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IDENTIFICATION OF ALTERNATIVE MEASURES FOR THE MANAGEMENT OF ROOT-KNOT NEMATODES ON SOLANACEOUS VEGETABLE CROPS IN SOUTHWEST BULGARIA

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Abstract

Vegetables from the Solanaceae family (eggplant, tomato, and potato) are among the crops in Europe which in terms of production rank first, and in Bulgaria their production is concentrated in the south-western part. The aim of the study was to identify alternative root-knot nematode control measures applicable in integrated pest management to improve plant health and reduce dependence on chemical pesticides in Solanaceae vegetable production. Based on the data of new research, a summary list of specific combinations of vegetable crops/species of root-knot nematodes in Bulgaria has been compiled. After analysing the problems and according to the innovative practices, methods to control a given root-knot nematode species in a certain crop were indicated. The studies enabled an inventory of potential alternative measures for integrated management and the creation of a dataset that will allow for the improvement of plant health and the reduction of dependence on chemical pesticides in the production of Solanaceae vegetables.

Key words: *alternative measures, root-knot nematodes, Solanaceae vegetable.*

INTRODUCTION

The economic damage caused by plant parasitic nematodes worldwide is estimated at 12.3% (Hassan et al., 2013; Singh et al. 2015). In heavily infected areas, yield losses of more than 50% are observed, in some cases the crop can be destroyed (Nicol et al., 2011). In addition, the presence of nematodes limits the cultivation of several crops on infected areas. Root-knot nematodes (*Meloidogyne* spp.) are distributed throughout the world and belong to the phytonematodes of greatest economic importance, followed by representatives of the genus *Pratylenchus* and the genus *Heteroder*a (Ravichandra, 2014). Nematodes of the genus Meloidogyne Goldi 1877 are pests of economic importance for many crops grown in greenhouses and fields (Samaliev and Stoyanov, 2008; Mesa-Valle, et al. 2020). They are obligate, sedentary root parasites, polyphages. The most common species are *M. incognita*, *M. arenaria*, *M. javanica* and *M. hapla*, which develop several generations during one growing season and are characterized by high population density and dynamics. The host range of these species includes over 2000 plant species, and species from the Solanaceae family can be defined as the main hosts. Many of the world's economically important agricultural crops belong to the family Solanaceae (*Solanum*, *Capsicum* and *Nicotiana*) with about 28 million hectares under cultivation worldwide (Motti, 2021). Vegetables from the Solanaceae family are among the crops in Europe that rank first in terms of production and production area (Santamaria & Signore, 2021). In recent decades, the consumption of fruits and vegetables has increased, with increased demand for tomato, pepper, and eggplant belonging to the family Solanaceae (Motti, 2021). However, losses are a major problem at all stages from production to consumption. Current chemical nematicides are insufficiently effective and must be optimized for specific pests and crops. The requirements to produce fresh vegetables without residual pollutants increase the interest of growers in the problems related to control measures and managing the impact of biotic stress factors. New and expanding trends in agriculture require research to provide cost-effective and easy-to-use alternatives to conventional synthetic pesticides or to identify measures compatible with integrated programs to protect and minimize the application of chemical agents. Information in the literature regarding the influence of alternative control measures on the development of vegetable crops to overcome nematode damage is insufficient, there is a lack of specific data on vegetable production in Bulgaria.

The aim of the study was to identify alternative root-knot nematode control measures applicable in integrated pest management to improve plant health and reduce dependence on chemical pesticides in Solanaceae vegetable production.

MATERIALS AND METHODS

The results of the present work were obtained based on the analysis of the scientific literature on the considered problem, the grouping of the obtained data, the monitoring of established root-knot nematode/crop combinations and laboratory analysis of soil and plant samples to determine the species composition of nematodes. The route studies and observations were conducted in the period 2021-2022. In the regions of Sofia, Pernik, Kyustendil, Samokov, Blagoevgrad, Gotse Delchev and Pazardzhik, 22 observation points were selected, which were planted by tomatoes and potatoes during the growing season. According to preliminary information and data of the Bulgarian Agency for Food Safety, root-knot nematodes were found on some of these areas. Investigations of the presence and distribution of root-knot nematodes of genus *Meloidogyne* included greenhouses and field areas of tomatoes, aubergines and potatoes intended for consumption and processing. The leading factor in choosing the plots with potatoes was that they should be grown on agricultural plots where monoculture tobacco was grown in the recent past. Figure 1 shows the surveyed regions.

Figure 1. Regions in Southwest Bulgaria that were subject to root-knot nematode monitoring

RESULTS AND DISCUSSIONS

The following species of root-knot nematodes have been established in Bulgaria: southern root-nematode *Meloidogyne incognita* (Kofoid & White, 1919), peanut root knot nematode *Meloidogyne arenaria* (Neal, 1889), northern root-knot nematode *Meloidogyne hapla* Chitwood, 1949, *Meloidogyne javanica* Treub., 1885, Chitwood, 1949, thames' root-knot nematode *Meloidogyne thamesi* Chitwood, 1952. The cereal root-knot nematode *Meloidogyne naasi* Franklin, 1965 has not been established, but there are conditions for its development.

In Bulgaria in 1925, Chorbadjiev made the first reports of root-knot nematode damage to tobacco seedlings in the region of Shumen District. Later in 1940 Kovachevski found it on cucumbers in the village of Varbitsa, Gorna Oryahovitsa. Trifonova and Gospodinov (1955), Stoyanov (1962), Choleva (1973) and others reported intensive research on the bioecology of the species of the genus *Meloidogyne* (Mateeva, 2004).

The results for the identification of model combinations of vegetable crop/nematode species for the different regions of Bulgaria were presented in a summary list. The data from modern research on the species composition are reflected in Table 1.

The results of our tests confirmed the presence of nematodes of the genus *Meloidogyne* in the studied samples, which confirms their wide distribution of these pests in the region of Southwest Bulgaria. The species *Meloidogyne arenaria*, *Meloidogyne incognita* and *Meloidogyne javanica* were identified. Mixed infection (*M. arenaria* + *M. incognita*) was observed from the greenhouse samples. In the field samples, the dominant species was *M. arenaria*.

The review done here aims to evaluation the latest studies to alternative methods of root-knot nematode control. Table 2 lists those that are easily applicable and effective.

Table 1. Summary list of specific combinations vegetable crop/species of root-knot nematode in Bulgaria

Area	Species of root-knot nematode	Crop	Reference sources
Kresna	*Meloidogyne* spp.,	tomato	Markova L.et al. (2014)
Southern Bulgaria	*Meloidogyne* spp.,	tomato	Trifonova & Vulkova (2007); Voulkova & Trifonova (2009); Tringovska et al. (2015)
	M. arenaria	tomato	Choleva et al. (2005); Yankova et al. (2006); Baycheva et al. (2018)
	Meloidogyne spp.,	greenhouse vegetable crops	Choleva et al. (2004)
Southern Bulgaria	*Meloidogyne* spp.,	greenhouse vegetable crops	Samaliev (2009b)
	M. hapla	potatoes, tomatoes	Trifonova & Voulkova (2008); Markova & Samaliev (2011)
	M. javanica	aubergine	Mohamedova et al. (2016)
	M. incognita	cucumbers, tomatoes	Trifonova & Voulkova (2008); Trifonova & Vachev (2010); Panayotova et al. (2016)
	M. incognita, M. arenaria, M. javanica, M. hapla и *M. thamesi*	greenhouse vegetable crops and arable crops	Samaliev et al. (2018)
	M. arenaria, M. incognita, M. javanica and M. hapla, races 1 and 2 of *M. incognita* and race 1 and 2 of *M. arenaria*	greenhouse vegetable crops	Samaliev (2009a).
Plovdiv, Troyan and Samokov	*M. hapla, M. arenaria* and *M. incognita*	potato	Samaliev & Baicheva (2010); Samaliev & Kalinova (2013)
	M. incognita, M. arenaria and *M. hapla*	over 30 host plants	Stoyanov (1980).
	M. arenaria	cucumbers	Yankova, et al. (2014).

Table 2. Alternative methods for control of root-knot nematodes (*Meloidogyne* spp.) in major vegetable crops

Alternative control measure	Species of root-knot nematode	Crop	Reference sources
cultural practices	*Meloidogyne* spp.	host plants	Azlay et al. (2022)
solarization	*Meloidogyne* spp.	tomato	Yücel et al. (2007); Samaliev (2009b).
resistant varieties	*Meloidogyne* spp., *M. incognita*	tomato	Lizardo et al. (2022); Trifonova & Voulkova (2008); Trifonova & Vulkova (2007); Yankova et al. (2006)
sanitation, heat-based methods	*Meloidogyne* spp.	vegetable crops	Collange et al. (2011)
plant extracts and essential oils	*M. incognita; M. incognita* race 2, *M. hapla*	tomato, cucumber,	Abo-Elyousr et al. (2009); Abo-Elyousr et al. (2010); Adegbite (2011); Azlay et al. (2022); Mostafa et al. (2017); Salim et al. (2016); Taniwiryono et al. (2009); Taye et al. (2012); Trifonova & Atanasov (2009); Trifonova (2012)

Alternative control measure	**Species of root-knot nematode**	**Crop**	**Reference sources**
aqueous extracts of garlic and *Ricinus* seeds	*M. incognita*	tomato	El-Nagdi & Youssef (2013)
organic amendment	*M. incognita*	tomato	Asif et al. (2016); Zakaria et al. (2013)
biological, chitinous material, seashell meal	*M. incognita*	tomato	Ladner et al. (2008)
biological management/ nematophagous bacteria and fungi	*Meloidogyne* spp.	host plants	Azlay et al. (2022); Moreira et al. (2015); Singh et al. (2019); Zakaria et al. (2013)
bioproducts of microbial origin	*M. incognita*	tomatoes	Radwan et al. (2012)
commercial products contain *bacteria Bacillus firmus* and *Pasteuria penetrans*, and *Purpureocillium lilacinus* mushroom	*Meloidogyne* spp.	host plants	Lamovšek et al. (2013); Samaliev, H. (1997); Samaliev & Baycheva (2004).
Bacillus thuringiensis /crystal protein/	*Meloidogyne* spp.; *M. arenaria*	tomato	Li et al. (2007); Mohamedova (2009)
rhizobacteria *Bacillus subtilis Bacillus altitudinis* AMCC1040 *Pseudomonas oryzihabitans* and *Xenorhabdus nematophilus*	*M. javanica*	aubergine	Mohamedova et al. (2016)
	M. javanica	host plants	Samaliev et al. (2000)
Fungi antagonists, *Acremonium strictum* и *Trichoderma harzianum*	*Meloidogyne* spp., *M. incognita*	tomato	Goswami et al. (2008); Jindapunnapat et al. (2013); Singh et al. (2019); Trifonova & Vachev (2010)
Nematophagous fungi, *Pochonia chlamydosporia*	*Meloidogyne* spp., *M. arenaria, M. incognita*	organic vegetable production	Atkins et al. (2003); Sosnowska (2007); Trifonova (2014)
stimulating plant growth; rhizobacteria strains	*M. incognita*	tomato	Cetintas et al. (2018)
grafting root stock / *Solanum sisymbriifolium*, cucurbitaceous rootstocks	*M. incognita,* *Meloidogyne* spp.	tomato	Baidya et al. (2017)
cover and biofumigant crops	*Meloidogyne* spp.	tomato and potato	Daneel et al. (2018)
cover crops and green manure crops of the genus Brassica; companion plants	*Meloidogyne incognita,* *Meloidogyne javanica*	vegetable plasticulture	Monfort et al. (2007); Stirling & Stirling (2003); Tringovska et al. (2015)
antagonistic plants (sorghum, crotalaria, mucuna, guandu bean and neem	*M. javanica*, *M. enterolobii*, *Meloidogyne* spp., *M. arenaria*	host plants,	Moreira et al. (2015); Yasmin et al. (2003); Al Body & Mateeva (2007).
African marigold, *Tagetes erecta*	*M. incognita*, *M. arenaria*	tomato, greenhouse vegetable	Natarajan et al. (2006); Al Body & Mateeva (2007)

Alternative control measure	Species of root-knot nematode	Crop	Reference sources
Tagetes minuta, *Datura metel*, *D. stramonium* and *Ricinus communi*	*Meloidogyne* spp.	tomato	Oduor-Owino (2003)
abamectin	*Meloidogyne* spp.	tomato	Qiao et al. (2012);
Chlorella vulgaris /algae/ and potassium húmate	*M. arenaria*, *M. incognita*, *M. hapla*	greenhouse tomato	Choleva et al. (2007); Choleva et al. (2004)
fertilization, compost, fertilizers, biostimulators	*Meloidogyne* spp., *M. javanica, M. incognita*	greenhouse tomato	Saeedizadeh et al. (2020); Markova, L. et al. (2014)
poultry manure	*Meloidogyne* spp.	tomato	Chindo & Khan (1990)
silver nanoparticles	*Meloidogyne* spp.		Bernard Monfort et al. (2019);
selenium	*M. arenaria*	tomato	Baycheva et al. (2018)

After analysis of literature data, we found large variations between studies. Many practices were listed as only partially effective; therefore, combining control methods in integrated nematode management is a challenge that requires a systematic approach and identification of key future research. There is growing concern among vegetable growers as registered chemical nematicides decline. Alternative methods, means and techniques based on innovative practices are needed to solve the problem.

Despite the availability of effective alternative methods for root-knot nematode control, limited progress has been made in their implementation. In part, this may be due to the fact that the plant parasitic nematodes control has traditionally been carried out primarily by chemical means.

The trend worldwide for a chemical pesticide-free agricultural production and environment minimizes the use of pesticides (Lykogianni et al., 2021). The application of crop rotation is economically unprofitable for farmers, and the main part of greenhouse vegetables in Bulgaria is grown as a monoculture. The breeding of resistant varieties against these enemies is a difficult task (Samaliev & Stoyanov, 2008). This necessitates the search for alternative methods of control of *Meloidogyne* spp. In this aspect, among the non-chemical control measures is the biological method, especially in the context of integrated plant management. One of the many options for biological control is to specifically obtain and support or colonize biological active agents that suppress the development of plant parasitic nematodes. In this regard, the availability of different types of biological agents with potential for successful control of these enemies is of interest. Nematode antagonists can be various types of bacteria, fungi, viruses, rickettsia, predatory nematodes such as mites, collembolans (Volpiano et al., 2019). Trifonova et al. (2009) conducted mycological studies in the southern regions of Bulgaria and found that *Fusarium oxysporum*, *Verticillium chlamydosporium* and *Gliocladium roseum* parasitized the eggs of female individuals of the genus *Meloidogyne*, with 7.6% to 23.5% of the eggs in subsequent generations died. Of the previously known microorganisms with an antagonistic effect against Meloidogyne, spp. non-pathogenic bacterial strains have increasingly been used in recent years (Sidhu, 2018). The use of rhizobacteria to biologically control plant diseases and to stimulate plant growth has been practiced with great success (Shaikh & Sayyed, 2015; Verma, 2019). The search for suitable rhizobacteria against plant-pathogenic nematodes started about 30 years ago (Zavaleta-Meija & VanGundy, 1982; Mohamedova & Samaliev, 2011; Sidhu, 2018), mainly the genera *Pseudomonas* spp. and *Bacillus* spp. They have properties that determine their success in practice - they inhibit egg hatching or produce metabolites toxic to nematodes but have no negative effects on soil and on plant growth and development. Studies carried out by Ahmed (1999) on the effect of the rhizobacterium *Bacillus subtilis* on damage caused by *Meloidogyne* spp. in tomato showed that even when the root-knot nematode population increased and damage was important, plant growth was enhanced (induced tolerance). There is data in the literature that suggests that treatment of plants with synthetic phytohormones and organomineral fertilizers also induced resistance to pests including nematodes (Guimarães, 2010; Bhattacharya, 2021; Dar et al., 2021).

In greenhouse experiments, the influence of using the following fertilizers Kendal, 18 Biopower, Nutriphite, Hortiplus MIQL 2826, Max Fitus as inducers of resistance to *Meloidogyne incognita* was studied. The authors reported proven differences in nematode population numbers between the control and variants treated with Biopower and Nutriphite fertilizers (Assunção et al., 2010).

In Bulgaria, Markova et al. (2014) studied the effect of the liquid root biostimulator Fertiactyl® GZ on damage by root-knot nematodes *Meloidogyne* spp. in tomato plants grown in greenhouse conditions.

The authors found that the liquid root biostimulator had a stimulatory effect on the growth and development of plants infected with Meloidogyne spp., and the tested tomato cultivars Raleigh and Matthias after treatment with Fertiactyl® GZ showed no symptoms of damage.

The cultivation of resistant varieties as well as the observance of crop rotation are methods that limit the use of the chemical control method. The protection of vegetable crops of the Solanaceae family against plant parasitic nematodes should ensure the health and potential of the crops through the extensive implementation of alternative control measures such as sanitary measures, phytosanitary monitoring, crop rotation, mixed cropping, breeding of resistant or tolerant varieties, application of biological agents and other innovative practices. The emergence of nematode resistance to nematicides, the negative impacts on human health and the environment, and the drastic decrease in the availability of existing and new chemical pesticides in Europe lead to an increased need for alternative control options. In addition, there are legal requirements to regulate agricultural production according to IPM principles and to market plant products with little or no pesticide residues (Directive 2009/128/EC).

CONCLUSIONS

The analysis of the species composition of root-knot nematodes in solanaceous vegetable crops in Southwest Bulgaria showed that the distribution and the population structure were relatively constant over time.

Limited progress has been observed in implementing alternative measures to control root-knot nematodes.

A range of data was created to enable the inclusion of alternative measures in integrated pest management (IPM) programs and to reduce reliance on chemical control.

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