

# First Report on the Effect of Aqueous Extracts of Hungarian Organic Mulch Materials on Entomopathogenic and Slug-Parasitic Nematodes

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Few researches address the compatibility of organic mulching and entomopathogenic (EPN) and slug-parasitic (SPN) nematodes, although organic mulching may provide favourable conditions for these beneficial organisms. Our aim was to examine the effect of different concentrations (0.1, 0.5, 1 and 5%) of aqueous extracts of green waste compost, the dry leaf litters of the common walnut (*Juglans regia*) and Norway maple (*Acer platanoides*) on EPN (*Heterorhabditis bacteriophora*, *Steinernema carpocapsae*, *Steinernema feltiae*, *Steinernema kraussei*) and SPN (*Phasmarhabditis hermaphrodita*) species. Experiments were set up in 96-well, flat-bottom microplates. After a 24-hour exposure time, the number of dead animals was counted under a transmission microscope. Green waste compost extracts caused quite low or no mortality in case of all examined species. Mortality caused by the 5% Norway maple leaf litter extract was moderate (34.6%) in the case of *S. carpocapsae* juveniles, while 100% of juveniles of other species died. The highest (5%) concentration of the common walnut leaf litter extract caused 100% mortality in all species. As a conclusion, green waste compost mulch seems to be more compatible with EPN and SPN species than common walnut or Norway maple leaf litter mulch.

**Keywords:** Biocontrol agent, common walnut, green yard waste compost, laboratory, leaf litter, Norway maple, toxicity.

As man becomes aware of the unfavorable side-effects of synthetic pesticides, more and more chemicals are withdrawn from use. Consequently, crop protection faces new challenges in the control of soil-dwelling pests. Biological control agents, such as entomopathogenic and slug-parasitic nematodes (EPNs and SPNs) are being researched and promoted to provide good control measures in an environment where there is a justified need to reduce the application of chemical insecticides.

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EPNs (Rhabditida: Heterorhabditidae and Steinernematidae) have a wide range of pest insect hosts (Askary and Abd-Elgawad, 2017). They are obligate parasites that kill their hosts by relying upon their association with a symbiotic bacterial species living in the alimentary canal of infective juveniles (Sankaranarayanan and Askary, 2017).

*Phasmarhabditis hermaphrodita* (Schneider) (Rhabditida: Rhabditidae) is a facultative SPN species that attack certain slug species of Limacidae, Arionidae and Milacidae families (Wilson et al., 1993; Askary et al., 2012).

Some advantages of applying these beneficial nematodes are: i.) they pose no known threat to human health or wildlife, ii) they are hard to overdose (Askary et al., 2012); in addition, iii) they are highly compatible with certain biological and chemical pesticides (Lacey and Georgis, 2012).

Application and effectiveness of EPNs depend on various abiotic factors including soil moisture, soil temperature and UV-radiation (Shapiro-Ilan et al., 2006, 2012). These factors can be influenced using organic mulching simultaneously with EPN and SPN species because mulch materials increase soil moisture, do not allow soil overheating (Sinkevičienė et al., 2009), and prevent light from reaching the soil surface (Bond and Grundy, 2001).

Both wheat straw and apple wood chips enhanced the effectiveness of *Heterorhabditis zealandica* (Poinar) against codling moth (*Cydia pomonella* L.) larvae (de Waal et al., 2011). On the other hand, while wood chips of various origins enhanced the larvicidal activity of *Steinernema feltiae* (Filipjev), the same mixture reduced the activity of *Steinernema carpocapsae* (Weiser) (Lacey et al., 2006). Discarded cane leaves in field had no effect on the viability of *Steinernema braziliense* (Nguyen, Ambros Ginarte, Leite, dos Santos and Harakava), but decreased the virulence of *Heterorhabditis* sp. (Poinar) (Leite et al., 2015). Higher *S. carpocapsae* effectiveness was noticed in case of soy crop residues (Shapiro et al., 1999). Different types of compost at different maturity stages could be appropriate as a carrier medium for *S. feltiae* (Herren et al., 2018).

*Phasmarhabditis hermaphrodita* was able not only to disperse in bark chips and leaf litter but to reproduce in leaf litter without its host animal (MacMillan et al., 2009). On the other hand, the reduced reproduction of *Ph. hermaphrodita* was noticed in leaf compost when compared to horticultural substrate and garden soil (Nermut, 2012).

Composts in agricultural use can be made of various materials from yard waste (Hartz et al., 1996) to sewage sludge (Wei and Liu, 2005). Therefore, their effects on soil organisms may be different. Green and yard waste composts seem to have no effect on the entomopathogenic *S. feltiae* (Shapiro et al., 1999) and on plant parasitic nematode species such as *Paratrichodorus minor* ((Colbran) Siddiqi), *Pratylenchus* spp., *Xiphinema* spp. (McSorley and Gallaher, 1995).

Leaves and other parts of walnut trees (*Juglans* spp. L.) due to their chemical composition are considered to have allelopathic or toxic effect on certain plants (Ercisli et al., 2005; Wang et al., 2014; Shi et al., 2017; Pardon et al., 2019) therefore those materials are generally not recommended as mulch materials. On the other hand, their effect on soil organisms is a less studied topic (MacDaniels and Pinnow, 1976; Summers and Lussenhop, 1976; Wang et al., 2014; Fekrat et al., 2016).

In our previous open-field experiment, leaf litter of maple trees (*Acer* spp. L.) decreased the number of galls on tomato roots caused by root-knot nematodes (*Meloidogyne*

*incognita* Kofoid and White) (Petrikovszki et al., 2016). However, no positive or negative effect of maple leaf litter on nematodes has been found in the literature yet.

The use of organic mulch may, however, increase the number of terrestrial pests such as wireworms and slugs. Wireworms for example, tend to stay closer to the surface, to the roots of plants because of the higher soil moisture induced and retained by mulching (Musick and Petty, 1974). In addition, it was observed that a 2 cm thick and light layer of leaf litter is needed to provide terrestrial molluscs with appropriate hiding place and habitat in a woodland (Millar and Waite, 1999). EPNs against wireworms and SPNs against slugs may be an appropriate choice in biological control, however the compatibility of different mulching materials and these beneficial organisms remained a less studied field up until the present.

The initial hypothesis was that certain organic mulch materials may have different levels of lethality on EPN and SPN species. Therefore, in this study, our aim was to examine the effect of aqueous extracts of green yard waste compost, as well as that of the dry leaf litter of the common walnut (*Juglans regia* L.) and Norway maple (*Acer platanoides* L.) on entomopathogenic (EPN) and slug parasitic (SPN) nematode species under laboratory conditions.

## Materials and Methods

### *Preparation of aqueous extracts*

Common walnut (*Juglans regia*) and Norway maple (*Acer platanoides*) leaf litters were collected on 17 and 30 October 2018. The common walnut tree is located in the Experimental Field Plant Protection Institute (47°35'22.35" N 19°22'04.32" E) of Szent István University, while the Norway maple tree is in the inner yard (47°35'33.86" N 19°21'43.02" E) of Szent István University in Gödöllő, Hungary. For our experiment, green yard waste compost ('Zöld Híd Komposzt' 04.2/3245-2/2017 Nébih, 2019) was produced and provided by the Zöld Híd B.I.G.G. Non-profit Ltd. Gödöllő, Hungary. All the above mulching materials were left to become air-dried at 25 °C and 20% RH for 2 days. Later, 2.5 g of mulching materials were ground by a coffee mill (Bosch MKM 6000) for 15 seconds. Powders were mixed with 50 ml Milli-Q (MQ) water, then covered with aluminium foil and let soaked at room temperature. After 24 hours, stock solutions (5% w/v) were filtrated through wadding and were diluted with MQ-water to obtain different concentrations (1, 0.5 and 0.1%).

### *Examined EPN and SPN species*

In this study, the following commercial products (Biobest, Belgium) were used: EPNs as *Heterorhabditis bacteriophora* (B-Green), *Steinernema carpocapsae* (Carpocapsae-System), *S. feltiae* (Steinernema-System), *S. kraussei* (Kraussei-System) and the SPN *Phasmarhabditis hermaphrodita* (Phasmarhabditis-System). These products were stored at 5 °C until the experiments.

### Experimental setup

Experiments were set up in 96-well, flat-bottom microplates (Kartell S.p.A., Italy). EPN and SPN products were previously dispersed in MQ-water in order to break inactive stages of infective juveniles (IJs). Into each well, 5 active IJs from every species were measured with 60  $\mu$ l MQ-water using a micropipette. In addition, 200  $\mu$ l of every concentration or the MQ-water as control were added to each well. Each treatment was replicated four times. In case of control, 8 replicates were used. Microplates were closed with parafilm and incubated for 24 hours in a thermostat in dark at 20 °C  $\pm$  1 °C. After 24 hours, the number of dead nematodes were counted under a transmission stereomicroscope (Olympus SZH 10) on  $\times$ 30 magnification. The movement of IJs was induced by adding 10  $\mu$ l 5% lactic acid (modified method based on Ciancio, 1995). A maximum mortality of 20% in control treatment was considered as a validity criterion for the tests (Kiss et al., 2018). When mortality of control animals was higher than 20%, the test was considered invalid, and the same test was repeated.

### Data elaboration and statistical analysis

Data were square root arcsine-transformed in MS Excel 2016 before being analysed by the PAST3 (Paleontological Statistics) statistical software (Hammer et al., 2001). Mortality values of all species were evaluated separately on the basis of the examined extracts. One-way ANOVA, Tukey's test and Mann–Whitney U test was used, depending on whether normality was fulfilled (Shapiro–Wilk test).

## Results

In the cases of the 0.1 and 0.5% of common walnut leaf litter extracts, mortality values were between 0 and 5%. A different sensitivity was noticed with the treatment of 1% common 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* (Steiner) died. In addition, the highest concentration (5%) caused 100% mortality for all species (Fig. 1.a–e).

The lethal effect of lower (0.1 and 0.5%) concentrations of Norway maple leaf litter extract was under 10% in all cases. The mortality of examined species caused by 1% Norway 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 (Fig. 1.f–j).

Neither the larvae of *H. bacteriophora* nor those of *Ph. hermaphrodita* died in any of the green waste 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 (Fig. 1.m, n).

A similar tendency was observed not only at steinernematids (Fig. 1.b–c, g–i), but at the larvae of *Ph. hermaphrodita* (Fig. 1.e, j, o) with the 0.1 and 0.5% of common walnut and Norway maple leaf litter extracts as well.

## Discussion and Conclusion

The common walnut leaf litter extract caused a general high mortality in our study. Juglone-like chemical compounds caused toxic effects on target organisms, like the plant parasitic *Meloidogyne hispanica* (Hirschmann) (Maleita et al., 2017) and non-target spe-

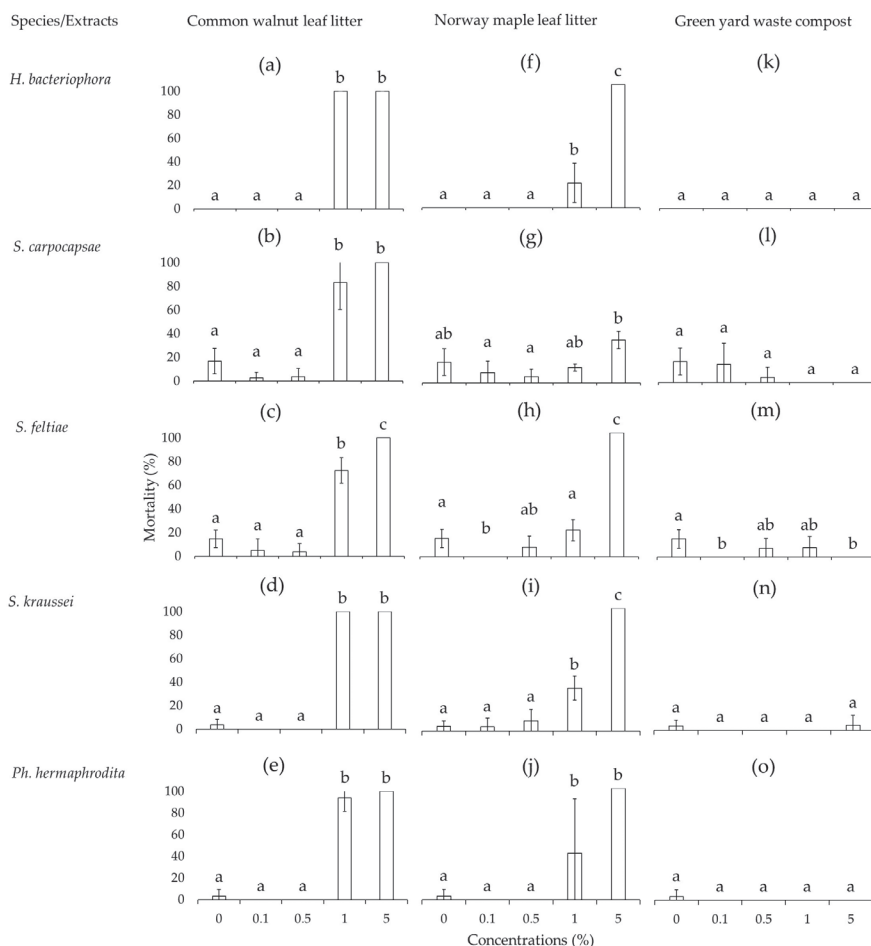


Fig. 1. Percentage mortality (mean  $\pm$  CI 95%) of EPN (*Heterorhabditis bacteriophora*, *Steinernema carpocapsae*, *Steinernema feltiae*, *Steinernema kraussei*) and SPN (*Phasmarhabditis hermaphrodita*) species after a 24-hour exposure to concentrations of 0.1, 0.5, 1 and 5% of the common walnut, Norway maple leaf litter and green waste compost extracts. (One-way ANOVA, Tukey's pairwise comparisons (g), Mann–Whitney U test (a–f, h–o); different letters in the same row indicate significant difference at  $p < 0.05$  level)

cies, like the model organism *Caenorhabditis elegans* (Maupas) nematodes (Wang et al., 2017). One of the reasons may be that our collected material consisted of freshly fallen leaves. In leaves, juglone content degrades with time (Coder, 1983), and its level diminishes during the composting process (Funt and Martin, 2000) by the aerobic metabolism by soil microorganisms (Ponder and Tadros, 1985). Besides juglone, walnut leaf extract may contain several other allelochemicals (Wang et al., 2014), which may be responsible for a nematocidal effect as well (Kokalis-Burelle and Rodríguez-Kábana, 2006; Soltys et al., 2013).

The effects of *Acer* species leaf extracts have not been studied on nematodes yet, but there are some observations on the antifungal effect of Norway maple (Dix, 1974) and the antibacterial effect of the sugar maple (*Acer saccharum* Marshall) (Anderson, 2005). Another explanation behind the nematocidal effect of our leaf extracts can be that both the common walnut and Norway maple leaf litter extracts were darker than the studied compost extract. This characteristic may be due to the higher tannin and lignin content of the leaf extracts (Anderson, 2005). The nematocidal effect of tannin-rich plant extracts was already observed in case of *H. bacteriophora* (Glazer et al., 2015).

The green yard waste compost extract involved in our studies did not decrease the viability of EPN and SPN species, similarly to a previous study with *S. feltiae* in a pot experiment in which different type of composts (from green wastes, wood chips and vermicompost) were used (Herren et al., 2018). On the other hand, certain types of composts, like solid compost could be harmful on different organisms, such as seedlings of cress (*Lepidium sativum* L.) and individuals of the red wiggler worm (*Eisenia fetida* Savigny) due to its pollutant content (Pivato et al., 2016). Not only the source materials of the compost but its maturity stage during the composting process may influence the toxicity rate (El Fels et al., 2016).

In the present study, different species showed different sensitivity to the treatments, in accordance with a study based on the effect of different fungicides on EPN [*Heterorhabditis downesi* (Stock, Griffin and Burnell), *Steinernema carpocapsae*, *S. feltiae*] species (Laznik et al., 2012). Similarly to the results of the previous study, *S. carpocapsae* and *S. feltiae* appeared to be the least sensitive to the common walnut and to Norway maple extract treatments as compared to the other examined species.

Both *Steinernema* species and *Ph. hermaphrodita* showed similar or even lower levels of mortality in the case of lower concentrations (0.1 and 0.5%) of all the three extracts when compared to control. These results may be explained by the phenomenon called hormesis, when certain materials that are toxic on test organisms in high concentration, have a stimulating effect in low doses (Hofbrucker-MacKenzie et al., 2019).

As a conclusion, our initial hypothesis was proven correct: our examined species showed different sensitivity (expressed in mortality values) to treatments. Certain composts can probably be used simultaneously with EPN and SPN application. However, further studies are needed to find measures to scale down the negative effects of leaf litters of the common walnut and Norway maple on EPN and SPN species. A solution can be the combination of these leaf litters with other organic materials including compost made of green waste. In addition, time may be a useful factor in decreasing leaf litter toxicity as proven in white mustard (*Sinapis alba* L.) (Tirczka et al., 2015), rape (*Brassica napus* L.) (Zhang et al., 2015), Chinese cabbage (*Brassica pekinensis* L.), mustard (*Brassica juncea* L.) Czern.) and radish (*Raphanus sativus* L.) (Zhang et al., 2016) tests.

We recommend further experiments with leaf litters of different stages of maturity on EPN and SPN species and suggest extending examinations to plant parasitic nematodes as well. In order to understand the complexity of the process, the determination of chemical compounds responsible for nematicidal effect is proposed. To test the viability of our *in vitro* results, open-field experiments are needed, where *Ph. hermaphrodita* against mollusc pests and, *S. carpocapsae*, the most promising EPN species of our studies, against wireworms can be applied with organic mulching.

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