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# Liming, phosphorus and zinc influence on soil nematode community structure at hot pepper

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**Abstract**: Monitoring the biological quality of soils often include nematodes as bioindicators. The aim of this study was determined the influence of liming, P and Zn effect on nematode community structure as an indicator of soil ecosystem health in hot pepper. Pot experiment with hot pepper was conducted in randomized block design as follows: control, P, Zn, P + Zn, lime, P + lime, Zn + lime and P + Zn + lime (25 mg/kg P, 1 mg/kg Zn and lime as 2.22 g/kg $CaCO_3$). The highest number of nematodes genera was with phosphorus application (17.25). The most common genera were *Rhabditis* and *Tylenchus*. Above all indices, only the Structure index (SI) shown significance ($P < 0.01$) in reflecting soil disturbance. The highest significant correlation was found between Maturity index 2–5 and Structure index ($r = 0.946^{***}$), Maturity index and Plant parasitic nematode and Maturity index ratio ($r = -0.919^{***}$) and between Ecological and Channel index ($r = -0.815^{***}$).

**Keywords**: ecological indices; hot pepper; nematode community; liming; soil

Biodiversity in soil systems is high relative to above ground systems and nematodes are first-rate bioindicators for monitoring the biological quality of soils. They have a significant impact on soil processes, especially through their influence on biomass and microbial populations and also they play an important role in soil food web, mineralisation and nutrient re-cycling (Chen et al. 2009; Landi et al. 2017; Brmež et al. 2018).

The direct or indirect disturbance as tillage, pesticide use, fertilization, liming, salinization etc., lead to alterations in community structure at all trophic levels and thereby disturb the functioning of the entire agroecosystem (Timper 2014; Moura, Franzener 2017; Kristek et al. 2018).

In agricultural soils along with free-living nematodes as fungal feeders, omnivores and predators (Yeates et al. 1993), there are an increased number of bacterial feeders (Neher et al. 2004; Grubišić et al. 2016) and plant parasitic nematodes. Bacterial feeders nematodes are more common in agricultural and grassland systems, whereas fungal feeders nematodes are more important in forests.

Soil acidity has determinal effect on plants and soil organisms, so the activities and functioning of soil organisms are generally reduced which results in inhibi-

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tion of biological functions such as organic matter decomposition and nutrient re-cycling. Liming is the the most widespread agricultural measure for correcting soil acidity (Li et al. 2010; Antunović et al. 2014).

The Maturity index (MI) for free-living nematodes may be viewed as a measure of disturbance, thus MI decrease with increasing disturbance e.g. stress conditions. The Maturity index 2–5 (MI2–5), which exclude nematodes with c-p 1 value (colonizer-persister groups, c-p 1 to 5), is often used for measure stress of soil nematode community (Bongers 1990). Nematodes with low colonizer-persister (c-p) values indicate communities where soil disturbance (tillage, pesticide use, mineral fertilization) are very common and there are more often present rapidly reproducing and small nematodes flourish.

Disturbance to the soil environment was more severe when Maturity index (MI), Maturity index 2–5 (MI2-5), and Shannon – Weaver diversity index (H') values were lower (Chen et al. 2009).

Nematode faunal indices including enrichment index (EI), basal index, Structural index (SI) and Channel index (CI) have been developed and applied in order to analyse the soil nematode community structure (Ferris et al. 2001; Ferris, Bongers 2006).

Even though the pepper is nowadays grown hydroponically (Vinković et al. 2016, 2018; Amalfitano et al. 2017) there are many farmers which grown pepper seedlings in soil substrate. Thus, the soil properties have a great importance because hot pepper fruits are directly transferred into food chain. The production of hot pepper in recent years increased, mostly due to its capsaicin content, which have several benefits for human health in nervous, cardiovascular and digestive system (Fattori et al. 2016; Vinković et al. 2017).

The aim of this paper was to determine the influence of lime, zinc and phosphorus application on nematode community structure in soil and their ecological indices under pepper cultivation.

## MATERIAL AND METHODS

**Treatments and experimental setup**. The pot experiment was conducted in spring on 27th April 2010. Each pot (volume 3.5 $dm^3$) was filled with 4 kg of soil taken from nearby arable land. Soil chemical characteristic were determined before pepper cultivation (Table 1) as follows: pH (ISO 10390:1994; Soil quality – determination of pH), organic matter (ISO 14235:1998; Determination of organic carbon by sulfochromic oxidation), P and K (AL-method, Egner et al. 1960). According to soil analysis, the soil acid pH reaction and poor with P and K (4.70 and 9.46 mg/100 g soil, respectively).

Hot pepper (*Capsicum annuum* L.) seedlings (sown previously on April 10) were planted into pots. The experiment (Table 2) was set up in 4 replication with: 2 different treatments of liming (without lime – A and with lime – B) and 4 different subtreatments: control (0), phosphorus (1), zinc (2) and phosphorus + zinc (3). The total number of 32 pots were set up in randomized block design.

**Nematode extraction and identification**. For this experiment soil samples were taken one month after setting up the experiment, on May 27, 2010. From each experimental pot soil samples (100 g of soil) were taken with 2 mm diameter probe, from 0–13 cm depth (i.e. depth of pot). In Laboratory of Nematology at the Faculty of Agriculture in Osijek nematode were extracted from 100 g of soil using Erlenmeyer method (Seinhorst 1956). After nematode extraction from soil and counting the nematodes, nematodes genera was determined using microscope (Olympus BX50, 400–1000×).

Nematodes were determined to genera level because identification to species level was not always possible, due to the absence of adult specimens. Nematode genera was identified according to: Mai and Lyon (1975), Zullini (1982), Andrassy (1984, 1988, 1993), Hunt (1993), and Bongers (1994). Trophic structure of soil nematodes was determined according to Yeats et al. (1993).

**Data analysis**. In order to conclude which indices better parameter is reflecting the nematode communities disturbance, diversity and ecological indices were used as follows:

(1) Maturity index of free living nematode:

$MI = \Sigma\, v_i f_i / \Sigma f_i$;

where: $v_i$ – the c-p value of free living nematodes of the $i^{th}$ taxon; $f_i$ – the frequency of the $i^{th}$ taxon (Bongers 1990)

(2) Modified maturity index: $MI2\text{-}5 = \Sigma\, v_i f_i / \Sigma f_i$

where: $v_i$ – the c-p value 2–5; $f_i$ – the frequency of the $i^{th}$ taxon (Bongers 1990);

Table 1. Chemical characteristic of the soil

| pH in | | Organic matter (%) | mg/100 g of soil (AL method) | |
|---|---|---|---|---|
| KCl | $H_2O$ | | $P_2O_5$ | $K_2O$ |
| 4.21 | 5.52 | 1.95 | 4.70 | 9.46 |

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Table 2. Treatments of lime, phosphorus and zinc application

| (A) No lime | | (B) With lime | |
|---|---|---|---|
| A0 | Control (0) | B0 | Control Ca (2.22 g/kg $CaCO_3$) |
| A1 | P (25 mg/kg $P_2O_5$ (triplex) | B1 | P (25 mg/kg $P_2O_5$ (triplex) + Ca (2.22 g/kg $CaCO_3$) |
| A2 | Zn (1 mg/kg Zn ($ZnCl_2$) | B2 | Zn (1 mg/kg Zn ($ZnCl_2$) + Ca (2.22 g/kg $CaCO_3$) |
| A3 | P (25 mg/kg $P_2O_5$ (triplex) + Zn (1 mg/kg Zn ($ZnCl_2$)) | B3 | P (25 mg/kg $P_2O_5$ (triplex) + Zn (1 mg/kg Zn ($ZnCl_2$) + Ca (2.22 g/kg $CaCO_3$) |

(3) Plant parasitic index: PPI = $\Sigma\, v_i f_i / \Sigma\, f_i$,
where: $v_i$ – the plant parasitic; $f_i$ – the frequency of the $i^{th}$ taxon (Bongers 1990);

(4) Maturity index ratio: PPI/MI (Bongers et al. 1997),

(5) Shannon-Weaver diversity index´:

H' = $-\Sigma\, p_i \ln p_i$;
where: $p_i$ – the proportion of individuals in the ith taxon (Shannon, Weavrater 1949);

(6) Fungal feeders and Bacterial feeders ratio: F/B (Hendrix et al. 1986);

(7) Enrichment index: EI = 100 $(e/(e + b))$ (Ferris et al. 2001);

(8) Structure index: SI = 100 $(s/(s + b))$;

(9) Channel index:

CI = 100 (0.8 FF2/(3.2 BF1 + 0.8 FF2)) (Ferris et al. 2001).

The functional guilds BF-*x* (*x* = 1–4), FF-*x* (*x* = 2–4), OM-*x* (*x* = 4–5), and PF-*x* (*x* = 3–5) were suggested by Bongers and Bongers (1998) where *x* represented cp-values and feeding groups were bacterial feeders (BF), fungal feeders (FF), omnivorous nematodes (OM), and predators nematodes (PD). These functional guilds were then used for the calculation of the indices EI, SI and CI according to Ferris et al. (2001). In EI and SI, *e* is the abundance of individuals in guilds BF1 and FF2 weighted by their respective weightings, and *b* is similarly calculated using the weightings assigned to BF2 and FF2, while *s* is similarly calculated using the weightings assigned to BF3–BF5, FF3–FF5, OM3–OM5 and PD2–PD5 (Ferris et al. 2001).

These values were combined to elucidate which indices were best in reflecting the indicative role of nematode communities. The influence of phosphorus and zinc application without liming on soil nematode community in hot pepper was determined in previous study (Brmež et al. 2014) using the MI, MI 2-5, PPI and their ratio (PPI/MI).

**Statistics**. Analysis of variance (ANOVA) was done with using GLM procedure and computer program for statistical data processing SAS 9.4 (SAS Institute Inc.). To evaluate the differences between separate means the LSD test was used. Error bars in figures show the 95% confidence interval.

## RESULTS

Diversity of 32 nematode genera were determined in this study. Nematode genera which were present in all analysed samples are: *Acrobeloides*, *Aphelenchoides, Aphelenchus, Dipterophora, Ditylenchus, Eucephalobus, Eudorylaimus, Paratylenchus, Plectus, Pratylenchus, Prodorylaimus, Rhabditis*, *Mesodorylaimus, Tylenchorhynchus* and *Tylenchus*. The genera *Rhabditis* and *Tylenchus* were the most abundant.

The average number of nematode genera (Fig. 1) did not differ significantly between treatments (lime and without lime), while statistically significant difference occurred between subtreatments. The nematode genera richness was significantly ($P < 0.05$) higher in subtreatments with phosphorus application (17.25).

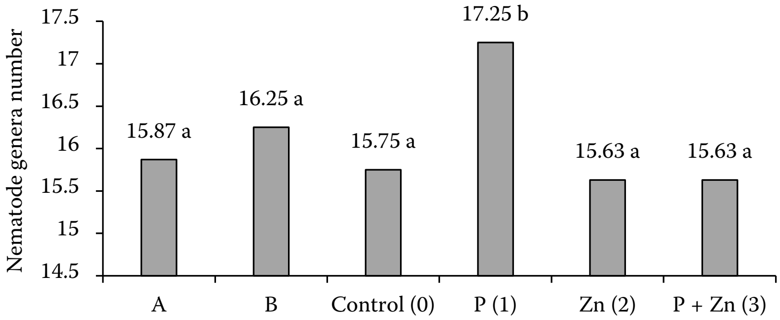


Figure 1. The average number of nematode genera in treatments of hot pepper experiment (the letters indicate difference for P < 0.05)

https://doi.org/10.17221/217/2017-HORTSCI

Table 3. The ANOVA for disturbance indices (MI, MI2-5, PPI and PPI/MI), diversity indeks (H'), fungal feeders and bacterial feeders ratio (F/B) and food web index (EI, SI, CI) in hot pepper experiment

| | MI | MI2-5 | PPI | PPI/MI | H' | F/B | EI | SI | CI |
|---|---|---|---|---|---|---|---|---|---|
| A0 | 2.20 | 2.58 | 2.05 | 0.94 | 1.18 | 0.54 | 63.18 | 64.09 | 34.81 |
| A1 | 2.15 | 2.30 | 2.13 | 0.99 | 1.18 | 0.55 | 43.88 | 38.69 | 58.40 |
| A2 | 2.13 | 2.25 | 2.07 | 0.98 | 1.14 | 1.27 | 50.24 | 34.63 | 63.90 |
| A3 | 2.23 | 2.30 | 2.07 | 0.93 | 1.19 | 0.93 | 40.84 | 38.94 | 66.78 |
| B0 | 2.20 | 2.32 | 2.14 | 0.98 | 1.15 | 0.49 | 40.94 | 43.45 | 62.01 |
| B1 | 2.14 | 2.28 | 2.05 | 0.97 | 1.14 | 0.60 | 47.32 | 36.70 | 46.98 |
| B2 | 2.20 | 2.35 | 2.04 | 0.93 | 1.16 | 0.38 | 46.73 | 44.12 | 33.54 |
| B3 | 2.31 | 2.52 | 2.11 | 0.92 | 1.15 | 0.44 | 45.86 | 55.03 | 50.73 |
| Mean | 2.19 | 2.36 | 2.08 | 0.95 | 1.16 | 0.65 | 47.37 | 44.46 | 52.14 |
| LSD 0.05 | ns | ns | ns | ns | ns | ns | ns | 17.83 | ns |
| LSD 0.01 | ns | ns | ns | ns | ns | ns | ns | 26.16 | ns |

Table 4. Pearson correlation coefficients between disturbance indices (MI, MI2-5, PPI and PPI/MI), diversity indeks (H'), fungal feeders and bacterial feeders ratio (F/B) and food web index (EI, SI, CI) in hot pepper experiment (N = 32)

| | MI | PPI | PPI/MI | MI25 | F/B | H' | EI | SI | CI |
|---|---|---|---|---|---|---|---|---|---|
| MI | 1 | | | | | | | | |
| PPI | –0.029 ns | 1 | | | | | | | |
| PPI/MI | –0.919 *** | 0.402 * | 1 | | | | | | |
| MI25 | 0.514 *** | –0.193 ns | –0.508 ** | 1 | | | | | |
| F/B | 0.064 ns | –0.136 ns | –0.139 ns | –0.345 ns | 1 | | | | |
| H' | 0.569 *** | 0.206 ns | –0.436 * | 0.363 * | 0.079 ns | 1 | | | |
| EI | –0.471 ** | –0.207ns | 0.363 * | 0.425 * | –0.133 ns | –0.157 ns | 1 | | |
| SI | 0.543 ** | –0.141 ns | –0.527 ** | 0.946 *** | –0.331 ns | 0.478 ** | 0.346 ns | 1 | |
| CI | 0.384 * | 0.175 ns | –0.289 ns | –0.439 * | 0.575 *** | 0.206 ns | –0.815 *** | –0.370 * | 1 |

***P < 0.001, **P < 0.001, *P < 0.001, ns – not significant

Bacterial feeders were most common trophic group of experiment (Fig. 2). In trophic group analysis without liming (Fig. 2a), the highest number of plant parasitic nematodes (47%) was found with Zn application, whereas the lowest number of plant parasitic nematodes was at P and Zn application (32%). The number of predators were the lowest in proportion of all trophic group of the experiment.

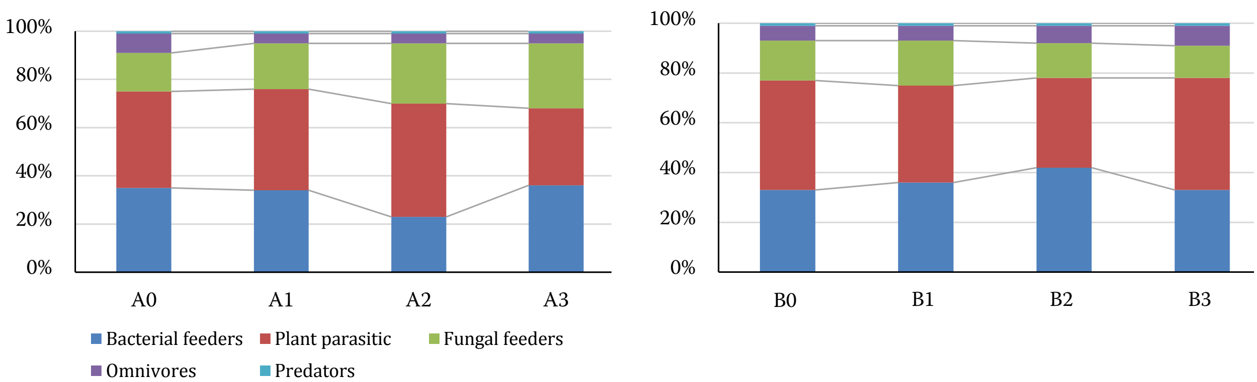


Figure 2. The trophic groups of hot pepper experiment without liming (a – on the left) and with liming (b- on the right)

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According to the analysis of variance (Table 3), in the structure index (SI) showed statistically difference in comparisson of mean values of treatments and subtreatments. A positive significant correlation (Table 4) was between MI 2-5 and SI ($r$ = 0.946 ***), while the negative significant correlation was between MI and PPI/MI ($r$ = –0.919***) and between EI and CI ($r$ = –0.815***).

## DISCUSSION

Comparison of ecological indices between limed and no limed indicates that number of genera and the maturity of the soil nematode community were smaller in no limed soil, indicating that soil health and function were adversely affected. Through those results it can be concluded that nematode community structure have been little influenced by changes in environmental conditions. Even though in this study the nematode genera was the highest with P application (Fig. 1), Sarathchandra et al. (2001) found that total nematode genera showed a general increase (statistically insignificant) as the application rate of P increased from 0 to 100 kg and that some of these results confirm that phosphate fertiliser at least has no significant (neither adverse nor beneficial) effects on soil microbial populations.

Even though there were found non-significant difference in F/B value (Table 3), the fungal feeders are more abundant in acid soils (no lime), which was confirmed in our study, as the CI value was smaller in limed soil due to decreased number of fungi.

The small share of omnivores in this study confirm their sensitivities, thus the omnivores and predatory nematodes proved to be sensitive to heavy metal contamination, but also for agricultural land it is common the low population of this trophic groups (Čermák et al. 2011; Franco-Navarro, Godinez-Vidal 2017). In this study the highest share of nematodes were plant parasitic nematodes, than bacterial feeders and fungal feeders. The omnivores and predators have share less than 10%. The family *Rhabditidae* (bacterial feeders) may increase following a resource pulse, in stressed, natural environments, but using the bacterial feeders as a trophic group is not very useful in assessing the effect of the disturbances (Georgieva et al. 2002). Sánchez-Moreno and Navas (2007) found that soil nematode community is almost fully recovered 46 months after sudden pollution with heavy metals as Pb, Ni, Cu and Zn. HU et al. (2014) reported that bacteria feeders nematodes and cp-1 functional group were the dominant trophic group in the greenhouse as against larger numbers of plant parasitic and cp-3 functional group which ones found in the farmland.

Changes in soil pH can affect nematodes directly by chemical changes, such as their playing ability, or indirectly, as changes in the availability of their main food supply of bacteria and fungi. Räty and Huhta (2003) reported that liming (6 g $CaCO_3$/kg soil) significantly ($P$ < 0.05) increased MI and ratio of bacterial feeders to fungal feeders nematodes (B/(B+F)). Also, authors reported that total number of nematodes genera increased from 11.8 (control) to 15.2 with lime application and that there is significant increasement of nematode genera *Acrobeloides*, *Plectus, Tetratocephalus*, *Tylenchus*, *Aphelenchoides* and *Tylencholaimus* with lime application.

Georgieva et al. (2002) conducted that genera richness of nematode communities decreased and that abudance of many colonizers as *Acrobeloides, Eucaphalobus* and *Chiloplacus* increase with Zn + Cu and Zn treatments. Eskmitt and Korthals (2006) found that nematode genera *Tylenchus* and *Cephalobusas* as good bioindicators of soil pollution with Zn. Zhang et al. (2006) conducted that various concentrations of mineral fertilizer $ZnSO_4$ (0, 100, 200, 400, 800 mg/kg Zn) was not significantly influenced on the number of plant parasitic nematodes.

## CONCLUSION

The influence of liming, phosphorus and zinc application on soil nematode community structure in hot pepper was studied using nematological indices. Biodiversity od nematode genera was not affected significantly by liming, while adding phosphorus had significantly increase biodiversity of nematode genera. Above all indices (MI, MI2-5, PPI, PPI/MI, F/B, H', EI, SI, CI), only Structural index (SI) showed statistically difference in interaction of treatments and subtreatments. Bacterial feeders and plant feeding nematodes were the most dominant trophic group in all treatments, while the predators were the rarest tc group in all treatments.

Even though adding of lime, phosphorus and zinc in the soil for hot pepper production depend on chemical soil properties of the given soil, biological component such as nematode community

https://doi.org/10.17221/217/2017-HORTSCI

structure should not be neglected and can be used an effective tool for analysing soil quality as well as other aspect of land use. However, index calculations were less sensitive at detecting fertilizer treatment effects, we suggest to use other community analyses such as indicator species analysis or diversity evaluations to detect fertilizer effects.

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