THE EFFECT OF TRIFENDER (*TRICHODERMA ASPERELLUM*) AND THE NEMATODE-TRAPPING FUNGUS (*ARTHROBOTRYS OLIGOSPORA* FRESENIUS) ON THE NUMBER OF THE NORTHERN ROOT-KNOT NEMATODE (*MELOIDOGYNE HAPLA* CHITWOOD) IN GREEN PEPPER

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Abstract: 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.

The effect of the microbiological plant protection product Trifender and the nematode-trapping fungus *Arthrobotrys oligospora* on the degree of damage caused by root-knot nematodes, has been investigated in greenhouse pepper. We set up our experiment in Pusztamonostor (Jászság region, Hungary) on a family farm, in 2008 and 2009. We carried out a preliminary evaluation (Zeck-scale; 0–10) in the pre-crop with symptomatic assessment. According to the preliminary evaluation, the infection in the case of Trifender showed rates of 2–3, and the *A. olygospora* showed rates of 3–4, on average.

The results of 2008 show that Trifender had no effect on the number of females, but plant height increased by 12% and 15%, and yield grew by 25–35%. In 2009, the number of females decreased by 33%, and plant height increased by 11% and 18%.

In 2008, in the case of *A. oligospora,* there was no effect on the number of females, but plant height increased by 6%. In 2009, the number of females decreased by 35%.

Key words: Trifender, *Arthrobotrys oligospora, Meloidogyne hapla,* green pepper, greenhouse, number of females

INTRODUCTION

Root-knot nematodes (Nematoda: *Meloidogynidae*) are mainly distributed in tropical and sub-tropical regions, but in temperate climates they are dangerous pests in greenhouses. Furthermore, species with high cold tolerance can also be found in the field. Their morphological characteristics are the egg-white colour of females. The eggs are pear-shaped in the fully developed state (Fig. 1). The larvae and males are worm-like. The root-knot nematodes are endoparasites. They belong to one of the most important families of plant-parasitic nematodes. The typical damage caused by root-knot nematodes are the different sized galls formed (Fig. 2) on the plant's root system that obstruct water and nutrient uptake (Andrássy and Farkas 1988; Budai *et al*. 2005). Second-stage juveniles invade the roots in the zone of elongation and then migrate intercellularly and sedentarise into the zone of differentiation of the vascular cylinder (Hemaprabha and Balasaraswathi 2008).

Protecting plants against these nematodes is made difficult because the nematodes cannot be eradicated totally from the field (Andrássy and Farkas 1988; Antal 2003; Budai *et al*. 2005; Budai and Varjas 2008). The environmentally friendly methods and biological protection methods are of great importance due to the lack of applicable pesticides. One of these methods is the application of microbiological products such as the investigated Trifender (active agent is *Trichoderma asperellum* antagonist fungus) and *A. oligospora*.

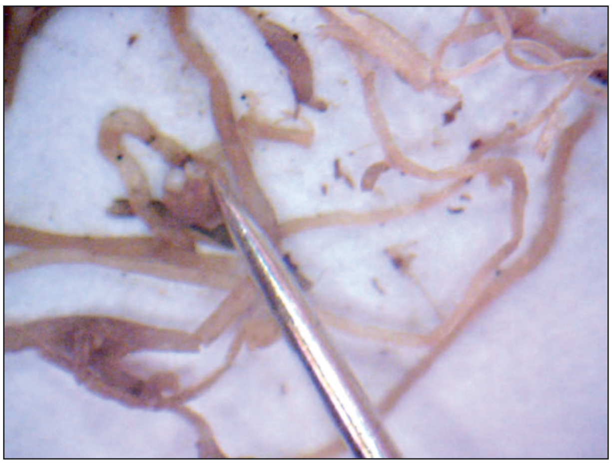

Fig. 1. *M. hapla* females on the green pepper root (Photo by Tímea Bíró-Stingli)

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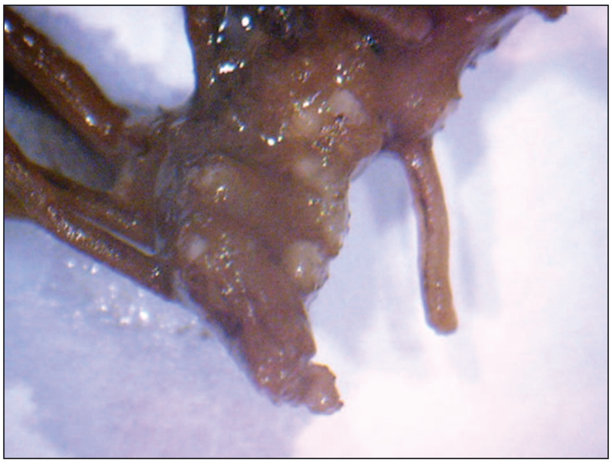

Fig. 2. Damage of the Northern root-knot nematode (*M. hapla*) on green pepper roots (Photo by Tímea Bíró-Stingli)

Trichoderma species belong to the most frequent soil microbes. They occur as saprobionts in soil; primarily on decomposing plant material. They can be isolated from the upper layer of soil containing a high amount of plant roots (Bohár 2003). Most isolates are able to parasitize other fungi in nature. As regards their ability for biological protection, it is favourable that none of the species of the genus are plant pathogens (Harcz 2004). The efficiency of the *Trichoderma* species has been proven by several authors (Harcz 2003; Spiegel *et al*. 2005; Sharon *et al*. 2007; Budai and Varjas 2008).

Most nematode-trapping fungi are dependent on specific hyphal structures. Nematodes can be trapped mechanically or by adhesion on or in these structures. These structures are a prerequisite for the ability of the fungus to invade a host and are thus crucial for survival as well as virulence. The diversity of trapping structures is large and highly dependent on the environment of the fungus. Within the one single species *A. oligospora*, not only are adhesive nets formed but also so-called conidial traps, hyphal coils around hyphae of other fungi, and appressoria in the rhizosphere of agricultural crops (Nordbring-Hertz 2004).

A. oligospora Fres. is a nematophagous fungus which captures nematodes by means of adhesive hyphal networks. When a nematode has been snared, the fungus pierces the animal's integument and produces a bulbous structure from which trophic hyphae grow throughout the body of the worm (Olthof and Estey 1963). The results of Amin and Budai (1993) are promising. They provided in their treatment that use of *A. oligospora* can decrease the number of *Meloidogyne* species.

MATERIALS AND METHODS

Our experiment was set up in Jászság region Pusztamonostor, Jász-Nagykun-Szolnok County, Hungary. The experiment took place in an unheated greenhouse (green pepper) of a family-run farm, in 2008 and 2009.

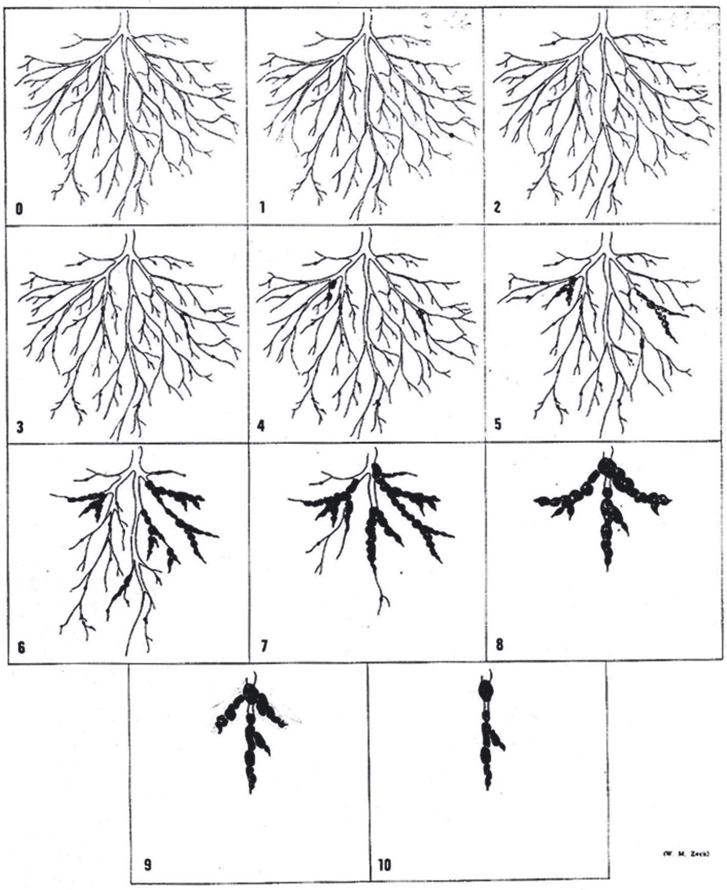

Fig. 3. The Zeck-scale of root-knot infection (Zeck 1971)

In the greenhouse we carried out our preliminary evaluation in the pre-crop (cocktail tomato and indeterminate growth tomato, variety Gaheris). Symptomatic assessment based on the Zeck-scale (0–10) (Zeck 1971) was used. On the Zeck-scale, a rate of 0 meant a healthy plant root system without infection, and 10 meant a destroyed plant with a destroyed root system (Fig. 3). During the symptomatic assessment, 120 (Trifender) and 122 (*A. oligospora*) samples (roots) were evaluated.

The Trifender experiment was set up in a randomized block design in 4 replications, right after planting the green peppers (variety Claudius 2703), on 25th April 2008 and 30th April 2009.

The *A. oligospora* treatment was done 9 days before (2nd May 2008) and 3 days before (23rd April 2009) the planting of the green peppers in a randomized block design in 4 replications (with treated and untreated parts).

Two plant protection products were used. One of the products was Trifender (Microbiological Plant Growth Promoter); wettable powder (WP). Trifender's active agent is approximately 5% w/w *T. asperellum* antagonist fungus T1 (NCAIM 68/2006) strain conidia in min. 5x108 conidia/g concentration. The other plant protection product was *A. oligospora* trial microbiological preparation, wettable powder (WP). The active agent of *A. oligospora* is the A O1 branch of nematophagous fungus.

The applications were carried out on the same first half part (175 m^2) of an unheated greenhouse (total area of 350 m^2), in both 2008 and 2009. Ten rows of green peppers were planted in both years.

The *A. oligospora* treatment was carried out in wet soil, in both years, also on the first half part (175 m^2) of the unheated greenhouse.

The Trifender plant protection product (0.25% dilution, rate of 1.15 l/m^2) was poured to the base of green pepper seedling stems. *A. oligospora* (0.25% dilution, rate of 0.2 l/m^2) was sprayed in a steady manner on the first half part of the greenhouse and immediately worked into the soil.

In the Trifender treated and control area, the samplings were done on 2nd July 2008 and 23rd June 2009. In the *A. oligospora* treated and control area, samples were taken on 13th July 2008 and 23rd June 2009 (at the time of full development of the 1st generation of *M. hapla*). Five samples were taken from each replication. Altogether, 40 green pepper roots were investigated in both years and both treatments (Trifender and *A. oligospora*).

After washing off the roots of the green peppers, they were placed in plastic sachets. A 2% formalin solution was poured into the sachets to conserve the samples. Stereo microscope was used to evaluate the roots and count the females.

Some of the samples were used to precisely identify the *Meloidogyne* species found in the greenhouse, on the basis of the international references of Whitehead (1968), Orton (1974), Jepson (1987) and Karssen (2002).

The microscopic image of *M. hapla* was taken by LEICA Image Maker Program.

The heights of the Trifender treated and control plants were measured on 2nd July and 25th October 2008, and on 12 June and 26th August 2009. The *A. oligospora* treated and control plants were measured on 26th May in 2008, and 12th June and 26th August in 2009. Ten plants were measured in each replication.

On the basis of the number of *Meloidogyne* females and plant height data, the statistical analysis of the differences of the results of treated and untreated (the control) area, were done by Welch test.

Yield measurements of Trifender treated and control plants were done 2–2 times in 2008 and 2009. The yield measurements of *A. oligospora* treated and control plants were done 2 times in 2009, on the whole area of the treated and untreated plots, where the farmer collected peppers from treated and untreated areas in different cases.

RESULTS

Preliminary evaluation of root-knot nematode infection

In the case of Trifender, according to the preliminary evaluation carried out in the pre-crop, the infection showed rates of 2–3 on the average, according to the Zeck-scale (0–10).

We also observed Zeck-scale rates of 4, 5 and 6, so the infection rate was considered to be at the medium level in the greenhouse.

In the case of *A. oligospora*, the infection showed rates of 3–4 on the average, and we also observed Zeck-scale rates of 5, 6 and 7.

Identification of the *Meloidogyne* species found

During the identification, *Meloidogyne hapla* Chitwood (the Northern root-knot nematode) was identified in both treatments (Trifender and *A. oligospora*). No males were found, which is common regarding *M. hapla* (Fig. 4).

The main characteristics of the *M. hapla* female are based on the pattern of the perineum (soft arches of the cuticle furrows over the vulva and punctates of anal part), blurred side section, and its sizes. The polyphagous *M. hapla* can be found in greenhouses and open fields in Hungary (Andrássy and Farkas 1988).

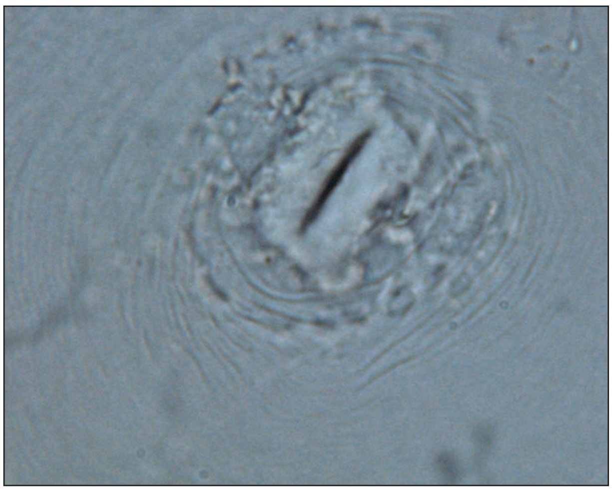

Fig. 4. The perineum of *M. hapla* female
(Photo by Mariann Kaminszky-Elekes)

Counting of *Meloidogyne hapla* females

Trifender

The results of 2008 determined that no significant difference ($p > 0.05$) was found regarding the number of females found on Trifender treated and untreated pepper roots. Trifender had no effect on the rate of females (Table 1). Table 1 shows that differences between certain replications are significant. One of the reasons may be that samples originated from different parts of the greenhouse (samples were collected from more infected spots, according to the preliminary evaluation), so the infection is not homogenous.

According to the Welch test, the results of 2009 showed a significant difference ($p < 0.001$) between Trifender treated and untreated samples (Table 2). According to the Abbott-formula (Abbott 1925) the efficiency was around 30%, which can be determined as side effect only.

Arthrobotrys oligospora

The results of 2008 showed that Welch test determined, there was no significant difference ($p = 0.08$) regarding the number of females found on *A. oligospora* treated and untreated pepper roots. *A. oligospora* had no effect on the rate of females (Table 3).

The results of 2009 show that a significant difference ($p = 0.02$) was found between *A. oligospora* treated and untreated samples. According to the Abbott-formula (Abbott 1925) the efficiency was around 35%, which can be determined as side effect only (Table 4). This is similar to the results proved by Amin and Budai (1993).

Table 1. Comparison of the damage of root-knot nematodes in treated (Trifender) and untreated green pepper (Pusztamonostor, 2008)

Treatment	Dose	Average number of *M. hapla* females [pieces/plant] (2008)				
		1st replication	2nd replication	3rd replication	4th replication	average of replications
Trifender	0.25%	932	622.6	144.8	161.2	465.15
Control	–	666.2	750.6	413.8	130.4	490.25

Table 2. Comparison of the damage of root-knot nematodes in treated (Trifender) and untreated green pepper (Pusztamonostor, 2009)

Treatment	Dose	Average number of *M. hapla* females [pieces/plant] (2009)					efficiency [%] (abbott)
		1st replication	2nd replication	3rd replication	4th replication	average of replications	
Trifender	0.25%	210.6	211	185.8	190.6	199.5	33.24
Control	–	290.8	374	311.4	219	298.8	

Table 3. Comparison of the damage of root-knot nematodes in treated (*A. oligospora*) and untreated green pepper (Pusztamonostor, 2008)

Treatment	Dose	Average number of *M. hapla* females [pieces/plant] (2008)				
		1st replication	2nd replication	3rd replication	4th replication	average of replications
A. oligospora	0.25%	364.2	668.4	528.6	512	518.3
Control	–	152.6	306.8	226.6	288.8	243.7

Table 4. Comparison of the damage of root-knot nematodes in treated (*A. oligospora*) and untreated green pepper (Pusztamonostor, 2009)

Treatment	Dose	Average number of *M. hapla* females [pieces/plant] (2009)					efficiency [%] (abbott)
		1st replication	2nd replication	3rd replication	4th replication	average of replications	
A. oligospora	0.25%	282.6	271.2	231	432	304.2	35
Control	–	482.2	404.8	443.4	546.8	469.3	

Plant height measurement of green pepper plants

Trifender

In 2008 during the 1st measurement, the Trifender treatted plants were 8 cm taller, during the 2nd measurement in which the plants were 12 cm taller on the average, compared to the untreated plants. The Trifender treated plants proved to be significantly taller ($p < 0.01$) compared to the untreated plants (Table 5), according to the Welch test.

In the 1st and 2nd measurement in 2009, the difference proved to be significant ($p = 0.015$ and $p = 0.019$) between Trifender and the untreated control (Table 6). During the 1st measurement, the treated plants were on the average, 6 cm, and during the 2nd, 13 cm taller.

Arthrobotrys oligospora

In 2008, the plants treated with *A. oligospora* were 2 cm (6%) taller on the average, than untreated plants.

In 2009, during the 1st and 2nd measurement the difference was not significant between the treated and untreated plants ($p = 0.35$ and $p = 0.13$).

During the 1st measurement, the treated plants were 1 cm taller, and during the 2nd, 5 cm shorter, on the average (Table 7).

Table 5. Effect of Trifender on the plant height of the infected green pepper by root-knot nematodes (Pusztamonostor, 2008)

Treatment	Average plant height/plant [cm]			
	1st measurement (02/07/2008)		2nd measurement (25/10/2008)	
	average of replications	in % of untreated control	average of replications	in % of untreated control
Trifender	71.4	112	90.9	115
Control	63.5	100	78.9	100

Table 6. Effect of Trifender on the plant height of the infected green pepper by root-knot nematodes (Pusztamonostor, 2009)

Treatment	Average plant height/plant [cm]			
	1st measurement (12/06/2009)		2nd measurement (26/08/2009)	
	average of replications	in % of untreated control	average of replications	in % of untreated control
Trifender	57.6	111	87.8	118
Control	51.8	100	74.1	100

Table 7. Effect of *A. oligospora* on the plant height of the infected green pepper by root-knot nematodes (Pusztamonostor, 2009)

Treatment	Average plant height/plant [cm]			
	1st measurement (12/06/2009)		2nd measurement (26/08/2009)	
	average of replications	in % of untreated control	average of replications	in % of untreated control
A. oligospora	45.3	102	72.4	93.2
Control	44.5	100	77.7	100

Yield measurement

Trifender

During the 3rd and 4th harvest in 2008, 25% and 35% increases were determined in the amounts of harvested yields from the treated area (Table 8). During the 3rd harvest on the Trifender treated area 31.6 kg, and during the 4th harvest, 48 kg more of green peppers were collected.

In 2009, during the 3rd and 5th harvest, a 24% and 23% decrease were determined in the treated area compared to the untreated. During the 3rd and 5th harvest on the Trifender treated area, 9 kg less of green pepper were collected (Table 9).

Arthrobotrys oligospora

In 2009, during the 3rd and 5th harvest, a 25% and 22% decrease were determined in the treated area compared to the untreated. During the harvest on the *A. oligospora* treated area, there were 8 kg less of green peppers collected (Table 10).

Table 8. Results of the 3rd and the 4th harvest of green pepper treated with Trifender (Pusztamonostor, 2008)

Treatment	3rd harvest		4th harvest	
	yield [kg/m^2]	yield in % of untreated control	yield [kg/m^2]	yield in % of untreated control
Trifender	0.7	135	1.2	125
Control	0.5	100	0.9	100

Table 9. Results of the 3rd and 5th harvest of green pepper treated with Trifender (Pusztamonostor, 2009)

Treatment	3rd harvest		5th harvest	
	yield [kg/m^2]	yield in % of untreated control	yield [kg/m^2]	yield in % of untreated control
Trifender	0.16	76	0.17	77
Control	0.21	100	0.22	100

Table 10. Results of the 3rd and 5th harvest of green pepper treated with *A. oligospora* (Pusztamonostor, 2009)

Treatment	3rd harvest		5th harvest	
	yield [kg/m^2]	yield in % of untreated control	yield [kg/m^2]	yield in % of untreated control
A. oligospora	0.14	75	0.16	78
Control	0.18	100	0.2	100

DISCUSSION

From the *Meloidogyne* species, *M. hapla* was identified in the greenhouses. In the 1st year, Trifender and *A. oligospora* had no effect on the number of females, as no significant difference was proved when compared to the control. But Trifender could increase plant height by 12% and 15%. Its additional pest/pathogen mitigating effect is significant, as yield increased by 25–35%.

In the year 2009, a significant difference was proved regarding the number of females from the Trifender and *A. oligospora* treated plants compared to the untreated. The efficacy was 30% and 35% that can be the side effect only.

Concerning plant height in the case of Trifender, significant differences were also proved during the1st and 2nd measurement. But in case of *A. oligospora*, there were no significant differences between the plant heights.

The results of research studies are promising. It can be concluded that *Trichoderma* species and *A. oligospora* are likely to be applied in treatments against *Meloidogyne* root-knot nematodes.

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