NON-CHEMICAL PROTECTION AGAINST ROOT-KNOT NEMATODES IN GREENHOUSE PEPPER

PhD theses

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1. BACKGROUND AND AIMS OF THE PHD WORK

The damage (irregular galls of different sizes on the plant roots) caused by root-knot nematodes (*Meloidogynidae*) is a general problem in greenhouses in Hungary, as well as in foreign countries. Their damage is significant in greenhouses and under field conditions, either. The most significant damage can be found among heat-demanding vegetables on cucumber, tomato and pepper.

It makes the protection against them more difficult, that they cannot be eradicated totally from the field. Nowadays, the continuous withdrawal and decreasing number of applicable plant protection products, makes the protection more difficult against root-knot nematodes.

During our research, we were focusing on the substitution of chemical products for biological plant protection methods. Out of the possible solutions, the effect of green manure plants, *Trichoderma asperellum* Samuels, Lieckfeldt et Nirenberg (Trifender) and *Arthrobotrys oligospora* Fresenius (Artis) fungi have been investigated regarding the rate of damage caused in greenhouse pepper by root-knot nematodes.

About protection methods against root-knot nematodes with green manure plants, *A. oligospora*, as well as *Trichoderma* species, sufficient amount of national and international references are available, but on the contrary, only a few national and international experimental results are known regarding the applicability in greenhouse pepper.

The greenhouse experiments were set up in Jászfényszaru by Ferenc Kollár, in Röszke by István Szalma, in Pusztamonostor by József Langa, as well as in Zsámbok by Géza Szabó growers. Laboratory evaluations were carried out in Central Agricultural Office, Directorate of Plant Protection and Soil Conservation, Central Laboratory of Pest Diagnostics, Laboratory of Nematology, in Budapest, as well as in Szent István University, Department of Plant Protection (today Institute of Plant Protection).

The green pepper pot experiments were carried out in the plant-house of Plant Protection and Soil Conservation Service of Budapest and Pest County, and in Zsámbok, in unheated greenhouse.

The aims set during my research are as follows:

- Selecting greenhouses, seriously infected by root-knot nematodes in Jászság region, evaluation of preliminary infection
- The investigation of root-knot nematode reducing effect of phacelia (*Phacelia tanacetifolia*), mustard (*Sinapis alba*), oil-radish (*Raphanus sativus*) and calendula (*Calendula officinalis*) in greenhouse (Jászfényszaru); and (Röszke) phacelia, mustard and calendula as catch-crop
- As a supplementary experiment of the previous, to investigate the root-knot nematode reducing effect of the aforementioned catch-crops in green pepper pot experiment (Plant Protection and Soil Conservation Service of Budapest and Pest County)
- During the visits of green pepper growers in Jászság region:
    - getting acquainted with the growing practices of growers, and the chemical application against terrestrial pests
    - evaluation of damage caused by root-knot nematodes, knowledge of protection methods against them, (biological protection methods, application of green manure plants as phacelia, mustard and oil-radish)
    - searching for growers, who are able to do efficient non-chemical protection against root-knot nematodes
- Investigation of root-knot nematode reducing effect of Trifender (microbiological yield improver) and *A. oligospora* (experimental microbiological product at the time of the experiment) in unheated greenhouse in Pusztamonostor
- Investigation of root-knot nematode reducing effect of Trifender and Artis microbiological products in unheated greenhouse in Zsámbok
- As a supplementary experiment of the previous, setting up a pot experiment in unheated greenhouse in Zsámbok

2. MATERIALS AND METHODS

2.1. Background of research

The locations of the greenhouse experiments in Jász-Nagykun-Szolnok County were, in Jászság region, in Jászfényszaru and Pusztamonostor, in Zsámbok (located at the border of Gödöllő Hills and Jászság region), in Pest County, and in Röszke, in Csongrád County **(Fig. 1)**.

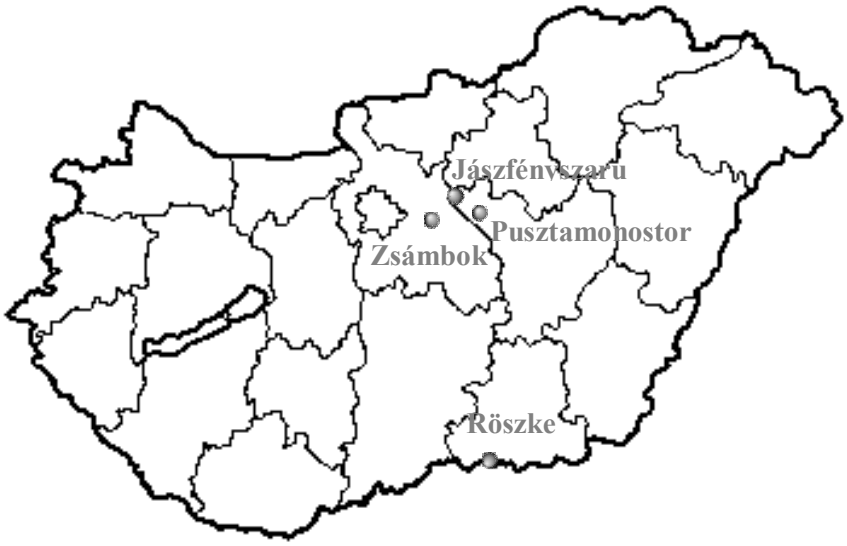

Fig. 1. The locations of the greenhouse experiments

Laboratory investigations and evaluations were carried out in Central Agricultural Office, Directorate of Plant Protection and Soil Conservation, Central Laboratory of Pest Diagnostics, Laboratory of Nematology, in Budapest, as well as in Szent István University, Institute of Plant Protection (formerly Department of Plant Protection).

The green pepper pot experiments were carried out in the plant-house of Plant Protection and Soil Conservation Service of Budapest and Pest County, and in Zsámbok, in unheated greenhouse.

2.2. Method of green manure- and repellent plant experiments in Jászfényszaru

The experiment was set up in May 2006, in unheated greenhouse in green pepper, in Jászfényszaru. Prior to the experiment, in November 2005, rye-strips had been sown, later shredded and incorporated into the soil. Mustard, calendula, oil-radish and phacelia were sown as catch-crops. The greenhouse was divided into 16 plots. 4 replications were formed, so each replication consisted of 4 plots (2 controls, 1 rye and 1 catch-crop strip).

During the evaluation (3 occasions) we determined the rate of infection per replication, so as the percentage of root-galls per root.

The determination of number of root-knots (rate of root damage) was carried out on the basis of the 0-5 scale used by Raviv et al. (2005) (cit. Garabedian and Van Gundy 1984).

The statistical evaluation of the experiment was carried out by one-way analysis of variance and Tukey's post-hoc test.

2.3. Method of green manure- and repellent plant pot experiment in Gödöllő

As a supplementary trial of the greenhouse experiment in Jászfényszaru, a pot experiment was set up in the plant-house of Plant Protection and Soil Conservation Service of Budapest and Pest County in Gödöllő. The effect of green manure- and repellent plants on root-knot nematodes was investigated under laboratory circumstances.

The trial was set up in July 2006, in plastic pots. Disease-free peat was distributed equally in the pots, after that green pepper seedlings were planted and finally, seeds of green manure- and repellent plants were sown. 10, root-knot nematode infected green pepper roots were placed in each pot. In the experiment, 5 applications were tested in 5 replications.

The determination of the number of root-knots was carried out on the basis of the 0-5 scale used by Raviv et al. (2005) (cit. Garabedian and Van Gundy 1984).

The statistical evaluation of the experiment was carried out by one-way analysis of variance and Tukey's post-hoc test.

2.4. Method of green manure- and repellent plant experiment in Röszke

In order to make a comparison with the trial carried out in Jászfényszaru, green manure plant experiment was set up in Röszke. The plants were sown simultaneously with the other experiment. The effect of mustard, phacelia and calendula as catch-crops have been evaluated in greenhouse pepper.

In October 2005, prior to the experiment, a preliminary root-knot nematode infection evaluation had been carried out. The pest species, according to Mándoki (2012), who carried out resistance-comparison tests there before, is *M. incognita.*

Setting-up of the experiment, as well as sowing of green manure- and repellent plants have been done in May 2006, in 3 unheated greenhouses in green pepper. The planting of green pepper was performed in April 2006. The sowing of green manure plants was carried out in all 3 greenhouses, in non-neighbouring inter-rows. The selected inter-row was split into 25 equal

sections in total length of the greenhouse, into which green manure plants were sown alternately, by keeping 2 m distance as control plots between all sections.

In October 2006, in green pepper belonging to catch-crops, *Meloidogyne* infection was evaluated on a 1-5 scale (Budai et al. 1997).

The statistical evaluation of the experiment was carried out by one-way analysis of variance and Tukey's post-hoc test.

2.5. Method of biological protection experiments in Pusztamonostor (Trifender and *A. oligospora* treatments)

The experiment was set up in Pusztamonostor, in unheated greenhouse in green pepper, in 2008 and 2009. In the greenhouse where treatment was applied, we carried out preliminary evaluation in the precrops.

The experiment has been set up, in case of **Trifender**, right after planting green pepper, in randomized block design in 4 replications, in April 2008 and 2009. In case of ***A. oligospora*** the treatment was done 9 days before green pepper planting in May 2008 and 3 days before planting in April 2009, in randomized block design in 4 replications.

The investigated products were Trifender (*T. asperellum*) microbiological plant growth promoter, and *Arthrobotrys oligospora* trial microbiological preparation.

The sampling was done in Trifender treated plots, as well as in case of *A. oligospora,* in the summer of 2008 and 2009 at the time of full development of 1st generation of *M. hapla.*

The green pepper roots collected in the trial, have been evaluated by using microscope, and partly used later for species determination.

In case of Trifender experiment, plant height measurements were done 2 times in 2008 and 3 times in 2009; in case of *A. oligospora* trial, once in 2008 and 2 times in 2009. In 2008, yield measurements were done in Trifender treatment 2 times, and 11 times in 2009, in *A. oligospora* treatment 11 times in 2009, either, on the whole area of the treated and untreated plots.

The statistical analysis was done by Two-Sample T-Test (Welch-test).

2.6. Method of biological protection greenhouse experiment in Zsámbok (Trifender and Artis treatments)

Our experiment was set up in unheated greenhouse in May 2014, in Zsámbok. The treatment was carried out in set out green pepper seedlings. The investigated products were, similarly to Pusztamonostor experiment, Trifender (*T. asperellum*) microbiological plant growth promoter and Artis (*Arthrobotrys oligospora*) microbiological product. Artis had been authorized already by the time of the experiment and launched at the market. The experiment was carried out in 8 replications, 8 plots were formed in each replication, Trifender, Artis and control alternately.

The experiment was evaluated in August 2014, on the basis of Zeck-scale (Zeck 1971). Samples have been collected in the greenhouse for microscopic species determination. Plant height was measured in the summer of 2014. The statistical evaluation of the experiment was carried out by one-way analysis of variance and Tukey's post-hoc test.

2.7. Method of biological protection pot experiment in Zsámbok (Trifender and Artis treatments in different combinations)

The pot experiment was set up in Zsámbok, either, in May 2014 green pepper seedlings were planted in pots in unheated greenhouse. 50% of the seedlings have been artificially infected by *Meloidogyne* spp. infected kohlrabi roots placed beside pepper roots.

Treatment was carried out one week after planting. The investigated products were similarly to greenhouse experiment, Trifender and Artis. 8 different treatments in 8 replications were applied, alltogether 64 plants were investigated.

The development of plant heights recorded at planting, was observed 4 more times during vegetation. The generative production of plants was recorded 2 times, during which 4 categories were established.

In November 2014, at the end of vegetation time, each green pepper root in the pots have been evaluated on the basis of 0-10 Zeck-scale (Zeck 1971).

Several washed-off green pepper roots were taken for microscopic species determination, in order to specify the occurring *Meloidogyne* species. The species determination was confirmed by PCR analysis. The statistical evaluation of the experiment was carried out by one-way analysis of variance and Tukey's post-hoc test.

2.8. Survey of green pepper growing in Jászság region and the protection against terrestrial pests

During our visit in Jászság region and surrounding villages (the most important green pepper growing region), in the summer of 2007, growers have been questioned about their growing practices. We have also been looking for symptoms related to root-knot nematodes in green pepper, by the investigation of greenhouses. We intended to have a global picture about the growing practices of growers participated in the survey and chemical application against terrestrial pests.

Our main goals were as follows, determining the rate of damage caused by root-knot nematodes, protection methods including biological protection and application of green manure plants (phacelia, mustard, oil-radish).

The aforementioned survey has been repeated in the summer of 2015, in order to compare with the experiences of 2007.

3. RESULTS

3.1. Results of green manure- and repellent plant experiments in Jászfényszaru

According to results, the vigour of emergence and growth was proved to be highest for oil-radish (in some spots suppressed even green-pepper), followed by mustard, calendula and phacelia. The % rate of the number of galls were varying mostly between 1 and 10%. The rate of damage was proved to be lowest or 0 at the edges.

On the basis of the % results of the 3 evaluations, regarding the effect of treatment on average number of galls, highest rate was found for rye (7,04%), lowest for mustard (0,62%).

Significant difference was proved in the number of galls between rye and oil-radish (1,58%), as well between rye and mustard ($p<0,05$).

By comparing the influences of green manure plants with different evaluation dates on average gall %, significantly higher difference was found for 5th October (5,55%), compared to 5th July (1,64%) ($p<0,05$).

3.2. Results of green manure- and repellent plant pot experiment in Gödöllő

During the evaluation of pot experiment, the recorded gall % values were varying between 0 and 60% in certain replications. In soils containing phacelia roots, no galls have been found. In the other treatments, the rate of galling was not proved to be significant compared to control.

3.3. Results of green manure- and repellent plant experiments in Röszke

In October 2005, the preliminary evaluation confirmed, that the soils of the three greenhouses chosen for the experiment, were moderately infected by root-knot nematodes.

In October 2006, the evaluation of root-knot nematode infection of harvested green pepper roots has shown moderate infection, either.

By investigating the rate of galling on certain sections, significant difference was shown between catch-crops (phacelia, calendula, mustard) and control plots. The highest gall % was recorded for the green pepper roots of control plots, the lowest were found in mustard and phacelia sections.

Yield results were similar in both pepper harvests. Beside the too dense and high white mustard section several pepper plants dried. Significantly lower yield was harvested during both harvests from green pepper plants beside mustard, compared to other plots.

3.4. Results of biological protection experiments in Pusztamonostor (Trifender and *A. oligospora* treatments)

During species identification we determined that females of *Meloidogyne hapla* were found in both treatments (Trifender and *A. oligospora*) in the greenhouses.

The results of Trifender treatments showed no significant difference in any of the years (2008 and 2009) regarding the number of females in Trifender treated and untreated pepper roots, but the number of galls was lower in treated roots in both years. Trifender treatment resulted in higher plants in both years, and moreover, in the first year yield was increased by 25 and 35%. In the second year yield was decreased. According to plant height measurements, both in 2008 and 2009, Trifender treated plants were proved to be significantly higher compared to control.

In 2008, significant difference was found between the *A. oligospora* treated and control plants, but the number of females was proved to be lower in control plots. Though, in 2009, the number of females was found to be significantly lower in treated plots.

On the basis of plant height measurements in 2008 and first measurement in 2009, treated plants were proved to be higher, however during second measurement in 2009, plant height was proven lower.

By comparing treatment results (Trifender and *A. oligospora* together) of the years of 2008 and 2009, regarding the average number of females, significantly more females were found in 2008, compared to 2009.

3.5. Results of biological protection greenhouse experiment in Zsámbok (Trifender and Artis treatments)

In the greenhouse experiment set up in 2014 in Zsámbok, the *Meloidogyne incognita* (Kofoid et White) was identified. Regarding Zeck-scale, highest rates (highest infection) were recorded for control, lowest scale rates (lowest infection) were shown for Artis treated plants, where significant differences were determined. Trifender treated plants showed significantly lower scale rates, compared to control. The treatments had no influence on plant height.

3.6. Results of biological protection pot experiment in Zsámbok (Trifender and Artis treatments in different combinations)

In Zsámbok pot experiment, *Meloidogyne incognita* (Kofoid et White) has been found, either, which was confirmed by PCR identification. According to Zeck-scale rates, artificial infection significantly increased root galling, though treatments with antagonists had no significant effect compared to infected control. Neither treatment, nor artificial infection had significant effect on plant height and generative production of plants.

3.7. Results of the survey of green pepper growing in Jászság region and the protection against terrestrial pests

In the visited farms, regarding growing practices, unheated greenhouses have been used most frequently, but in some cases heated greenhouse could also be found. Mainly green pepper is grown, but in lower quantities vegetables are also cultivated for their own consumption.

Regarding terrestrial pests, root-knot nematodes are present in most of the places, but mole cricket and wireworm damage can also be found sometimes. Ipam 40 and Basamid G are used against them generally.

Against root-knot nematodes mainly Nemathorin 10 G is used, but in some places Vydate 10 G and Vydate 10 L are applied, either. Non-chemical protections are not frequent, so as the application of green manure plants.

According to the survey carried out in 2015, can be concluded that growing practices has not changed compared to 2007. Thrips damage was mentioned in almost all cases, as well as problems caused by soil-borne fungi.

The fact that more and more growers are using Trifender, counts as novel experience.

The growers are still not applying green manure plants, as they cannot afford eliminating the most profitable part of the vegetation period.

3.8. New scientific results

1. I demonstrated that rye, as precrop of greenhouse pepper, does not reduce the damage and number of *Meloidogyne hapla.*

2. I demonstrated that the application of green manure plants as companion crops in greenhouse pepper, reduces the number of *M. hapla* and *M. incognita*, but the quantity of yield is also reduced, therefore practical use is not recommendable.

3. I demonstrated that stronger root-knot nematode reducing effect can be forecasted during the application of green manure plants in pots, than during soil application in the greenhouse. In the pot, on the roots of green pepper planted beside phacelia, totally gall-free status can be achieved by the application of phacelia.

4. In Jászság region, I tested antagonist fungi against root-knot nematodes first under real growing circumstances. As a result, I demonstrated that both *Arthrobotrys oligospora* nematode trapping-, and *Trichoderma asperellum* antagonist fungus reduces the rate of root galling under appropriate circumstances in greenhouse pepper.

5. In Jászság region I conducted first a comprehensive survey among growers regarding the occurrence of damage of root-knot nematodes, the rate of damage, the applied protection methods against nematodes, and the reasons of application or ignoration of protection.

I stated, that the main difficulty of the application of green manure plants as precrops is, growers cannot afford eliminating the most profitable part of the vegetation period.

At the same time, for regular application of antagonist microorganisms (*Trichoderma asperellum* and *Arthrobotrys oligospora*), as well as towards introducing them into technology, conditions are ensured.

4. CONSEQUENCES, RECOMMENDATIONS

4.1. Consequences based on the results of green manure- and repellent plant experiments in Jászfényszaru and green manure- and repellent plant pot experiment in Gödöllő

Based on experimental results, it can be concluded that the conditions for homogenous and weed-free emergence of phacelia, are not ensured everywhere. We have not such experience with oil-radish and mustard due to favourable emergence characteristics, but we have to prevent green pepper from being overgrown by trimming plants.

In almost all cases high infection was recorded for green pepper plants beside rye, therefore rye cannot be recommended as protection against root-knot nematodes.

In previous years much higher infection rate was observed in the mentioned greenhouse. The reason can be due to the accumulation of natural enemies, as the grower eliminated the regular soil disinfection for the purpose of the experiment.

The outstanding efficiency (100%) of phacelia against nematodes, experienced in laboratory experiment, cannot be expected under real growing conditions. The explanation is that root exudates can pass through the whole depth of available soil volume in the pot, but in the case of soil of growing structures it is impracticable.

4.2. Consequences based on the results of green manure- and repellent plant experiments in Röszke

According to our opinion, if the grower has the opportunity for sowing green manure plants in the greenhouse between two growing seasons, it is advisable to take advantage of green manure plants. The intercropping of mentioned green manure plants in green-pepper with the applied method, raised several practical questions.

It would be worth investigating the incorporation of intercropped catch-crop residues into soil at an earlier development stage. In this case the unfavourable effect of catch-crops on the development of green-pepper could be eliminated, and after their effect on reducing the number of root-knot nematodes, such as yield can be investigated.

4.3. Consequences based on the results of biological protection experiments in Pusztamonostor (Trifender and *A. oligospora* treatments)

By comparing treatment results of the two years (2008 and 2009), regarding the average number of females, significantly more females were found in 2008, compared to 2009.

It can be explained by the followings, the fungi have more time for spreading in the 2nd year and in the case of *Arthrobotrys*, the grower has given more attention to the timing of irrigation, avoiding drying up of the soil.

4.4. Consequences based on the results of biological protection greenhouse experiments (Trifender and Artis) in Zsámbok

On the basis of the results of statistical analysis, it can be concluded that nematode infection could not reach the threshold, which could be resulted in serious growth problems.

But on the contrary, the applied microbiological products succesfully reduced root galling in the greenhouse.

4.5. Consequences based on the results of biological protection pot experiment in Zsámbok (Trifender and Artis treatments in different combinations)

The antagonist fungi applied against root-knot nematodes, are likely developing, reproducing and acting in a different way in limited space (container, bucket, pot) generally, as well as in our experiment, compared to soil of growing structures (glasshouse, greenhouse).

4.6. Consequences of the survey of green pepper growing in Jászság region and the protection against terrestrial pests

According to the results of comprehensive survey conducted among green pepper growers in Jászság region, can be concluded that growing practices and protection methods have not changed basically during 2007-2015. In order to have more secure yield, chemical protection is preferred in the future, too. The main difficulty of the application of green manure plants as precrops is, growers cannot afford eliminating the most profitable part of the vegetation period. At the same time, for regular application of antagonist microorganisms (*Trichoderma asperellum* and *Arthrobotrys oligospora*), and introducing them into technology, conditions are ensured.

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2. **Bíró, T.**, Tóth, F. 2009. The effect of Trifender (*Trichoderma asperellum*) on the number of root-knot nematode (*Meloidogyne hapla Chitwood*). *Acta Phytopathologica et Entomologica Hungarica* 44 (2): 363-371
3. **Bíró, T.**, Tóth, F. 2009. A Trifender (*Trichoderma asperellum*) hatása a szabadföldi gyökérgubacs-fonálféreg (*Meloidogyne hapla* Chitwood) paprikában okozott kártételének mértékére. *Növényvédelem* 45 (10): 535-541
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