, carrot, lettuce, okra and tomato, yet both studies dealt with just a few vegetables. It is usually assumed that the presence of the host plant is the main factor determining the population density of nematodes infecting on vegetable crops (Ali et al., 2018; Sabeh et al., 2019; Bozbuga et al., 2018) noted that most vegetable crops were registered as hosts for at least 1 nematode species. The current study supports this observation and shows not only the polyphagous status of many nematode species; but also the variability in host susceptibility (expressed as the percentage of infected crops and nematode population density, respectively). The most cultivated and consumed vegetable crops (Diab et al., 2019; Gomes et al., 2003) are good hosts for the 2 most common nematode genera, Meloidogyne and Helicotylenchus. Therefore, it is not surprising that these 2 genera are widelt distributed throughout the country. Among these genera, Meloidogyne was identified as the main systemic pest in agroecosystems, affecting 95.7% of the crops examined, with a population density of up to 50 nematodes per gram of sample volume. The dominance of Meloidogyne on vegetable crops was previously reported by other researchers (Almohithef et al., 2018; Altaibaeva et al., 2016), who observed that these nematode species are abundant in vegetable farming. This situation poses a serious threat to vegetable production worldwide and explain the widespread use of chemical pesticides on vegetable crops (Sikandar et al., 2020). Among the nearly 2000 genera of nematodes, which include 4100 phytoparasitic nematode species (Jones et al., 2013), the one most commonly seen is Meloidogyne. This observation aligns with the results of this study.

Among the greenhouse vegetables examined, onion was the only one uninfected with *Meloidogyne*. In addition, a low population density of *Meloidogyne* has been reported for bell peppers, green beans, parsley, green peppers, pumpkins, and parsnips. The low susceptibility of some vegetables (especially onions) to *Meloidogyne* seems to have been subconsciously exploited by vegetable growers in crop rotations as a tactic against this particular nematode (Pang et al., 2009). In some areas, however, parsley was found to be seriously infested with *Meloidogyne*, which suggests the existence of different *Meloidogyne* species and necessitates the deployment of alternative nematode management solutions (Dutta et al., 2019).

Several studies carried out on soils with different texture and chemical composition show how the physicochemical properties of soil affect the population density, distribution and structure of nematode communities. These findings support the assertion that nematodes can potentially act as bioindicators of soil quality (Moura and Franzener, 2017). In general, however, the effect of soil texture on nematode population density varies between the nematode species.

Nematodes living in the soil require an aerobic aquatic environment; therefore, particular attention should be paid to fluctuations in the soil moisture content. When investigating the effect of soil moisture on nematodes, the following should be considered: the soil phase (mineral composition and organic matter), the liquid phase (soil moisture content), the gaseous phase (soil air), and the nematode species. According to the results of the present study, moist soil environments have the highest population densities of nematodes, which aligns with the previous research (Treonis and Wall, 2005), and a slower growth of nematode numbers in arid conditions may be related to the exacerbation of moisture limitation (Bastow, 2020). The present study confirms that nematodes *Meloidogyne*, *Pratylenchus* and *Helicotylenchus* can potentially cause great damage to vegetables, indicating the need to pay more attention to these nematodes in order to increase the productivity of most vegetable crops grown in Russia. The findings obtained during the study may be helpful in developing appropriate strategies for integrated nematode management.

# 5. Conclusions

During the study on vegetable crops grown in greenhouses, 11 genera were detected, namely: Scutellonema, nematode Helicotylenchus, Aphelenchoides, Hemicriconemoides, Ditylenchus, Meloidogyne, Rotylenchulus, Xiphinema, Quinisulcius, Pratylenchus and Tylenchulus. Of these, Meloidogyne turned out to be the most common one with the average population density of 50 nematodes per gram of root volume, followed by Helicotylenchus (5 nematodes per gram), Pratylenchus (4 nematodes per gram) and Scutellonema (2 nematodes per gram). As for other nematode species, there was less than 1 nematode per gram detected in the sample. The highest Meloidogyne densities were observed in cucumbers, green peppers, carrots, eggplant, basil, and celery in all repetitions. Among the greenhouse vegetables examined, onion was the only vegetable without the Meloidogyne infection. Plant diseases caused by Pratylenchus, Scutellonema and Helicotylenchus were present in 29.7%, 51.5% and 81.6% of all crops examined, respectively. All crops under consideration (except for parsley, onions and cucumbers) were hosts of mixed populations of nematodes, with 9 species found on tomato and hot peppers.

Investigation of 180 root and soil samples revealed 81% of *Meloidogyne* infection cases, whilst the prevalence of *Helicoty-lenchus* was approximately 69.8%. The prevalence of other genera was less than 15%. *Xiphinema* was found exclusively in carrots and celery, whilst *Ditylenchus* was only detected in tomatoes and carrots (for each, the prevalence was 2.1%). The relative abundance of genera *Meloidogyne*, *Helicotylenchus*, and *Pratylenchus* was 58.3%, 10.4%, and 2.1%, respectively. For other genera encountered, it was less than 1%. These findings show that besides the host plant, soil properties also play an important role in the abundance, distribution and structure of plant-parasitic nematode communities. These findings may serve as a framework for further research aimed at the improvement of vegetable pest controls.

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Data Availability

Data will be available on request.

#### **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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