DOI 10.2478/s11687-009-0043-6

HELMINTHOLOGIA, 46, 4: 230 – 240, 2009

Influence of arbuscular mycorrhizal fungi on the nematicidal properties of leaf extracts of *Thymus vulgaris* L.

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Summary

The effect of arbuscolar mycorrhizal fungi (AMF) on the nematicidal activity of *Thymus vulgaris* against the root-knot nematodes *Meloidogyne incognita* and *M. javanica* was investigated in two *in vitro* experiments. In the first experiment egg masses of *M. incognita* and *M. javanica* were immersed for 3 weeks in aqueous leaf extracts of thyme plants non-inoculated or previously inoculated with *Glomus mosseae* or mixed AMF strains (*Sclerocystis sinuosa*, *Glomus claroideum*-1, *G. claroideum*-2 and *G. claroideum*-3). Thereafter the hatching test continued in distilled water for five more weeks. In the second experiment egg masses of both *Meloidogyne* species were exposed to the different thyme extracts for 4, 8 and 16 hours and then incubated in distilled water for 8 weeks. Distilled water and 5 mg/ml aqueous solution of fenamiphos nematicide were used as controls. Numbers of second stage juveniles emerging weekly were expressed as cumulative percentages of the total egg content of the egg masses. In the first experiment juvenile emergence from eggs of both *Meloidogyne* species immersed in thyme extracts for three weeks was completely suppressed since the first week. Hatching of eggs of *M. incognita* in all the extracts was significantly lower than that in water control, although emergence in the extract from uninoculated thyme plants was significantly higher than the others and no statistical different from that of aqueous fenamiphos solution. Emergence of *M. javanica* juveniles was significantly lower after immersion in all the extracts than in distilled water control and aqueous fenamiphos solution. In the second experiment a 4-hour exposure to the extract from thyme inoculated with *G. mosseae* and mixed AMF population significantly reduced the final hatch of *M. incognita* in comparison to distilled water. A 16-hour exposure to the extract from mixed AMF inoculated plants resulted in a significantly lower egg hatch than the shorter exposure times, whereas no statistical difference was found between 4 and 8 hour exposure to both extracts. Emergence of *M. javanica* juveniles was statistically lower than in water only after 16 hours exposure to the extracts from mixed AMF strains inoculated plants, but no difference was found among the different exposure times. Growth of *T. vulgaris* was significantly increased only by the infections of mixed AMF strains.

Keywords: *Glomus* spp.; *Meloidogyne* spp.; nematicidal properties; *Thymus vulgaris*; *Sclerocystis sinuosa*

Introduction

Environment and human health concerns require to reduce nematicide use and to develope alternative safer and effective compounds for nematode control (Noling & Becker, 1994). Plants may represent a source of natural nematicides, as a high number of nematicidal compounds are already reported in many species (Chitwood, 2002).
Active principles from Lamiaceae plants and/or their constituents showed a broad spectrum of activity against insects, mites, plant pathogenic fungi and also nematodes (Isman, 2000).
Investigations focused mainly on the nematicidal properties of essential oils from many lamiaceous species (Oka *et al*., 2000; Pandey *et al*., 2000), whereas only few studies regarded the nematicidal activity of crude aqueous extracts or green biomass of these plants (Chatterjee *et al.*, 1982). Nematicidal properties of *Thymus* spp. was previously investigated on the virus-vector nematode *Xiphinema index* Thorne *et* Allen, which was consistently suppressed either by the aqueous extracts and the green biomass of different *Thymus* species (Insunza *et al*., 2001a; Aballay *et al*., 2004). The main active component of Thymus spp. is the phenolic monoterpene thymol, which demonstrated to reduce drastically population of various phytoparasitic nematode species soil either in vitro and in the soil (Soler-Serratosa *et al*., 1996).
Arbuscular mycorrhizal fungi (AMF) are obligatory

biotrophic symbionts living in the roots of most terrestrial plants (Gerdemann & Nicolson, 1963; Sieverding, 1991), among which also lamiaceous plants (Mago & Mukerji, 1994). Positive effects of AMF on plant nutrition and growth are well known (Marschner, 1997; Takács & Vörös, 2003), as well as other aspects such as biocontrol toward plant pathogens, tolerance to water stress, and adverse environmental conditions, but little is known about the potential of AMF to affect the content of active compounds generated by secondary metabolism pathways. Several studies reported the effect of mychorrizal symbiosis on the content of bioactive plant metabolites like phenols (Zhu & Yao, 2004), terpenoids (Akiyama & Hayashi, 2002) and also volatile compounds (Guerrieri *et al*., 2004), whereas only few reports are available on the influence of mycorrhizal colonization on essential oil content and biocidal properties of lamiaceous species (Copetta *et al*., 2006) and no specific investigation on thyme.
This paper reports the results of two *in vitro* assays aimed to verify the potential influence of root colonization by different strains of AMF on thymol content of *T. vulgaris* plants and, consequently, on the nematicidal activity of aqueous extracts of this lamiaceous plant on the root-knot nematodes *M. incognita* and *M. javanica*.

Materials and Methods

Plant growth and mycorrhizal parameters
Two months old thyme seedlings, previously sown in steam sterilized (6 hrs at 100 °C) sandy soil, were transplanted (2 plants per pot) in thirty clay pots filled with 4,000 g sterilised sandy soil (64.4 % sand, 18.5 % silt, 16.8 % clay, 1.1 % OM). Chemical characteristics of the soil were: $pH_{(H2O)}$ 8.25, $pH_{(KCl)}$ 7.55, AL-P_2O_5 888 mg.kg^{-1}, AL-K_2O 397 mg.kg^{-1}, KCl-NH_4-N 2.91, KCl-NO^3-N 11.6 mg kg^{-1}, humus content 0.9%. After one month, batches of ten pot s were inoculated with *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe or with a mixed population of AMF strains (*Sclerocystis sinuosa* (Gerdermann & Bakshi) Almeida & Schenck, *Glomus claroideum*-1, *G. claroideum*-2 and *G. claroideum*-3 Schenck & Smith), both at a density of 10 % w/w inoculum pot^{-1}). AMF species were isolated from a calcareous chernozem soil at Nagyhörcsök (Hungary). Ten pots with non-inoculated plants were used as control.
Four months after the AMF inoculation, plants of each group were uprooted and the effect of mycorrhizal infection on plant growth was evaluated recording total plant weight and top dry weight. Mycorrhizal dependency of thyme plants was calculated by expressing the difference between the total plant weight or top dry weight of the mycorrhizal inoculated and the non-inoculated plants as the percentage of the total plant weight or top dry weight of mycorrhized inoculated plants (Plenchette *et al*., 1983). Root samples collected from each plant were cleaned and stained by Phillips and Hayman's method (1970) and indigenous AMF colonizations were counted on the intact root systems by a stereoscopic dissecting microscope. Frequency of mycorrhizal infection and quantity of arbuscules on thyme roots were estimated by scoring the density of infection on five 30 cm root segments by a five class system (Trouvelot *et al*. 1986).

Experiments
Thyme extracts were prepared by soaking green leaves from the uprooted plants in distilled water (1:4) for 24 hrs. Leaves were then comminuted in a blender (8,000 rpm) and the suspension filtered through Non Waven N° 261/A circular filter paper. Extracts were then stored in plastic bottles and kept in a freezer until used.
Batches of 25 egg masses (averaging 400 eggs per mass) of *M. incognita* (Kofoid *et* White) Chitw. and *M. javanica* (Treub) Chitw., previously reared on tomato cv. Roma in glasshouse at 25 $\pm$ 2 °C, were handy picked up from infested roots and placed in 2 cm diam sieves (215 mm aperture). Each sieve was put in a 3.5 cm diam Petri dish and three ml of each test solution were added to submerge the egg masses. Dishes were arranged in a complete randomized block design with four replicates per treatment and incubated in a growth cabinet at 20 °C.
In the first experiment the egg masses were removed from the test solutions after three weeks and then the incubation continued in distilled water for five more weeks (Sasanelli & Di Vito, 1991).
In a second experiment the batches of egg masses were immersed for 4, 8 and 16 hours in the leaf extracts and then transferred to distilled water and incubated as in the first experiment. There were four replications for each treatment.
Distilled water and 5 mg/ml aqueous solution of fenamiphos (ethyl 4-methylthio-m-tolyl isopropylphosphoramidate) (Greco & Thomason, 1980) were used as controls in the first experiment and only distilled water in the second.
Emerged second stage nematode juveniles were removed and counted at weekly intervals, renewing distilled water at the same time. The hatching test was run for eight weeks.
At the end of both experiments, the egg masses were dissolved by shaking in a 1 % sodium hypochlorite aqueous solution (Hussey & Barker, 1973) and the unhatched eggs were counted. Numbers of second stage juveniles emerging weekly were expressed as cumulative percentages of the total egg content of the egg masses (hatched + unhatched eggs). Percentage hatches from the second experiment were referred to control (100 %) in order to allow comparison of data of the two nematode species.
Data from the first experiment were statistically analysed, after transformation in arcsen root square values, by ANOVA and means compared by Least Significant Difference's Test and Student's *t* Test. In the second experiment the effects of leaf extracts, exposure times and nematode species and their interactions were examined by statistic analysis of variance for a 4 x 3 x 2 factorial design.

Analysis of thymol in thyme leaves and their aqueous extracts
1. Fresh leaves were chopped in small pieces and they were put in HS tubes (150 – 200 mg) and subjected to GC

analysis.
2. Leaf aqueous extracts. Chopped leaves of thyme plants were homogenized in a mortal, diluted in distilled water (1:4, fresh weight ratio) and shaken overnight.
Thyme leaves and aqueous extracts were sealed in vials and stored at -20 °C until analysis.

Table 1. Extent of the AMF infection (F%) and arbuscular richness (A%) in roots of thyme in soil inoculated with *Glomus mosseae* or a mixture of AMF strains

Treatment	Frequency of the AMF infection (F%)*	Extent of the AMF arbuscularity (A%)*
T vulgaris + *G. mosseae*	94.6[a] A[b]	31.1 A
T vulgaris + Mixed AMF	85.3 A	11.3 B
T vulgaris	0.0 B	0.0 C

Thymol content was measured by gas chromatography (Perkin Elmer Clarus 500 gas chromatography and Headspace). Quantification was based on multiple headspace extraction (MHE) and total vaporization technique (TVT) with FID detector, eliminating the matrix effects. Thymol content was determined in comparison to Thymol std. (Reanal) and each measure was repeated three times for each sample.
Data from GC analysis were statistically analysed by ANOVA and means compared by Least Significant Difference's Test.

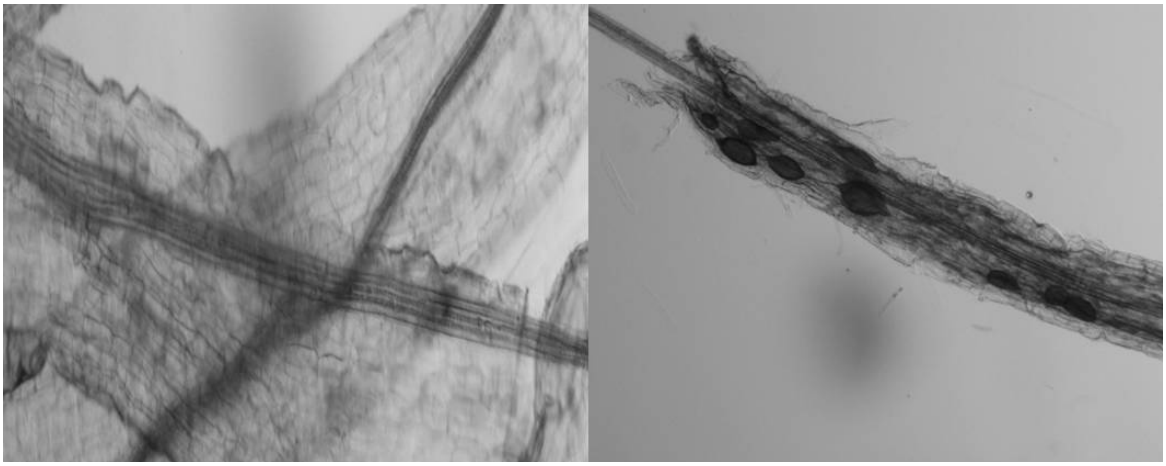

Fig. 1. Segments of thyme roots non inoculated (on the left) and vesicules in *Glomus mosseae* infected thyme root (on the right)

Results

Microscopic observation of thyme root samples did not reveal AMF colonization in sterilized soil neither before and after transplanting. A high root colonization was observed in the plants inoculated with *G. mosseae* or mixed AMF strains, as frequencies of infection of 95 and 85%, respectively, were calculated, without significant differences between the two treatments (Table 1; Fig. 1). Extent of the AMF arbuscularity, indicative of the symbiotic efficiency of endomycorrhizal fungi, was significantly higher in the roots inoculated with *G. mosseae* (31 %) than in samples from plants infected with mixed inoculum (11 %).
Biomass production of mixed AMF inoculated plants was significantly increased by AMF colonization. Total plant weight and top dry weight of mycorrhized thyme plants were 38 – 113 % and 11 – 17 % higher than non-inoculated plants (Table 2). No significant difference was observed between the two AMF treatments, whereas plants inoculated with mixed AMF strains resulted significantly heavier than non inoculated control. Mycorrhizal dependency was 28 and 10 % in *G. mosseae* and 53 and 15 % in mixed AMF strains, respectively, on the base of total plant weight and top dry weight.
In the first experiment, most of the eggs from the egg masses in distilled water hatched within three weeks (Table 3 and 4). Aqueous solution of fenamiphos significantly reduced hatching of both *Meloidogyne* species compared to distilled water. Emergence of *M. incognita* and *M. javanica* was almost completely suppressed in *T. vulgaris* leaf extracts from uninoculated plants since the first week, resulting significantly lower than that in water control and fenamiphos solution.
After the third week and until the end of the experiment emergence of juveniles of *M. incognita* in all the extracts was not different from fenamiphos, although emergence in the extract from uninoculated plants was significantly higher than that in the other extracts. Eggs of *M. incognita* previously exposed to fenamiphos solution showed a significantly hatch increase in the last week of the experiment (Table 3).
Emergence of *M. javanica* juveniles in the extracts from mychorrized thyme plants was suppressed since the first week in comparison with that in water and fenamiphos solution. Egg hatch was always significantly lower in all thyme extracts than in fenamiphos solution, but mortality of eggs exposed to the extracts from *G. mosseae* inoculated plants was significantly higher than that in the other two extracts (Table 4).
Egg hatch of both *Meloidogyne* species was not furtherly increased after removing egg masses from the thyme extracts, thus suggesting that these extracts were lethal to the

Table 2. Effect of AMF inoculation on the growth of *Thymus vulgaris*

Treatment	Total plant weight (g)	% Increase*	Mycorrhizal dependency	Top dry weight (g)	% Increase	Mycorrhizal dependency
T.vulgaris + *G. mosseae*	116.7 AB[a]	38	28	13.6 A	11	10
T.vulgaris + Mixed AMF	180.0 B	113	53	14.4 A	17	15
T.vulgaris	84.6 A	---		12.3 A	---	

Mean of five replicates.
[a]Data flanked in each column followed by same letters are not statistically different according to LSD's Test (P=0.01);
* Compared to non-inoculated thyme

eggs. At the end of the experiment for both *Meloidogyne* species finally cumulative hatching percentage from the egg masses exposed to the all extracts and fenamiphos solution was significantly lower than that of control in distilled water.

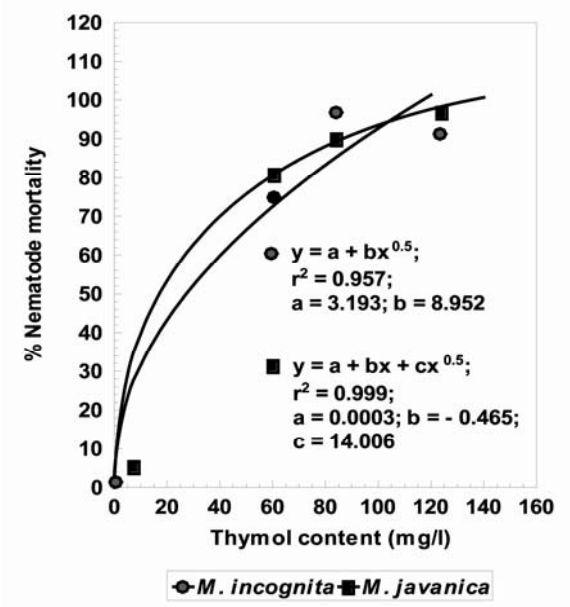

Fig. 2. Relationship between *M. incognita* and *M. javanica* per cent egg mortality (*y*) and thymol content in leaf aqueous extract (mg/l).

Mortality of *M. incognita* and *M. javanica* eggs exposed to thyme extracts resulted also significantly higher than fenamiphos with the exception for extract from uninoculated plants on *M. incognita*. Moreover, extract from thyme plants inoculated with *G. mosseae* was significantly more effective than those from mixed AMF strains inoculated plants and, for *M. javanica*, also than extract from non inoculated plants.

In the second experiment the final hatch of *M. incognita*, expressed as percentage of the control (100 %), was significantly reduced after 4 hour exposure to the extract from thyme inoculated with *G. mosseae* and mixed AMF population in comparison to distilled water (Table 5). No statistical difference was found between 4 and 8 hour exposure to both extracts, whereas there was a significant decrease after 16 hour exposure to the extract from mixed AMF population inoculated plants compared to the shorter exposure times. The hatch of *M. incognita* in the extract from non-inoculated thyme plants was lower than that in water control after 8 hour immersion of the egg masses. In this extract a significant difference was found only between 4 and 8 hour exposure times.

Juvenile emergence of *M. javanica* in thyme extracts was statistically lower than the water control only after 16 hours exposure to the extracts from mixed AMF strains inoculated plants. No difference was found among the different exposure times.

Factorial analysis of variance showed highly significant main effects for the factors type of extract, exposure time and nematode species. Significant interactions were found between nematode species and the other factors (extracts and exposure time), whereas there was no interaction of extracts with exposure time. Interaction among the three factors resulted significant at P = 0.01.

A significant difference was found in thymol content of leaves and aqueous extracts of *Thymus vulgaris* plants inoculated and uninoculated with AMF (Table 6).

Based on the results of the first experiment nematode egg mortality was positively correlated with leaf thymol content (Fig. 2), as well as a mathematical relationship between nematode egg mortality and exposure time to different extracts was fitted to the experimental data from the second experiment (Table 7). The equations reasonably explain the above relationships, as indicated by the high values of the correlation coefficient R^2.

Discussion and Conclusions

Experiments *in vitro* evidenciated the strong biocidal effect of thyme extracts on both *Meloidogyne* species although this effect was evident only after a 16 hour exposures. Nematicidal activity of crude extracts from lamiaceous plants was already reported in previous experiments. Insunza *et al.* (2001a; 2001b) found that a 24 hours exposure to leaf and flower aqueous extracts of *Thymus serpyllum* L. was lethal to *Xiphinema index* and *X. americanum s. l.* Chatterjee *et al*. (1982) reported that exposure of *M. incognita* juveniles to the crude extracts of *Ocimum sanctum* L. and *O. basilicum* L. resulted in 100% mortality within 120 and 160 min.

Table 3. Effect of leaf extracts of *Thymus vulgaris*, inoculated or uninoculated with AMF, on the percentage cumulative hatch of *Meloidogyne incognita*

Treatment	Incubation period (weeks)																	
	In test solutions						In distilled water											
	1		2		*t**	3		*t*	4	*t*	5	*t*	6	*t*	7	*t*	8	*t*
T.vulgaris + *Glomus mossae*	5.6 (±4.1)	AB	6.0 (±4.6)	AB	-	6.2 (±4.8)	A	-	6.2 (±4.8) A	-	6.2 (±4.8) A	-	6.2 (±4.8) A	-	6.2 (±4.8) A	-	6.2 (±4.8) A	-
T. vulgaris + mixed AMF	0.8 (±0.5)	A	1.0 (±0.3)	A	-	2.5 (±1.8)	A	-	2.5 (±1.8) A	-	2.5 (±1.8) A	-	2.5 (±1.8) A	-	2.5 (±1.8) A	-	2.5 (±1.8) A	-
T.vulgaris	2.1 (±1.4)	AB	2.2 (±1.5)	AB	-	17.7 (±7.5)	B	**	17.7 (±7.5) B	-	17.7 (±7.5) B	-	17.7 (±7.5) B	-	17.7 (±7.5) B	-	17.7 (±7.5) B	-
Fenamiphos (5 µg/ml)	6.2 (±2.1)	B	6.9 (±2.2)	B	-	7.2 (±2.1)	AB	-	7.3 (±2.1) AB	-	8.1 (±2.3) AB	-	9.9 (±0.8) AB	-	10.9 (±1.6) AB	-	22.7 (±4.9) B	**
Distilled water	22.0 (±5.5)	C	46.2 (±5.9)	C	**	70.0 (±3.6)	C	**	74.7 (±4.8) C	-	75.7 (±5.1) C	-	75.9 (±5.1) C	-	75.9 (±5.1) C	-	75.9 (±5.1) C	-

Values are means of four replications. Means followed by the same letters on the same column are not significantly different according to Least Significant Difference (LSD) Test (P = 0.01).
* Significance of differences compared to the previous week according to Student's *t* Test (* for P = 0.05; ** for P = 0.01).

Table 4 . Effect of leaf extracts of *Thymus vulgaris*, inoculated or uninoculated with AMF, on the percentage cumulative hatch of *Meloidogyne javanica*

Treatment	Incubation period (weeks)															
	In test solutions						In distilled water									
	1		2	*t**	3	*t*	4	*t*	5	*t*	6	*t*	7	*t*	8	*t*
T. vulgaris + *Glomus mossae*	0.9 (±1.1)	A	1.0 (±1.2)	A -	1.0 (±1.2)	A -	1.0 (±1.2)	A -	1.0 (±1.2)	A -	1.0 (±1.2)	A -	1.0 (±1.2)	A -	1.0 (±1.2)	A -
T. vulgaris + mixed AMF	1.7 (±1.4)	A	2.1 (±1.8)	A -	6.7 (±2.1)	B *	6.7 (±2.1)	B -	6.8 (±2.1)	B -	6.8 (±2.1)	B -	6.8 (±2.1)	B -	6.8 (±2.1)	B -
T. VULGARIS	4.4 (±3.5)	A	5.3 (±4.5)	A -	13.0 (±7.7)	B -	13.2 (±7.9)	B -	13.2 (±7.9)	B -	13.2 (±7.9)	B -	13.2 (±7.9)	B -	13.2 (±7.9)	B -
Fenamiphos (5 µg/ml)	25.8 (±3.4)	B	28.4 (±2.7)	B -	29.0 (±2.4)	C -	29.1 (±2.4)	C -	29.1 (±2.4)	C -	29.7 (±2.4)	C -	30.9 (±2.0)	C -	30.9 (±2.0)	C -
Distilled water	23.5 (±5.8)	B	45.1 (±8.1)	C **	63.7 (±5.9)	D *	65.8 (±6.1)	D -	66.6 (±5.8)	D -	67.0 (±5.6)	D -	67.1 (±5.6)	D -	67.1 (±5.6)	D -

Values are means of four replications. Means followed by the same letters on the same column are not significantly different according to Least Significant Difference (LSD) Test (P = 0.01).
* Significance of differences compared to the previous week according to Student's *t* Test (* for P = 0.05; ** for P = 0.01).

Table 5. Hatch of *Meloidogyne incognita* and *M. javanica* after different exposure times to leaf extracts of *Thymus vulgaris*, inoculated and uninoculated with AMF

Treatment	Exposure times (hours)												LSD (P=0.01)	
	4				8				16					
	M. incognita		*M. javanica*		*M. incognita*		*M. javanica*		*M. incognita*		*M. javanica*		*M. inc.*	*M. jav.*
T. vulgaris + AMF (*Glomus mossae*)	86.9 (± 6.8)	A[(1)]	93.8 (± 8.0)	A	76.0 (± 11.1)	A	92.0 (± 6.0)	A	71.2 (± 9.1)	A	85.3 (± 6.9)	AB	21.1	16.1
T. vulgaris + AMF (Mixed population)	88.2 (± 5.6)	A	94.2 (± 6.6)	A	85.7 (± 4.5)	AB	89.7 (± 11.1)	A	70.1 (± 6.0)	A	79.3 (± 5.6)	A	12.3	18.6
T.vulgaris	91.3 (± 3.7)	AB	93.8 (± 7.4)	A	73.8 (± 6.6)	A	92.5 (± 7.6)	A	79.2 (± 10.5)	A	83.8 (± 12.7)	AB	17.1	21.9
Mean	88.8		78.6		78.5		91.4		73.5		82.8		---	---
Distilled water (Control)	100.0	B	100.0	A	100.0	B	100.0	A	100.0	B	100.0	B		

ANOVA F values	
Factor A – Type of extract	55.7 **
Factor B - Exposure times	14.5 **
Factor C – Nematode species	24.1 **
A x B	2.2
A x C	3.0 *
B x C	0.7 **
A x B x C	0.5 **

Values are means of four replications. (1) Means followed by the same letters on the same column are not significantly different according to Least Significant Difference (LSD) Test (P = 0.01).
* = F values significant at P = 0.05; ** = F values significant at P = 0.01.

Table 6. Thymol content in leaves and aqueous extracts of *Thymus vulgaris* plants inoculated and uninoculated with AMF

Treatment	Thymol content			
	In leaf tissues (mg/Kg f.w.)		In aqueous extract (mg/l)	
T. vulgaris + *G. mosseae*	1 570[1]	A[2]	126	A
T. vulgaris + Mixed AMF	1 077	B	86	B
T. vulgaris	742	C	59	C

[1]Mean of three replications.
[2]Data flanked in the column followed by same letters are not statistically different according to LSD's Test (P=0.01)

Essential oils in thyme plant tissues and their components may be considered as main responsible of the biocidal activity of this species. Thyme contains a 1 – 2 % of essential oils, mainly represented by the isomeric monoterpenoids thymol (30 – 70 %) and carvacrol (3 – 15 %) (Zambonelli *et al*., 2004; Reddy *et al*., 1998; Rustaiyan *et al*., 2000). However, other compounds are present as cymol, terpinene-4-ol, thymol-methyl-ether and caryophyllene (Fig. 3).

Nematicidal properties of thyme essential oil and of thymol and carvacrol are largely demonstrated, as Oka *et al*. (2000) found that essential oil of *T. vulgaris* reduced juveniles mobility and egg hatching of *M. javanica* and 250 ml/l thymol and carvacrol inhibited egg hatch on the same nematode. At the same concentration, thymol and carvacrol caused 100 % mortality of *Caenorhabditis elegans*, but were less potent against the root lesion nematode *Pratylenchus penetrans* (Tsao & Yu, 2000). A 161 ppm lethal concentration (LC_{90}) of thymol in soil was found against *M. arenaria* on soybean (Soler-Serratosa *et al*., 1996).

Moreover, thyme essential oil and its main components have been also reported for fungicidal and antibacterial activities (Panizzi *et al*., 1993; Muller-Ribeau *et al*., 1995; Janssen *et al*., 1987; Kurita *et al*., 1981; Paster *et al*., 1990; Daferera *et al*., 2000; 2003), but showed also herbicidal effects (Angelini *et al*., 2003; Tworkoski, 2002) and insecticidal properties (Duke *et al*., 1992; Regnault-Roger *et al*., 1993).

As a mechanism of interruption of nervous system was found for thymol and carvacrol in insects (Ryan & Byrne, 1988), a similar mode of action could be supposed for the nematicidal action of thyme (Korayem *et al.*, 1993).

Broad spectrum of action of thyme makes this plant a valuable species for the preparation of environmentally safe biopesticides. In a small pot experiment in soil infested by *M. javanica* thymol (75 mg/kg soil) and especially carvacrol reduced formation of galls on tomato roots, whereas in a 3 l pot experiment on cucumber 200 mg/kg soil of essential oils containing carvacrol were more effective than those containing thymol (Oka *et al*., 2000).

However, possible negative effect of essential oils on soil microrganisms and particularly mychorrizae should be also evaluated. Mycorrhizal colonization by *Glomus intraradices* Schenck & Smith on tomato was negatively affected by treatments with 250 ml carvacrol and 250 mg thymol/100 ml distilled water, whereas infectivity of *G. mosseae* was inhibited only by thymol (Calvet *et al*., 2001).

A possible alternative to liquid formulations could be represented by the use of thyme as green manure. Soil incorporation of the aerial parts of *T. vulgaris* as green manure significantly suppressed population of *X. index* on

Table 7. Relationship between *M. incognita* and *M. javanica* per cent egg mortality (y) and exposure time (x), in different thyme extracts

Treatment	Nematode species	Equation	R^2
T. vulgaris + *G. mosseae*	*M. incognita*	$\ln y = 3.7814 + 1.6963/x^{0.5} - 53.9746^{-x}$	1.00
	M. javanica	$\ln y = 1.9521 + 0.0007\ x^{2.5} - 22.3489^{-x}$	0.99
T. vulgaris + Mixed AMF	*M. incognita*	$\ln y = 2.557 + 0.0002\ x^{3} - 15.1476^{-x}$	0.99
	M. javanica	$\ln y = 2.1925 + 0.0008\ x^{2.5} - 68.393^{-x}$	1.00
T. vulgaris	*M. incognita*	$y = 0.5059 + 6.0799\ x^{0.5}$	0.75
	M. javanica	$\ln y = 1.9069 + 0.0002\ x^{3} - 14.258^{-x}$	0.99

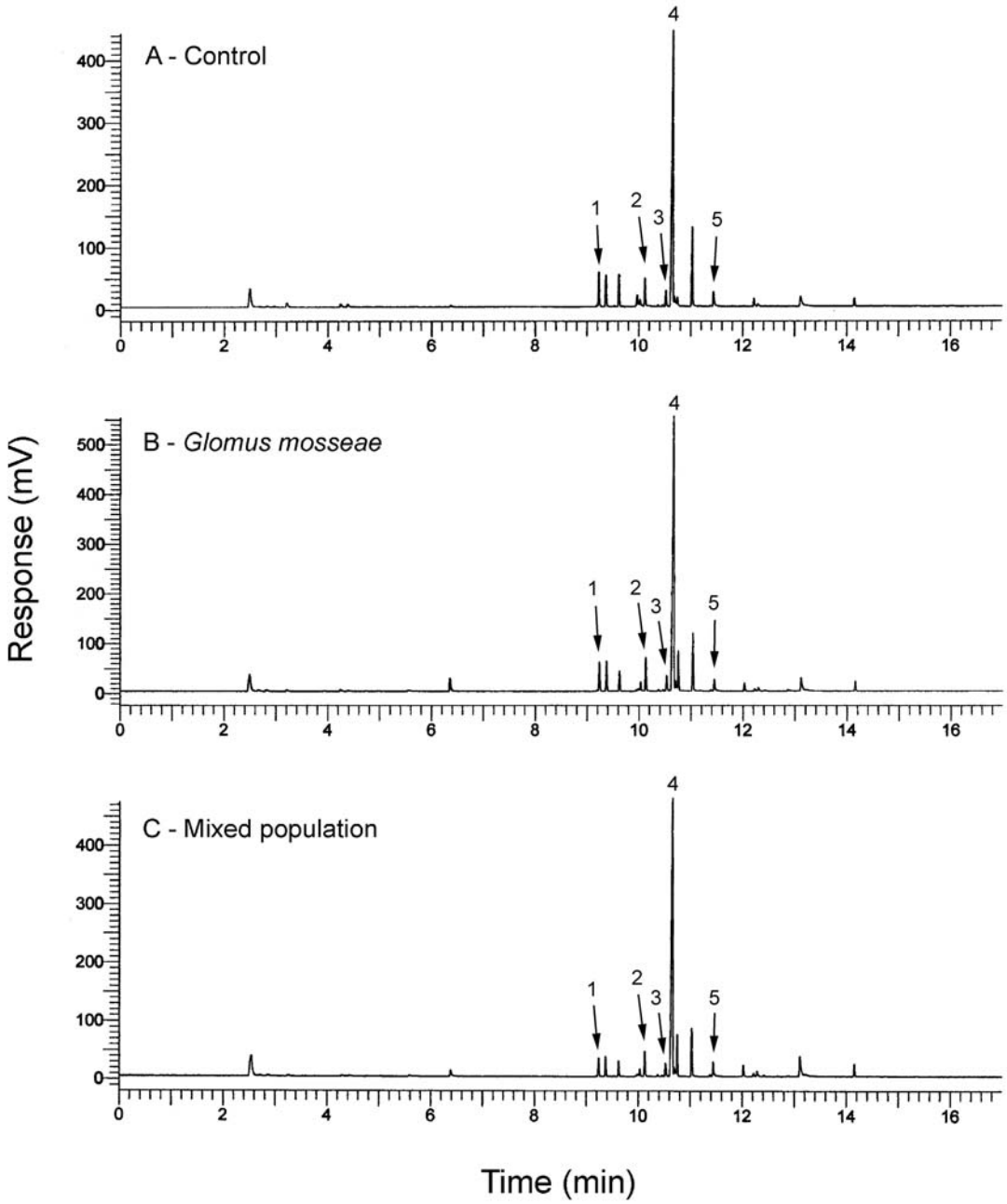

Fig. 3 Chromatogram of the main volatile compounds (1=cymol; 2=terpin-4-ol; 3=thymol-methyl-ether; 4=thymol; 5=caryophillene) in leaves of uninoculated thyme plants (A) or inoculated with *Glomus mosseae* (B) or mixed population (C).

grapevine when added at 2 % w/w in a greenhouse pot experiment (Aballay *et al*., 2004), but was not suppressive on *X. index* and *X. americanum s.l.* in field trials (Aballay & Insunza, 2002; Aballay *et al*., 2001).

Chemical analyses showed that thymol content was increased by mychorrization of thyme roots. Few reports are available about the effects of AM fungi on the production of essential oils in a limited choice of lamiaceous species. A relation between the presence of AM fungi, increased growth, essential oil accumulation, and improved mineral uptake was reported for *Mentha arvensis* (Khaliq & Janardhanan 1997; Gupta *et al*., 2002; Freitas *et al*., 2004). Khaosaad *et al*. (2006) showed that *G. mosseae* increases the concentration of essential oils in two genotypes of *O. vulgare* but not in P-fertilized nonmycorrhizal plants. Moreover, in a comparative analysis of the effects induced by three AM fungi, Copetta *et al*., (2006) found that inoculation with *Gigaspora rosea* Nicolson & Schenck BEG 9 increased biomass, root branching and length, and total amount of essential oil in basil *O. basilicum* L. and that increased oil yield was associated to a significantly larger number of peltate glandular trichomes (main sites of essential oil synthesis) in the basal and central leaf zones.

Increased growth and development in AM plants, com-

pared to nonmycorrhizal ones, was reported for many different species (Smith & Read, 1997). The increase of biomass production observed in AMF infected thyme plants would be useful in the perspective of using these plants as a green manure or producing nematicidal formulations. The results of the present work concerning *T. vulgaris* are in agreement with such reports. However, effects of mychorrization on plant development may be different, depending on the fungal species (Copetta *et al.*, 2006).
Therefore, in conclusion, mychorrized thyme plants soil could be suggested for integrated nematode management strategies, as it provides a good pest suppressivity reducing the impact on soil - plant equilibrium compared to chemical nematicides.

Acknowledgements

Work was carried out in the framework of the Bilateral Agreement between CNR (National Research Council - Italy) and MTA (Hungarian Academy of Sciences - Hungary) 2007-2009.
The Authors contributed equally in conduction of the experiments.

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RECEIVED JULY 8, 2009 ACCEPTED SEPTEMBER 3, 2009