Hungarian University of Agriculture and Life Sciences

Effect of different mulching materials on *Meloidogyne incognita* and beneficial nematodes

Thesis of Doctoral (PhD) dissertation

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1. Scientific background and objectives

Soil surface mulching has a very complex function and offers various advantages, most of which are in line with the principles of integrated pest management. Mulching regulates the effects of soil physical and chemical parameters as well. From a crop protection point of view, mulching influences indirectly pests, acts as a kind of physical barrier and more diverse and numerous predator communities appear in the mulched areas.

A large amount of raw material is produced every year, which is excellent for use as a soil cover material. However, a significant part of the fallen leaves in the autumn, or other municipal green waste produced in the home gardens, serves energy purposes, although it could be used as mulching material and applied locally.

According to a comprehensive study, smallholder farmers often misdiagnose the symptoms of plant-parasitic nematodes as a plant response to biotic or abiotic factors. Only few farmers can identify the damage and are dissatisfied with the available crop protection methods against plant-parasitic nematodes. The use of available control procedures is often limited by their effectiveness, cost, and the impact on non-target organisms or human health. These problems pose challenges the controlling of plant-parasitic nematodes not only in tropical areas (like in African countries) but also in Hungary.

An alternative and environmentally friendly solution could be the use of green waste of municipal origin for crop protection purposes in the control strategy against plant-parasitic, especially root-knot nematodes (*Meloidogyne* sp.). Numerous scientific reports deal with the reduction of *Meloidogyne*-populations and their damage by organic mulching, but this process is not part of the current crop protection strategies.

The aim of my research work was the examination of the effect of leaf litter mulching on the rootdamage caused by artificial *Meloidogyne incognita*-infestation in an open-field tomato experiment. Mononchida predatory nematodes could be a potential natural enemies of plant-parasitic nematodes. Therefore, the helping and enhancing of mononchids is an essential task. To that effect, the density of mononchids nematodes was followed in every year under mulched and unmulched plots.

During the yield evaluations of the tomato plants, a new pest problem occurred in the mulched plots: the damage of snails, slugs and wireworms was recorded. This phenomenon has suggested that entomopathogenic (Heterorhabditidae, Steinernematidae) and snail parasitic (Phasmarhabditidae) nematodes may play a significant role in the biological control of new pests. In order to successfully apply these beneficial organisms together with organic mulching in the future, it is important to find out whether the mulching materials have a negative effect on these nematodes.

Over the years, the main experiment was expanded with field and laboratory experiments and studies. In the case of the open field experiment, additional mulch materials were included in the experiments. In addition, the direct (lethal and repellent) effect of the extracts derived from the mulch materials was also examined in order to find out how mulching can affect *M. incognita* individuals. Moreover, pH and tannin content of the extracts were also determined as additional background data.

In parallel with, yet in connection with this research area, a series of experiments was began with the aim of the biological disposal of *Meloidogyne*-infested roots. As a matter of fact, these infectious plant materials should not be placed in the compost because compost can be a source of infection. To test this, tests were performed with earthworms and woodlice, representing two animal groups in the composter. These studies were sorted into three main topics: suppressivity study, food preference, and decomposition experiments.

The aims of my PhD work were the following:

Investigation of the effect of mixed leaf litter mulching on the damage of southern root-knot nematode (*M. incognita*) in an open-field microplot experiment on tomato plant.

Investigation of the effect of mixed leaf litter mulching on changes in the number of mononchid preadtory nematodes.

Investigation of lethal and repellent effects of extracts derived from mulch materials on *M. incognita* juveniles under laboratory conditions.

Investigation of the lethal effect of extracts derived from mulch materials on the juveniles of entomopathogenic (Heterorhabditidae, Steinernematidae) and slug-parasitic (Phasmarhabditidae) nematodes under laboratory conditions.

Investigation of the effect of animals (earthworms, woodlice) in compost on the damage of *M. incognita* in pot and laboratory experiments.

2. Materials and methods

Experiments can be grouped into three categories:

- Field experiments with muching (Table 1),
- Laboratory experiments with extracts derived from mulch materials (Table 2),
- Pot and laboratory experiments with organisms living in compost (Table 3).

2.1 Additional examinations for experiments

- Yield measurement and fruit health assessment;
- Determination of *Meloidogyne* damage based on three rating scales: Zeck (1971), Garabedian, and Van Gundy (1984), and Mukhtar et al. (2013) scales;
- Identification to species of *Meloidogyne* females based on the preparation method described by Hartman and Sasser (1985);
- Extraction of free-living nematodes from soil samples according to the modified version of the Baermann funnel method (Szakálas et al. 2015);
- Identification to species of predatory nematodes as described by Andrássy (2009) and Ahmad and Jairajpuri (2010);
- Physical characteristics of soil of the open-field experiment: soil compaction with LD-Agro penetrometer, soil moisture content with Stelzner Tensiometer, soil temperature with puncture thermometer, air temperature with Voltcraft DL-101-T data logger thermometer;
- Chemical characteristics of soil of the open-field experiment: determination of pH by the method of Buzás (1988), and organic matter content by the method of Walkley-Black (1947);
- Biological characteristics of soil of the open-field experiment: density of earthworms according to the method ISO 23611-1 2006, mycorrhizal root colonization according to the ink-vinegar staining technique described by Vierheilig et al. (1998);
- Evaluation of tannin content of mulch extracts by UV-VIS spectrophotometry on behalf of the Corvinus-Fitolabor Kft. Laboratory of the Corvinus University of Budapest in accordance with MSZ ISO 9648: 1994.
- pH measurement of mulch extracts with Voltcraft pH-212 pH tool;
- Identification to species of woodlice based on the descriptions of Farkas and Vilisics (2013).

Table 1 Detailed data of open-field microplot mulching experiments

Location	Year	Treatments	No. of combinations	No. of replications/ combinations	Plot size	No. of plots	Area	Related student
Gödöllő	2016 2017	Mulched with mixed leaf litter or unmulched Irrigated or non-irrigated Infested with *M. incognita* artificially or not Inoculated with mycorrhiza or not	16	6	2 × 2 m	24	96 m^2	Máté Czuppon Mónika Erdei Mónika Erdélyi
	2018	Mulched with mixed leaf litter or unmulched Irrigated or non-irrigated Infested with *M. incognita* artificially or not	8	12				
	2019	Mulched with mixed leaf litter or unmulched Infested with *M. incognita* artificially or not	4	24				
Gödöllő	2019	Mulched with mixed leaf litter Mulched with walnut leaf litter Mulched with yard-waste compost Mulched with wheat straw Unmulched control Infested with *M. incognita* artificially or not	10	16 (mulched) 8 (control)		36	144 m^2	Nándor Rózsa
Szolnok	2019	Mulched with wheat straw or covered with plastic mulch Treated with fertilizer or sheep manure Treated with walnut leaf litter extract or garlic extract Infested with *M. incognita* artificially or not	24	4		24	96 m^2	Nataly Ftaimi

Table 2 Detailed data of laboratory experiments with aqueous extracts of organic mulch materials

<table>
<tr><th>Aim</th><th>Examined species</th><th>Extract</th><th>Concentration (%)</th><th>Exposure time</th><th>No. of indiv./ replication</th><th>No. of repl./ control</th><th>No. of repl./ extract</th><th>Induction with lactic acid</th><th>Medium</th><th>Related student</th></tr>
<tr><td rowspan="6">Mortality test</td><td rowspan="2">Heterorhabditis bacteriophora
Steinernema carpocapsae
Steinernema feltiae
Steinernema kraussei
Phasmarhabditis hermaphrodita</td><td>Walnut leaf litter
Compost
Maple leaf litter</td><td>0.1; 0.5; 1; 5</td><td rowspan="6">24 hours</td><td rowspan="2">5 juveniles (J_3)</td><td rowspan="6">8</td><td rowspan="6">4</td><td rowspan="6">+</td><td rowspan="6">Microplate</td><td></td></tr>
<tr><td rowspan="2">Neem leaf litter</td><td>0.1; 0.3; 0.6; 1</td><td rowspan="2">Pratik Doshi</td></tr>
<tr><td rowspan="2">Meloidogyne incognita</td><td>0.1; 0.3; 0.5; 1</td><td rowspan="2">5 juveniles (J_2)</td></tr>
<tr><td>Walnut leaf litter
Oak leaf litter
Sycamore leaf litter
Maple leaf litter
Compost
Straw</td><td>0.1; 0.5; 1; 5</td><td></td></tr>
<tr><td>Phasmarhabditis hermaphrodita</td><td rowspan="2">Walnut leaf litter</td><td rowspan="2">0.78; 1.56; 3.125; 6.25; 12.5; 25</td><td>5 juveniles (J_3)</td><td rowspan="2">Robin Jakusovszky</td></tr>
<tr><td>Meloidogyne incognita</td><td>5 juveniles (J_2)</td></tr>
<tr><td>Area choice</td><td>Meloidogyne incognita</td><td>Walnut leaf litter
Oak leaf litter
Sycamore leaf litter
Maple leaf litter
Compost
Straw</td><td>5</td><td>8 hours</td><td>20–30 juveniles (J_2)</td><td>10</td><td>10</td><td>-</td><td>Petri dish (6 cm)</td><td></td></tr>
</table>

Table 3 Detailed date of pot and laboratory experiments with organisms living in compost

Aim	Examined species	Tested plant	Treatments	Length of exp.	No. of combinations	No. of replications/ combination	No. of indiv./ replication	Medium	Related student
Suppressivity study	*Dendrobaena veneta*	Tomato 'Dány'	Mulched with mixed leaf litter Presence or absence of earthworm Infested with *M. incognita* artificially or not	18 weeks	8	5	20	Pot (11 l)	Mónika Erdei
	Dendrobaena veneta var. Compastor	Basil	Vermicompost or potting soil Infested with *M. incognita* artificially or not	17 weeks	4	10	-		Domonkos Bognár
Food preference	*Porcellio scaber*	-	Egg masses of *M. incognita* on root gall	24 hours	1	10	3	Petri dish (9 cm)	Eszter Somogyi
			Fresh cucumber roots infested by *M. incognita* Composted cucumber roots infested by *M. incognita*		2			Petri dish (9 cm)	
			Healthy cucumber roots Cucumber roots infested by *M. incognita*						
			Healthy large-leaved lime leaves Composted cucumber roots infested by *M. incognita*						
Decomposition experiments		-	Presence or absence of woodlouse Infested roots by *M. incognita*	10 weeks	2	20	20	Bucket (800 ml)	
		Cucumber 'Monolit F1'	Potting soil (negative control) Soil infested by *M. incognita* (positive control) Soil infested by *M. incognita* composted by woodlouse	8 weeks	3		-	Pot (2.5 l)	

2.2 Statistical analyses

Microsoft Excel 2016 was used to process the data and create graphs and tables. Furthermore, PAST statistical program was used for the evaluations. In the case of one-way analysis of variance (ANOVA), Tukey's post hoc test or Mann-Whitney U test was used.

Welch test was used in the case of two-sample paired test. $P \leq 0.05$ significant level was determined for every statistical analysis.

Percentage data were square root arcsine-transformed before statistical analysis.

Three different scales were used for the determination of root damage caused by *M. incognita*. For all three scales, infested and non-infested values was compared in pairs and the scale showing the strongest significance level was presented in the dissertation.

For the mortality data of *M. incognita* juveniles, correlation analysis was performed with the following data pairs: tannin-pH, tannin-mortality, and pH-mortality.

3. Results

3.1 Identification to species of *Meloidogyne* females

During the examination of the perineum patterns showed only morphological characteristics of the species of southern root-knot nematode (*Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949).

3.2 Open-field experiments

3.2.1 Mulching with mixed leaf litter, Gödöllő (2016-2019)

The amount of **harvested fruit of tomato** was significantly increased every year by mulching treatment only. However, in 2018, artificial *M. incognita*-infestation increased yield, while irrigation and mycorrhiza treatment did not affect yield positively.

The **root damage caused by artificial *M. incognita*-infestation** was evaluated with three scales, but Zeck scale was used in further evaluations. Mulching treatment significantly reduced the root damage in two years (2017 and 2019), but was not affected by either irrigation or mycorrhiza treatment in either year.

In the case of the examined soil parameters, **soil moisture content** was the lowest in the case of unmulched non irrigated treatment, while there was no significant difference between the other treatments. In addition, there was no strong correlation between *M. incognita*-damage and soil moisture. In the case of unmulched treatments, regardless of irrigation, **soil temperature** followed the fluctuation of the air temperature much more, while in the case of the mulched plots this tendency was more balanced. There was a significant correlation between the change in soil temperature and the decrease in root damage. In the case of **soil compaction**, unmulched plots were significantly more compacted than mulched areas, however, this was not related to the decrease of root damage.

The values of **organic matter content** were not affected by either mulching or irrigation in the first year (2016). In the second year, however, the amount of organic matter in the soil increased significantly as a result of the mulching treatment. The **pH value** of the plots shifted slightly alkaline, mainly due to irrigation.

There were no differences in **mycorrhizal root colonization** values between mycorrhized and non-mycorrhized tomato plants. The number of **earthworms** did not affect the root damage caused by *M. incognita* in 2017, despite the fact that their number increased significantly due to soil cover.

The number of **free-living, non-predatory nematodes** was significantly affected by year, but not by the applied treatments.

The occurrence of **Mononchida predatory nematode** individuals was significantly influenced by year effect and mulching treatment. In the period between 2017 and 2019, mulching increased, while in 2017, irrigation treatment reduced their numbers. However, they were not affected by either mycorrhiza-inoculation or artificial *M. incognita*-infestation.

The presence of **four predatory genera** was identified from the study area: *Clarkus*, *Mylonchulus*, *Prionchulus*, and *Iotonchus*. The frequency of individuals varied greatly in relation to mulching: the genus *Clarkus*, *Mylonchulus*, *Prionchulus* was characterized by a higher number of individuals in the mulched plots than in the unmulched ones. The members of the genus *Prionchulus* were found only in mulched plots in both years, however, their numbers decreased from 2018 to 2019. Only one specimen of the genus *Iotonchus* was detected on unmulched plots in 2019.

A similar trend can be observed within genera. A higher density can be observed in the mulched plots than in the unmulched areas. However, irrigation and artificial *M. incognita*-infestation did not affect the reproduction of predatory species.

Within these genera, three species were identified: *Clarkus papillatus*, *Mylonchulus brachyuris* and *Prionchulus punctatus*.

3.2.2 Mulching with different mulching materials, Gödöllő (2019)

Tomato yield of the plots mulched with straw did not differ significantly from neither the unmulched plots nor the plots mulched with mixed leaf litter. The amount of average yields of the plots mulched with walnut leaf litter significantly differed from the straw mulched plots only. As a result of the compost mulching, yield was higher than in the unmulched, mulched with straw or mixed leaf litter plots.

Artificial *M. incognita*-infestation was around the lowest Zeck scale value, and there was no difference between mulching materials.

3.2.3 Mulching with straw and covering with plastic mulch, Szolnok (2019)

The combination of plastic mulch and fertilizer treatment provided significantly higher **average yield** than straw mulching with or without manure.

The **root damage caused by *M. incognita*-infestation** was highest on the roots of the non-fertilized plants besides plastic mulch, which was significantly higher value than in the case of plastic mulched and fertilized plants. Within straw mulching treatments, nutrient replenishment was not an influencing factor, and straw mulching treatments did not differ from the plastic mulch treatments.

3.3 Laboratory experiments with aqueous extracts of organic mulch materials

3.3.1 Mortality tests

Neem leaf litter

In the case of *Heterorhabditis bacteriophora*, a steep slope could be noticed; 0.1% of neem leaf extract did not cause any lethal effect, while 0.3% concetration resulted in 97.5% mortality. Neem leaf extract of 0.3% concentration did not cause any effect on the viability of *Phasmarhabditis hermaphrodita* juveniles. Only at higher concentrations (i.e., 0.6 and 1%), could 85% and 95% mortality be observed, respectively. The 13.75% of *Steinernema carpocapsae* juveniles died by 0.3%, while 0.6% and 1% concenctrations caused 80.36% and 79.64% mortality, respectively. In the case of *Steinernema feltiae*, there was a slight stepwise increase but no significant difference between mortality at control, concentrations of 0.1%, and 0.3%, with the highest average of mortality being 19.4%. Efficacy of 0.6 and 1% neem leaf extract concentrations showed between 70.5% and 90.8% mortality. Neem leaf extract of 0.1% did not have any effect on the survival of *Steinernema kraussei* juveniles, while 0.3% caused 46.5% mortality, whereas 95% and 100% mortality was observed in higher (0.6% and 1%) concentrations.

In the case of *M. incognita* juveniles, a relatively low (34.3%) mortality was observed at 0.1% compared to 0.3% (96.1%). However, higher concentrations (0.5 and 1%) also caused total mortality in this species.

Extracts of organic mulch materials used in open-field experiments

In the cases of the 0.1 and 0.5% of walnut leaf litter extracts, mortality values were between 0 and 5%. A different sensitivity was noticed with the treatment of 1% walnut leaf litter extract: 72.5% of *S. feltiae*, 83.3% of *S. carpocapsae*, 93.8% of *Ph. hermaphrodita* was dead, while all individuals of *H. bacteriophora* and *S. kraussei* died. In addition, the highest concentration (5%) caused 100% mortality for all species.

The lethal effect of lower (0.1 and 0.5%) concentrations of maple leaf litter extract was under 10% in all cases. The mortality of examined species caused by 1% maple leaf litter extract in increasing order was the following: *S. carpocapsae* (12.6%), *H. bacteriophora* (20%), *S. feltiae* (21.5%), *S. kraussei* (35%) and *Ph. hermaphrodita* (41.7%). Similarly to the 1% treatment, *S. carpocapsae* was the least sensitive to the 5% concentration: only 35% of its larvae died as compared to the 100% mortality of other species.

Neither the juveniles of *H. bacteriophora* nor those of *Ph. hermaphrodita* died in any of the compost extract treatments. Mortality values caused by all the concentrations of green waste compost extract were similar or lower (between 0 and 14.6%) than in the control in the case of the three *Steinernema* species.

The 5% concentration of all leaf litter extracts (walnut, oak, sycamore, maple, straw) induced 100% mortality in *M. incognita* juveniles after 24 hours. At concentrations of 1%, however, sycamore had a lethal effect of only 29.6% and straw had a lethal effect of 8.3%, while the other leaf litter extracts achieved 100%. The juveniles were the least sensitive to compost extract: even at the most higher concentration (5%), only 22.4% of the animals died.

The 0.78% of walnut leaf litter extract did not cause mortality in the *Ph. hermaphrodita* individuals tested. At concentrations of 3.125% and 25%, walnut leaf litter extract was 100% mortality, and other concentrations resulted in an average mortality of more than 80%. For *M. incognita* juveniles, the concentration of 0.78% caused more than 79.2% and the other concentrations caused 100% mortality.

3.3.2 Area choice test

M. incognita juveniles chose the sides of Petri dish in the case of Milli-Q aqueous control in almost the same percentage (46%–54%) and no difference was made between the compost extract and the Milli-Q water treatment pairs (50%–50%). However, they were repelled by the presence of leaf litter extracts: walnut leaf litter 63%, maple leaf litter 68%, sycamore leaf litter 75%, oak leaf litter 59%, and straw extract 63% avoided the applied juveniles.

3.3.3 Tannic acid content and pH

Among the extracts used in the experiments, the tannin content of the maple leaf litter extract was the highest, followed by the extract of oak leaf litter, sycamore leaf litter, walnut leaf litter, and finally straw extract. No tannin content could be measured from the compost extract.

The pH of the 5% concentrations of the tested extracts, with the exception of compost extracts, fell into the slightly acidic category. Within this, the most acidic value was given by the 5% concentration of maple leaf litter, then oak leaf litter, sycamore leaf litter, walnut leaf litter, and finally straw extract. The compost extract was in the slightly alkaline category with a pH of 7.79.

After further examination of the measured parameters, it can be concluded that the pH value of the extracts or their tannin content had no effect on n the mortality of *M. incognita* juveniles in most cases. In the compost extract, pH may have significantly affected larval mortality. In addition, the effect of pH on mortality was observed in the case of sycamore leaf litter extract, and the tannin content of straw extract affected the mortality of juvenile individuals. There was no correlation between tannin content and pH in the tested extracts.

3.4 Pot and laboratory experiments with organisms living in compost

3.4.1 Suppressivity test

Use of earthworm

In the case of the tomato pot experiment, neither the mulching nor the presence of *Dendrobaena veneta*, nor their combinations, affected either the yield of the tomato or the root the damage caused by the artificial *M. incognita*-infestation.

Use of vermicompost

In the case of basil herb, there was no difference in either vermicompost treatment or artificial *M. incognita*-infestation. However, the value of root damage was significantly higher in plants without vermicompost, planted only in potting soil with artificial *M. incognita*-infestation, than in basil plants planted in vermicompost medium.

3.4.2 Food preference

Egg mass consumption

Woodlice chose egg masses significantly more times compared to the root galled pieces, as 4.8 and 3.5 of the 5-5 pieces were available.

Food preference

Woodlice preferred composted root galls than leaf discs, which showed in both the number of fecal pellets and the percentage of consumed food as well. There was not difference between the consumption of healthy or infested cucumber roots neither in the number of fecal pellets, nor in the food consumption. Woodlice did not distinguish between the conditions of root galls.

3.4.3 Decomposition experiment

No *M. incognita* root damage was observed in the negative control plants that did not have artificial infestation. The rate of infestation with a Mukhtar scale was 2.1 on the roots of the positive control plants. In contrast, roots that developed in a medium mixed with root galls consumed by woodlice showed a significantly lower root damage value.

4. Conclusions and suggestions

4.1 Open-field experiments

4.1.1 Mulching with mixed leaf litter, Gödöllő (2016-2019)

Mulching increased the yield of tomato. In 2018, however, the artificial *M. incognita*-infestation seemed to have a stimulating effect on yield in case of low infestation.

Root damage caused by artificial *M. incognita*-infestation was able to be suppressed by mixed leaf litter mulching. Only in the case of soil temperature, a close correlation with the decreased root damage was observed. It can be assumed that the more balanced and lower soil temperature did not offer suitable environmental conditions for the plant-parasitic species.

The density of free-living, non-predatory nematodes has fluctuated greatly over the years in unmulched areas, with the year effect strongly striking. However, as a result of mulching, the number of non-predatory nematodes has been balanced over the years.

In contrast, the number of predatory nematodes in unmulched plots remained low throughout. Soil cover can provide more stable ecological conditions by increasing the moisture content of the soil and balancing the soil temperature, in addition, the undisturbed area may also have helped them to multiply.

4.1.2 Mulching with different mulching materials, Gödöllő (2019)

The most suitable mulching material is compost, then mixed leaf litter, walnut leaf litter, then straw. Allelopathic effects of walnut leaf litter was no detected. It is likely that the amount of substances harmful to plant growth reduced during composting *in situ*.

The different mulching materials did not significantly affect the root damage of *M. incognita*, in addition to the very low damage values.

4.1.3 Mulching with straw and covering with plastic mulch, Szolnok (2019)

Plastic mulch cover with fertilizer was stronger effect on yield than straw mulching.

Plastic mulch combined with nutrient replenishment reduced the root damage of *M. incognita* the most.

For plots with straw mulch, there was no significant difference according to nutrient replenishment. It is likely that the organic matter decomposed from the straw alone was sufficient to control *M. incognita*.

Although the nematicidal effect of garlic extract is known, it was not seen in the present experiment. It can be assumed that the amount and the frequency of application of the extract and the method of its application were not appropriate.

4.2 Laboratory experiments with aqueous extracts of organic mulch materials

4.2.1 Mortality tests

Neem leaf litter

In the case of the mortality test with neem leaf litter extract, differences in susceptibility was observed between the entomopathogenic and slug-parasitic nematode species. Based on the results, *H. bacteriophora* was the most sensitive of the studied species.

Extracts of organic mulch materials used in open-field experiments

In the case of entomopathogenic and slug-parasitic nematodes, a very high lethal effect of walnut leaf litter extract was observed. One explanation for this may be that the collected leaf litter consisted of freshly fallen leaves. In leaves, juglon decomposes over time and its amount decreases during the composting process due to aerobic metabolism of soil microorganisms.

Both walnut and maple leaf litter extract were darker than the compost extract, which can be explained by the higher tannin and lignin content. Extracts of plant with high tannin content can be lethal to *H. bacteriophora*, for example.

Compost extract had no negative effect on the viability of the examined species. With the exception of compost, all extracts contain tannin, so it can be assumed that tannin and other compounds are significantly degraded during composting processes and therefore have no negative effects on nematodes. In my studies, 1% straw and walnut leaf litter extracts had the lowest tannin content (0.02 and 0.08%). Mortality was very low for straw extract (8.3%) and 100% for walnut leaf litter extract. As the correlation study shows, not tannin or this compound alone may be responsible for the nematicidal effect.

In current experiments, *S. carpocapsae* and *S. feltiae* proved to be less sensitive than the other species. Furthermore, in the case of *S. feltiae* species, the mortality rates were lower at lower concentrations of maple leaf litter than in the control. This may be explained by the phenomenon of hormesis.

Even the lowest concentration of walnut leaf litter extract (0.78%) in *M. incognita* juveniles resulted very high mortality. However, in the case of *Ph. hermaphrodita*, this concentration did not cause any mortality at all, suggesting that the range of concentrations in which a dose-response relationship can be established is very narrow in this species as well.

4.2.2 Area choice test

The results of the area choice test are in tune with the mortality test. Compost extract did not affect the preference of *M. incognita* juveniles. The tannin content of the 5% extracts had no effect on this results.

4.3 Pot and laboratory experiments with organisms living in compost

4.3.1 Suppressivity test

Use of earthworm

Neither mulching nor the presence of *D. veneta* earthworm reduced the root damage, which is a different result from previous studies. It was quite hot in the summer of 2017, which caused the black pots to heat up quickly. As a result, the medium also warmed up quickly, on which the mulch material could not shade. As *M. incognita* prefers warm conditions, this environment was favorable for it, while *D. veneta* favoured less.

Use of vermicompost

D. veneta var. Compastor vermicompost reduced the root damage caused by *M. incognita*-infestation. According to studies, vermicompost has a beneficial effect on the growth and the herb of basil plants, however, this effect was not clear in the present experiment.

4.3.2 Food preference

Egg masses of *Meloidogyne* species is a gelatinous material that is presumably more digestible than roots for woodlice. This could be the reason why woodlice chose egg masses first, then root galls.

In food preference experiments, woodlice preferred root galls over the leaf of the large-leaved lime. The explanation is presumably to be found in the composition of the food. Decomposed, therefore, soft-textured plant materials were preferred over fresh plant materials, and as the experiment showed, regardless of whether they were healthy or infected.

4.3.3 Decomposition experiment

Composting and the decomposing work of woodlice may reduce the infectivity of galled root residues, but further studies are needed.

4.4 Suggestions

Plastic mulch can be an effective control method, especially in Mediterranean regions, as high temperatures can cause the soil to heat up that it makes inappropriate conditions for pests.

Organic mulching, on the other hand, prevents excessive soil warming and large temperature fluctuations, so environmental conditions will not be ideal for heat-demanding pests.

It is worthwhile to study the changes in the number of predatory nematodes according to different mulching materials, and the effect of certain soil parameters (like temperature, moisture content, pH) in long-term.

The scales used complemented each other occasionally, however, their application depends the type of experiments.

Experiments with mulch material extracts have shown that certain compost materials may be suitable as a carrier medium for the application of entomopathogenic and slug-parasitic nematodes. However, further studies are needed to eliminate the negative effects of walnut and maple leaf litter. Further experiments needed with leaf litters in different stages of ripening and the identification of chemical compounds.

It may be worth setting up a targeted experiment in which the beneficial *Ph. hermaphrodita* and the plant-parasitic *M. incognita* are present simultaneously. In this way, practical control methods could be developed in which a plant-derived extract and an animal organism can be used together and safely against a soil-dwelling (nematode) and a soil-surface (slug) pest.

It may be worthwhile to examine additional extracts or concentrations to determine their potential repellent effect.

Based on these studies with compost-living organisms, it may be appropriate to investigate the involvement of mixed species assays and other organisms in further experiments, as nutritional synergies between species may further enhance the disposal of infested plant parts.

5. New scientific results

1.) Mixed leaf litter mulching on *Meloidogyne incognita*-infested tomato plants was investigated for the first time under field conditions. Mixed leaf litter mulching decreased the root damage caused by *M. incognita* in open field, while it increased the yield of tomato.
2.) It was shown that among the measured soil parameters changed as a result of organic mulching, soil temperature showed the strongest correlation with the decreasing root damage caused by *M. incognita*.
3.) The numbers of individuals of the studied Mononchida predatory nematode species, *Clarkus papillatus*, *Mylonchulus brachyuris* and *Prionchulus punctatus* increased by organic mulching.
4.) The effects of aqueous extracts of walnut, maple, sycamore, oak and wheat straw was examined for the first time on the mortality of entomopathogenic (*Heterorhabditis bacteriophora*, *Steinernema carpocapsae, Steinernema feltiae, Steinernema krausei*) and slug-parasitic (*Phasmarhabditis hermaphrodita*) nematode juveniles. It was shown that the juveniles of *S. carpocapsae* and *S. feltiae* are less sensitive to the studied extracts than the juveniles of the other examined species.
5.) The juveniles of *M. incognita* proved to be more sensitive than the juveniles of the entomopathogenic and slug-parasitic nematode species in the case of the studied extracts.
6.) Nematicidal effect of the aqueous extracts of organic mulch materials was shown, but not in the case of the compost made from those materials.
7.) During the study of the biological disposal of infested root residues, woodlice (*Porcellio scaber*) consumed the roots infested by *M. incognita*, preferring the egg masses over the root galls.

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