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Bioagent and plant extract strategy of control against root-knot nematodes (*Meloidogyne* spp.) of greenhouse cucumber

Dima Markova[1,2*], Vinelina Yankova[2], Zhenya Ilieva[3], Iliyan Iliev[4], Donka Draganova[5] and Boyan Arnaoudov[2]

[1] *Agricultural University, 4000 Plovdiv, Bulgaria*
[2] *Agricultural Academy, Maritsa Vegetable Crops Research Institute, 4003 Plovdiv, Bulgaria*
[3] *Agricultural Academy, Institute of Soil Science, Agrotechnology and Plant Protection "Nikola Poushkarov", 1331 Sofia, Bulgaria*
[4] *Sofia University "St. Kliment Ohridski", 1000 Sofia, Bulgaria*
[5] *Biodinamika Ltd, 4000 Plovdiv, Bulgaria*
[*]*Corresponding author:* dimamarkova@abv.bg

Abstract

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The devastating root-knot nematodes (*Meloidogyne* spp.) can cause severe damage to greenhouse crops. Due to high economic losses, alternative products are essential to replace banned, or strictly regulated nematicides that affect human health and/or the environment. Studies were carried out at the Maritsa Vegetable Crops Research Institute, Plovdiv, to determine the biological activity of the product Nemguard and the microbioagent *Trichoderma asperellum* Bulgarian strain T6, as well as the combination between them against root-knot nematodes (*Meloidogyne* spp.) in cucumber varieties Defense F_1 and Kiara grown in greenhouses. The combined application of Nemguard + *Trichoderma asperellum* strain T6 according to the scheme is distinguished by the best effectiveness against root-knot nematodes, the best biometric indicators and yield of cucumbers in greenhouse conditions.

Keywords: plant-parasitic nematodes; biological control; cucumber; *Trichoderma*; garlic

Introduction

Root-knot nematodes of the genus *Meloidogyne* are severe plant pathogens that cause significant economic losses by inducing the development of root knot galls, altering the plant vascular system and draining essential nutrients from the host. The severe plant damage caused by these sedentary plant parasites can result in wilting, stunted growth, leaf discoloration, root deformation, reduced yield and crop quality (up to the total crop loss). Root-knot nematodes infection can also contribute to reduced plant resistance to other biotic and abiotic stresses (Jones et al., 2013).

Chemical nematicides carries risks of residues in plant production and environmental negative impact. Alternative solutions, such as garlic extract and some soil bioagents is an opportunity for successful control.

Garlic based products have been studied as environmentally nematicides and their active substances, diallyl polysulfides exist as formulated nematicides on the market (Eder et al., 2021). Studies have been performed to determine the nematicidal activity of products with different garlic composition against *Meloidogyne* spp. (Jardim et al., 2020).

The suppressive effect of garlic was pronounced after rotation with the crop for three years. Garlic can be successful-

ly used as an alternative economical crop for management of root-knot nematode in organic cultivation of vegetable crops in infested soil (Dhillon et al., 2019).

NemGuard®Granules is a new nematicide based on garlic extract containing polysulfides with nematicidal action. Studies with this product have been conducted to determine its effectiveness to reduce the damage caused by nematodes and it has been evaluated in different crops compared to a conventional control strategy and untreated control. The results obtained with carrots, tomatoes, lettuce and melons confirm the effectiveness of this plant extract as a nematicide and show its long-term activity over time. NemGuard®Granules is easy to use, leaves no residues (no MRL restrictions) and can be used alone, or in combination with conventional and microbial nematicides (Ladurner et al., 2014).

Garlic extract is available in both granular (GR) and concentrated suspension (CS) for use in transplanting, or sowing (Sasanelli et al., 2021). The active ingredient is not an ordinary garlic extract it comes from a process, in which specific polysulphide compounds are derived from allicin (the substance that causes the typical pungent smell of garlic). Garlic extract guarantees consistent and reproducible results. These polysulfide compounds act by contact and uptake (Eder et al., 2021). The nematicidal action continues 25–30 days. After treatment with GR, watering is recommended. The liquid CS formula can be used at different stages of culture development. The shelf life of the product is about 14 days, therefore after the first application, the product CS should be applied at intervals of 2 weeks (Label authorized by the Italian Healthy Ministery, 2021).

Species of the genus *Trichoderma* are free-living fungi that are common in soil and root ecosystems. Some strains establish root colonization and enhance growth and development, crop productivity, resistance to abiotic stress and uptake and use of nutrients. *Trichoderma* species can antagonize and control a wide range of economically important plant pathogens and nematodes. Root-knot nematodes, *Meloidogyne* spp. obligatory root endoparasites of great economic importance, and polyphagous species, such as *M. incognita* and *M. javanica* are among the major limiting factors of crop production worldwide. Therefore, these nematodes have been the main target for nematode biocontrol by *Trichoderma*. Several *Trichoderma* species and isolates have been evaluated as biocontrol agents against the nematodes in different crops under experimental conditions. Significant results of nematode control and plants growth were achieved. In order to improve the biocontrol process, modes of action of the fungus against root-knot nematodes have been studied. Mechanisms, such as parasitism, enzymatic lysis, antibiosis and induced resistance have been studied. Understanding the fungus-nematode-plant interactions and the mechanisms of the biocontrol process might contribute to improve the implementation of this biocontrol agent (Sharon et al., 2011). *Trichoderma asperellum* are known to be used in many cases for biocontrol of phytopathogenic fungi, bacteria and nematodes (Harman et al., 2004; Lorito et al., 2010; Rivera-Méndez et al., 2020; Muniz et al., 2022).

The aim of the study was to determine the biological activity of the product Nemguard and the microbial agent *Trichoderma asperellum* strain T6, as well as the combination between them against root-knot nematodes (*Meloidogyne* spp.) in greenhouse cucumber.

Material and Methods

The study was conducted during the period 2020-2021, at Maritsa Vegetable Crops Research Institute – Plovdiv, in greenhouse and vegetation facilities, which are naturally infested with root-knot nematodes (RKN) *Meloidogyne* spp. Test products: Nemguard (Garlic extract) – 400 ml/da (4 ml/10 m^2) 6 times treatment at intervals of one week, start of application immediately after transplanting; *Trichoderma asperellum* Bulgarian strain T6 titer $0.5*10^9$ CFU (colony forming units)/cm^3, consumption rate 1 cm^3/plant, 6 times treatment at intervals of one week, start of application immediately after transplanting. Three variants of treatment plus untreated control were followed. Each variant consisted of four replicates randomly situated in the trial plot.

Production trial

- Cucumbers variety Defense F_1:
  Planting scheme – 50 × 130 × 130 × 50 cm/45 cm;
  Number of plants per ha – 22000;
  Experimental area 160 m^2;
  Number of repetitions 4×10 m^2;
  Drip irrigation.
- Treatments:
  Nemguard;
  Trichoderma asperellum strain T6;
  Nemguard + *Trichoderma asperellum* strain T6;
  Control (untreated).

Trials in vegetation facilities

- Cucumber variety Kiara:
  Five facilities (10 m^2) x 28 number of plants;
  Gravity irrigation.
- Treatments:
  Nemguard;

Trichoderma asperellum strain T6;
Nemguard + *Trichoderma asperellum* T6;
Rotation: Nemguard – 6 times; *Trichoderma asperellum* strain T6 – 6 times;
Control (untreated).

Biometric indicators

At the end of the crop vegetation, the following indicators were reported: plant height (cm); stem diameter (mm); weight of the aboveground part (kg); root length (cm); root weight (g).

Root gall index (RGI)

Severity of root galling was assessed on a 10 rating scale of Bridge & Page (1980), where: 0 – Complete and healthy root system, no infection, 1 – few small galls can only be detected upon close examination, 2 – Small galls/knot only, but clearly visible main root clean, 3 – Some larger knot visible, main root clean, 4 – Larger knot predominate, but main root clean, 5 – 50% of root infested, knotting on some main roots, reduced root system, 6 – Knotting on main roots, 7 – Majority of main roots knotted, 8 – All main roots, including tap root, knotted, and few clean root visible, 9 – All roots severely knotted, and plant usually dying; 10 – all roots severely knotted, plants usually dead.

Soil invasive stages of RKN

Evaluation of treatment effects on the level of soil infestation of *Meloidogyne* was made. Soil samples were collected with soil auger (d = 1.5 cm) in 0–20 cm horizon. Bulk sample of 10 cores was taken per replicate. Nematodes of two subsamples of 100 cm^3 per bulk sample were extracted by centrifuge in sucrose after Coolen (1979) for semi-production trial and, respectively, 4 subsamples from vegetation. Extracted nematodes were counted *in vivo* of under inverted microscope Olympus IX and fixed in FAA.

Identification of nematodes was made in mass slides under microscope Olympus BX60 with DIC.

Effectiveness

Effectiveness (%) of the tested products was calculated according to the formula of Abbott (1925) on the basis of the reported root gall index at the end of the vegetation.

Compatibility test of product Nemguard and Trichoderma asperellum strain T6

Agar well diffusion method was used to determine the effect of the Nemguard product on Bulgarian strain T6 of *Trichoderma asperellum*. For this purpose, petri dishes with sterile culture medium (potato dextrose agar, Merck) were inoculated superficially with 100 microliters of spore suspension. The spore suspension was prepared by washing a petri dish of 7-day fungal culture with 10 ml of sterile water and diluting to 10^6 spores per ml. After inoculation, 10 mm wells were made in the medium using a sterile cork borer. Then, 50 µl of the product diluted with sterile water in a ratio of 1:1, 1:10 and 1:100, was added to wells. The petri dishes prepared in this way were incubated in a grow chamber at 28°C for 5 days. The experiment was done with three replications.

Statistical analysis

All data of the trials *were analyzed using analysis of variance (ANOVA).* The significance of differences between treatments at biometric indicators and root gall index was tested using Duncan's multiple range test (1955). *The di*fferences of abundance of invasive juveniles of RKN were outlined after Tukey's test by pairs ($p \leq 0.05$).

Results and Discussion

Production trial

The best biometric indicators in the greenhouse naturally infested with root-knot nematodes were found in combined application of Nemguard + *Trichoderma asperellum* strain T6. In this treatment, the lowest root gall index of 0.33 was reported, while the root gall index in the Control (untreated) was 1.08, followed by the separately application of Nemguard and *Trichoderma asperellum* strain T6. The values of the reported gall index in the three treatments are extremely close with insignificant differences between them (Table 1). Our results confirm the findings of other authors that *Trichoderma asperellum* reduced the root-knot nematode population and root gall index (Alori et al., 2020). The same was observed to garlic extract, which can be used to control *Meloidogyne* spp. The reported yield in the experiments was highest again in Nemguard + *Trichoderma asperellum* strain T6 – 116 t/ha (Figure 1). In this treatment, a significantly higher quantity of cucumbers was of the first quality. Some microbial inoculates have been found to improve plant growth and yield and act as biocontrol agents for some pests. They are environmentally friendly (Alori and Babalola, 2018). The effectiveness was highest in Nemguard + *Trichoderma asperellum* strain T6 – 69.44%, in the other two treatments it is 61.11% (Table 2). The established effectiveness was good, given that the tested products are organic. They can be used as an alternative to control root-knot nematodes in greenhouse cucumbers, and the combined application of Nemguard + *Trichoderma asperellum* strain T6 provides better protection.

Table 1. Biometric indicators and root gall index in cucumber variety Defence F_1 grown in greenhouses

Treatment	Plant height, cm	Stem diameter, mm	Weight of the above-ground part, kg	Root length, cm	Root weight, g	RGI (0–10)
Nemguard	372.08 a	15.13 a	1.554 a	22.54 a	16.38 a	0.42 b
Trichoderma asperellum strain T6	374.17 a	15.25 a	1.567 a	23.21 a	16.14 a	0.42 b
Nemguard+*Trichoderma asperellum* strain T6	375.00 a	15.31 a	1.571 a	23.29 a	16.30 a	0.33 b
Control (untreated)	297.92 b	11.62 b	0.750 b	14.33 b	15.36 a	1.08 a

Significant differences were marked with different letters after Duncan's multiple range test ($p \leq 0.05$)

Table 2. Effectiveness of the tested products against *Meloidogyne* spp. in cucumber variety Defense F_1 grown in greenhouses

Treatment	Effectiveness, %
Nemguard	61.11
Trichoderma asperellum strain T6	61.11
Nemguard +*Trichoderma asperellum* strain T6	69.44

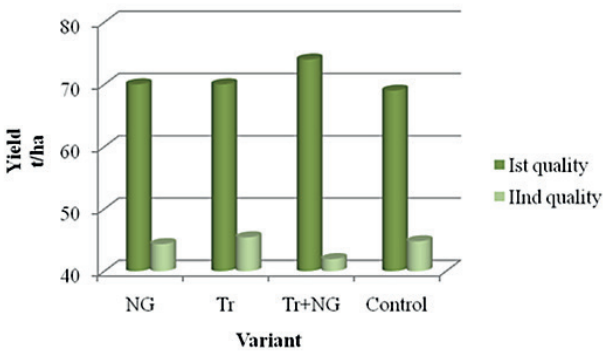

Fig. 1. Yield of 1st and 2nd quality of cucumber variety Defence F_1 in different trial variants

During the trial 69 746 soil nematodes were extracted and 5409 *Meloidogyne* invasive juveniles were identified in April sampling and 4797, respectively, in August. RKN were dominant group in all trial variants before and after treatments with 80% of total abundance. Results are presented in (Figure 2).

A significant reduces of abundance of RKN invasive J2 was observed in the variants of *Trichoderma* and combined treatments (e.g. over 5.5 times for *Trichoderma* and 14 times for combined treatment). Decrease in Nemguard variant was 4 times less than in control plot but insignificant.

Our results imply that combined treatment of Nemguard and *Trichoderma asperellum* strain T6 could be included in IPM, or biological control of root-knot nematodes in protected cultivation.

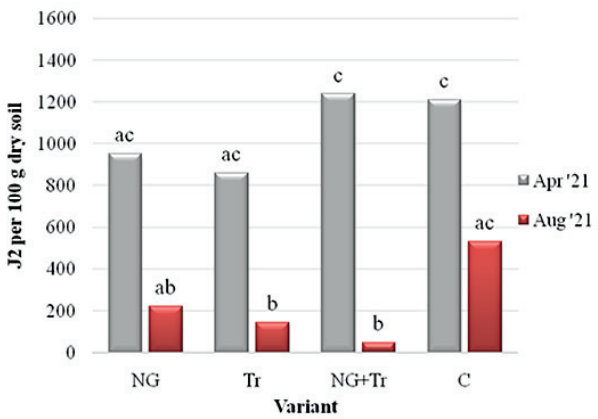

Fig. 2. Abundance of invasive juveniles of RKN in different trial variants of production trial before (22 April) and at the end of vegetation season (11 August). ANOVA results – (Wilks lambda = 0.5337, F (6.54) = 3.3192, p = 0.0074). Significant differences were marked with different letters after Tukey's test ($p \leq 0.05$)

Trials in vegetation facilities

In the experiments, performed in vegetation facilities, the best biometric indicators were observed again in Nemguard + *Trichoderma asperellum* strain T6 (Table 3). In this treatment, the highest yield was reported 14 t/ha, respectively (Figure 3). The lowest root gall index 0.4, was reported in the treatment Nemguard + *Trichoderma asperellum* strain T6 and in the treatment with Rotation, while the root gall index in the Control (untreated) was 3.4 (Table 3). The best effectiveness was observed in Nemguard + *Trichoderma asperellum* strain T6 and in the treatment with Rotation – 88.24% (Table 4).

Abundance of invasive J2 in soil in the beginning of trial was generally low (Figure 4). Reduces of invasive J2 abundance in soil was observed in all the trial variants, but untreated control. Untreated control doubled significantly abundance from starting population. Only Nemguard treat-

Table 3. Biometric indicators and root gall index in cucumber variety Kiara grown in vegetation facilities

Treatment	Plant height, cm	Stem diameter, mm	Weight of the aboveground part, kg	Root length, cm	Root weight, g	RGI (0-10)
Nemguard	380.00 b	11.30 b	574 a	28.20 ab	25.77 b	1.4 b
Trichoderma asperellum strain T6	398.00 ab	12.78 a	634 a	32.60 ab	27.93 ab	0.8 b
Nemguard +*Tr. asperellum* strain T6	419.60 a	13.90 a	686 a	38.20 a	37.64 a	0.4 b
Rotation	395.00 ab	10.87 b	526 a	29.00 ab	24.00 b	0.4 b
Control (untreated)	372.00 b	10.50 b	488 a	24.40 b	21.64 b	3.4 a

Significant differences were marked with different letters after Duncan's multiple range test ($p \leq 0.05$)

Table 4. Effectiveness of the tested products against *Meloidogyne* spp. in cucumber variety Kiara grown in vegetative facilities

Treatment	Effectiveness, %
Nemguard	58.82
Trichoderma asperellum strain T6	76.47
Nemguard +*Trichoderma asperellum* strain T6	88.24
Rotation	88.24

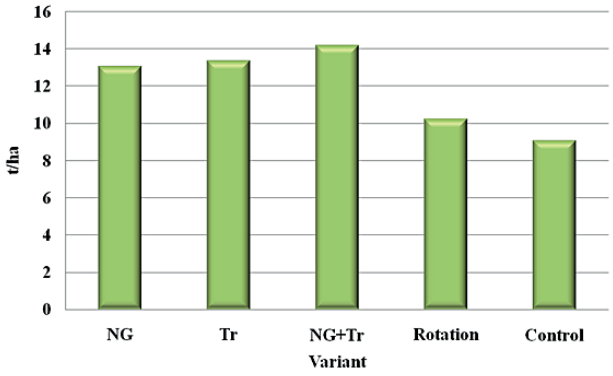

Fig. 3. Yield of cucumber variety Kiara in experience in vegetation facilities

ment showed significant decrease in comparison with start population. All other treatments kept low level of invasive J_2 of *Meloidogyne*.

Compatibility test of product Nemguard and Trichoderma asperellum strain T6

From Figure 5, it can be seen that the Nemguard product does not restrict the growth of the fungus *Trichoderma asperellum* strain T6 at any of the concentrations used. At higher dilutions, growth of the fungus is also observed inside the wells where the product is placed. This suggests that at these dilutions the fungus can use it as a nutrient substrate.

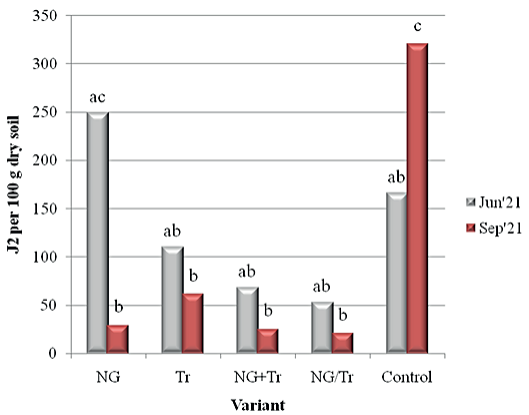

Fig. 4. Abundance of invasive juveniles of RKN in different trial variants in vegetation facilities trial before (23 June) and at the end of vegetation season (14 September). ANOVA results – (Wilks lambda = 0.08725, F (8.28) = 8.3492, p = 0.00001). Significant differences were marked with different letters after Tukey's test ($p \leq 0.05$)

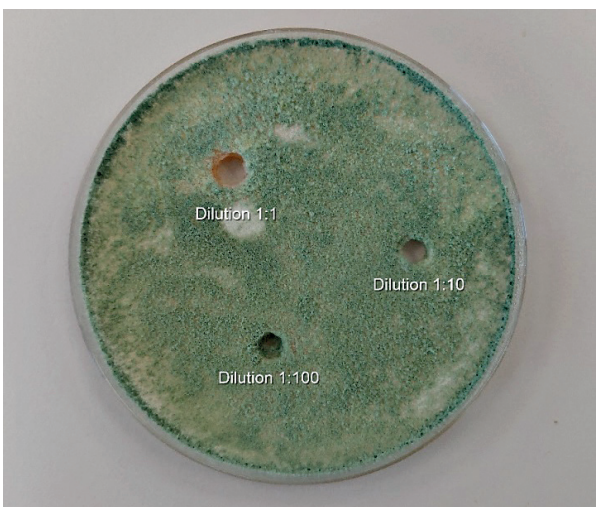

Fig. 5. Compatibility test of product Nemguard and *Trichoderma asperellum* strain T6

The treatment Nemguard and *Trichoderma asperellum* strain T6 studied by us applied alone and in combination reduced the population of *Meloidogyne* spp. and could be used as an alternative to control in integrated and organic cucumber production in greenhouses.

Conclusions

The treatment Nemguard and Bulgarian strain T6 of *Trichoderma asperellum* studied by us applied alone and in combination kept low nematode abundance in soil, reduced the population of *Meloidogyne* spp. They could be used as an alternative to control in integrated and organic cucumber production in greenhouses.

The combined application of Nemguard + *Trichoderma asperellum* strain T6 by scheme stands out with the best effectiveness against root-knot nematodes, the best biometric indicators and yield of cucumbers in greenhouse conditions.

Acknowledgments

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