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INFLUENCE OF LIQUID CHICKEN MANURE PREPARATION ON SOIL HEALTH AND AGROCHEMICAL SOIL PROPERTIES

Brmež, M.[(1)]***, Puškarić, J.***[(1)]***, Siber, T.***[(1)]***, Raspudić, E.***[(1)]***, Grubišić, D.***[(2)]***, Popović, B.***[(1)]

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SUMMARY

The aim of this study was to determine the influence of liquid chicken manure preparation on nematode biodiversity as an indicator of soil health and agrochemical soil properties. The field experiment was conducted during two years into two treatments and three samplings per year. One plot was treated with liquid chicken manure preparation (LCMP) while the other plot, without manure application, was control (C). In the first year of this study, the treatments were conducted in the wheat plantation, while in the following year the culture was rapeseed. Results indicate that genus biodiversity was significantly higher in treatments with LCMP (18% in wheat, 28% in rapeseed). Indices of disturbance (MI, MI [2-5], PPI/MI) show statistically significant differences during the two years indicating ecosystem stability, reduction of plant parasitic nematodes and the multitude of beneficial nematodes in treatment with LCMP compared to C. The analysed agrochemical properties showed the increasement of organic matter content (60%) and P and K content in treatment with LCMP compared to C. It can be concluded that the use of LCMP significantly increases the nematode diversity and the stability of the soil ecosystem as well as a nutrient content in the soil which results in a reduced economic investment, primarily in the use of mineral fertilizers.

Key-words: nematode biodiversity, wheat, rapeseed, ecological indices, agrochemical soil properties

INTRODUCTION

In order to overcome demands of food production for constant growing population, improving and preserving soil fertility is of a great importance, especially in developing countries (Liang et al., 2009). The great importance lies in healthy and productive soil because healthy soil is able to provide essential nutrients for plant growth, supports many biotic communities and activities and balances ecosystems (Mäder et al., 2002). Soil microorganisms have a major role in mineralization and decomposition of organic matter (defining soil quality and health), which makes soil biology directly linked to agricultural sustainability. Soil quality is related to soil fertility and it can be measured through evaluation of physical, agrochemical or microbial parameters (Nair and Ngouajio, 2012).

Nematodes are an important biotic component of the soil and assessment of nematode communities can provide insight into the biological and functional state of the soil (Ritz and Trudgill, 1999) and processes within different ecosystems. They are good bioindicators since they are closely related to the environment and react shortly after the slightest changes in the soil (Brmež et al., 2004). Population and functional diversity of nematodes are influenced by soil environment directly and/or indirectly. Crop residues and other applied organic amendments can enhance activities and abundance of these decomposers in the soil (Bulluck et al., 2002). Creating conditions favourable for biological activity of nematodes leads to enhanced concentration of mineral N available to the crops. Application of liquid chicken manure preparation is a good agronomic measure that provide favourable

(1) Prof. Dr. Mirjana Brmež, Josipa Puškarić, M. Eng. Agr. (josipa.puskaric@pfos.hr), Tamara Siber, M. Eng. Agr., Prof. Dr. Emilija Raspudić, Assoc. Prof. Brigita Popović - Josip Juraj Strossmayer University of Osijek, Faculty of Agriculture in Osijek, Vladimira Preloga 1, Osijek, Croatia, (2) Assoc. Prof. Dinka Grubišić - University of Zagreb, Faculty of Agriculture, Svetošimunska cesta 25, Zagreb, Croatia

conditions for microbiological activity (Bernal, 1998). Chicken manure is a readily available organic source of essential plant nutrients. It is used primarily as a source of plant nutrients (Mullins, Bendfeldt and Clarik, 2002). Moreover, manure is a source of energy for soil biota and thus influences many of the biological processes of soil (Woomer and Swift, 1993).

Abundance of bacterivore nematodes can be an indicator of microbial grazer activities and N mineralisation rates from soil fauna (Ferris, 2004). Free-living nematodes, such as bacterivorous and fungivorous contribute up to 27% of the available nitrogen in the soil (Ekschmitt et al., 1999) and also promote colonization of beneficial rhizobacteria in the rhizosphere (Knox et al., 2003). Considering negative correlation between free-living and plant parasitic nematodes, goals of sustainable agriculture should be population enhancement of free-living nematodes and mitigation of plant parasitic nematodes (Briar et al., 2007)

The aim of this research was to show how an application of liquid chicken manure preparation (LCMP), as an organic amendment, in different cover plants affects the nematode population dynamic in the soil and the number of genera through different nematological indices.

MATERIAL AND METHODS

The field experiment was set up on 9th March 2016 at the site near Osijek, Croatia (45°50′ N, 18°64′ E) in winter wheat (*Triticum aestivum* L.) fields for the first year, subsequently in rapeseed (*Brassica napus* L.) for the second year. Both years included two treatments on 2 ha altogether, from which one was control treatment (C) without any fertilizer application while other plot was treated with LCMP. Chemical composition of LCMP was: 21% organic matter, 10% N, 4% K and 1% P. The experimental design was randomized block and field experiment consisted of six samplings while within each were four repetitions for both treatments, 48 samples accordingly.

The samplings were conducted from 9th March 2016 (sampling I), before application of LCMP, one month after application (6 June 2016 - sampling II) and two months after application (5 July 2016 – sampling III). Wheat was harvested and the field was prepared for rapeseed sowing. New samples were taken before repeated application of LCMP (sampling IV), and the experimental field was left untouched over winter until spring 27th April 2017 (sampling V). The last sampling (VI) was conducted before rapeseed harvest (28 June 2017) in order to determine the influence of above-mentioned preparation (LCMP) on biodiversity of nematode community as bioindicators of „soil health".

All the samples were taken in arable layer depth of 1-25 cm with the probe (2 cm diameter), in zig-zag line. Each sample mass weighted 500 g, from which 100 g was segregated for extraction. The samples were marked, saved in plastic bags separately and delivered to Department for Plant Protection, Faculty of Agriculture in Osijek, for analysis.

Nematodes were extracted from each soil sample with Baermann funnels method (Baermann, 1917). After extraction nematodes were counted under the stereoscope (Olympus SZX) but nematode determination to genera was carried out under the microscope (Olympus BX50) according to Andrassy (2005, 2007, 2009), Bongers (1994) and Mai and Lyon (1975).

Changes in the nematode community related to the treatments were analyzed through the number of genera, genera structure through trophic groups by Yeates et al. (1993) as well as calculated indices of disturbances (MI, MI [2-5], PPI and PPI/MI) by Bongers (1990, 1994) and ecological indices (EI, SI) by Ferris et al. (2001). The results of the research were statistically processed, analysis of variance and LSD test were done using SAS 9.1.

Chemical characteristics of the soil were also determined. Analyses of major soil chemical properties (soil pH, organic matter, the content of P and K) in the arable layer (0-30 cm) of soil were done at Department for Agroecology, Faculty of Agriculture in Osijek. The samples were taken on 28th June 2017 in order to compare changes in the agrochemical properties in soil (C and LCMP). The pH of the soil was measured in the supernatant suspension of 1:5 (volume fraction), 0.01 mol/l KCl solution in water for pH($CaCl_2$) and deionised water for pH(H_2O) (ISO, 1994). The organic soil substance was determined by bicarbonate method (ISO, 1998), and plant-available P and K extracted by the ammonium-lactate (AL) solution (Egner et al., 1960).

RESULTS AND DISCUSSION

The impact of liquid chicken manure preparation (LCMP) on nematode communities in the soil (biological soil property) and agrochemical soil properties was examined throughout two years of trials. The percentage share of determined genera varied in association with different treatments (Table 1). Many genera of bacterivores (Ba) (*Cephalobus*, *Chiloplacus*, *Metateratocephalus*, *Prismatolaimus* and *Teratocephalus*) appeared in both treatments. In the treatment with LCMP there were no noted genera of bacterivorous nematodes that disappeared throughout sampling period. Van Diepeningen et al. (2006) noted that in organic farming higher species richness of nematodes provided a better capacity to recover quickly from the dry-wet disturbance compared to conventional farming. Gomez et al. (2006) reported higher microbial diversity in amended soils (both vermicompost and chicken manure) compared to the control.

Correlation between soil pH and trophic groups of nematodes is undisputable. Table 4 displays that pH level in both treatments are in a range of slight (LCMP) to moderate (C) alkalinity which can be related to a percentage share of fungivorous (Fu) nematodes in samples (Table 1). Some fungivores were more represented in the treatment with LCMP than C treatment in the last sampling, such as *Aphelenchoides*, *Diphtherophora* and *Ditylenchus.*

Table 1. Percentage share of determined nematoda genera

Tablica 1. Postotni udio utvrđenih rodova nematoda

Sampling *Uzorkovanje*	I	VI		**Sampling** *Uzorkovanje*	I	VI	
Treatments *Tretmani*	IS	C	LCMP	**Treatments** *Tretmani*	IS	C	LCMP
Acrobeles			0.41	*Metateratocephalus*			0.41
Acrobeloides	7.14	32.98	7.75	*Microdorylaimus*	0.65		
Acrolobus		1.05		*Panagrobelus*		0.52	1.63
Alaimus			0.40	*Panagrolaimus*		6.81	4.08
Aphelenchoides	13.64	7.85	12.65	*Plectus*	1.30	0.52	0.82
Aphelenchus	4.54	4.20	3.67	*Pratylenchus*	2.60		
Cephalobus			1.23	*Prismatolaimus*			9.39
Chiloplacus			0.41	*Psilenchus*	0.65		
Diphtherophora			0.41	*Pungentus*			0.41
Ditylenchus	11.40	10.99	11.83	*Rhabditis*	41.55	1.05	2.45
Eucephalobus	0.65	20.94	20.41	*Rotylenchus*	0.65		
Eudorylaimus	1.95	2.10	2.45	*Teratocephalus*			2.45
Filenchus		0.52		*Tylenchorhynchus*	0.65	0.52	
Malenchus			0.41	*Tylenchus*	12.99	4.71	4.49
Mesodorylaimus		5.24	11.83				
				Total number of genera *Ukupan broj rodova*	**14**	**15**	**22**

IS - initial state, C - control treatment, LCMP - treatment with liquid chicken manure preparation / *IS - početno stanje, C - kontrolni tretman, LCMP – tretman pripravkom tekućega pilećega stajnjaka*

Except for the beneficial nematodes, the group of plant parasitic (PP) nematodes can cause a great damage in agriculture while feeding directly on host plant which affects humans and their nourishment. Indirect damage they induce is also important as open wounds on plant tissues can allow numerous pathogens dwelling in the soil to enter the roots or nematodes can transmit viruses which get released while feeding (McSorley, 1997). Some plant parasitic nematodes from the family Tylenchidae, such as *Malenchus* and *Tylenchus* are dominant in each type of soil (Bongers at al., 1989) belonging to c-p group 2 since they cause insignificant damage considering their weak and short stylet. Results in Table 1 prove that *Tylenchus* is dominant since it is the only plant parasitic nematode genus that occurred in each sampling period, while *Malenchus* spp. appeared only in the treatment with LCMP. On the other hand, genus *Tylenchorhynchus*, belonging to c-p group 3, and cause severe damage compared to *Malenchus*, disappeared in LCMP. Value of the plant parasitic index (PPI) show that less damages to plants were observed in the treatment with LCMP due to lack of higher c-p group of plant parasitic nematodes (Table 2 and 3).

According to Akhtar and Malik (2000) organic amendments can enhance different microorganism activities including those that are hostile to plant parasitic nematodes. However, this action of plant disease control is complex. Lower PPI in treatment with LCMP could be possibly explained by the greater number of omnivorous (Om) nematodes which feed on other nematodes as well as on whole microfauna. *Eudorylaimus*, *Mesodorylaimus* and *Pungentus* had the highest frequencies of occurrence in the latter treatment that can be seen in Table 1 as well as in Tables 2 and 3 presented as percentage share in trophic groups. Since *Mesodorylaimus* belongs to the highest c-p group (5) and is found in the highest number, it greatly influenced many indices of disturbances such as maturity indices (MI and MI [2-5]) and ratio PPI/MI.

Table 2. Indices of disturbance, trophic groups distribution and indices of food web determined in field experiment in wheat, 2016

Tablica 2. Indeksi uznemirenja, postotni udio trofičkih grupa i indikatori funkcije ekosustava utvrđeni u poljskome pokusu u pšenici, 2016.

Number of sampling *Broj uzorkovanja*	I		II		III	
Treatments *Tretmani*	**IS (C)**	**IS (LCMP)**	**C**	**LCMP**	**C**	**LCMP**
Number of genera *Broj rodova*	8.25^{A}	8.25^{A}	13.00^{B}	15.25^{D}	11.00^{C}	14.75^{D}
MI	1.65^{A}	1.65^{A}	2.14^{B}	1.97^{B}	1.72^{A}	2.73^{C}
MI (2-5)	2.23^{A}	2.23^{A}	2.29^{AB}	2.16^{A}	2.43^{B}	3.26^{C}
PPI	2.27	2.27	2.20	2.01	2.64	2.50
PPI/MI	1.38^{B}	1.38^{B}	1.03^{A}	1.02^{A}	1.54^{B}	0.91^{A}
% Ba	49.25	49.25	35.00	42.75	54.75	39.25
% Fu	29.25	29.25	33.00	41.25	12.75	21.75
% Pp	16.50^{B}	16.50^{B}	25.50^{C}	12.75^{A}	25.50^{C}	11.00^{A}
% Om	5.00^{A}	5.00^{A}	6.50^{A}	3.25^{A}	6.00^{A}	28.00^{B}
% Pr	0	0	0	0	1.00	0
EI	82.35	82.35	53.22	58.35	83.99	76.89
SI	27.03	27.03	37.71	26.96	48.98	85.90
BI	16.37	16.37	36.52	36.01	13.71	9.58
CI	15.70	15.70	49.28	42.46	8.18	15.88

IS - initial state, C - control treatment, LCMP - treatment with liquid chicken manure preparation / *IS - početno stanje, C - kontrolni tretman, LCMP – tretman pripravkom tekućega pilećega stajnjaka*

*Values marked with different letters in rows refer to statistically significant differences (P<0.05) depending on treatment / *Vrijednosti označene različitim slovima u redovima odnose se na statistički značajne razlike (P<0,05) ovisno o tretmanu*

Table 3. Indices of disturbance, trophic groups distribution and indices of ecosystem function determined in the field experiment in rapeseed, 2017

Tablica 3. Indeksi uznemirenja, postotni udio trofičkih grupa i indikatori funkcije ekosustava utvrđeni u poljskome pokusu u uljanoj repici, 2017.

Number of sampling *Broj uzorkovanja*	IV		V		VI	
Treatmens *Tretmani*	**C**	**LCMP**	**C**	**LCMP**	**C**	**LCMP**
Number of genera *Broj rodova*	13.50^{B}	15.00^{C}	13.00^{B}	18.00^{D}	10.00^{A}	13.75^{B}
MI	2.28^{BC}	2.41^{CD}	1.89^{A}	2.09^{AB}	2.13^{B}	2.56^{D}
MI (2-5)	2.57^{BC}	2.70^{C}	2.15^{A}	2.45^{B}	2.23^{A}	2.67^{C}
PPI	2.04	2.09	2.23	2.12	2.06	2.00
PPI/MI	0.90^{B}	0.87^{AB}	1.18^{D}	1.03^{C}	0.99^{C}	0.79^{A}
% Ba	44.50^{AB}	38.50^{A}	53.25^{BC}	40.00^{A}	64.25^{C}	45.25^{AB}
% Fu	22.25^{A}	35.25^{B}	22.50^{A}	27.00^{AB}	23.25^{A}	33.00^{B}
% Pp	15.75	6.50	21.00	25.50	5.00	4.75
% Om	15.00	17.25	2.75	7.50	7.50	17.00
% Pr	2.50^{B}	0^{A}	0.50^{A}	0^{A}	0^{A}	0^{A}
EI	61.06	63.57	62.72	68.37	39.82	49.52
SI	60.07^{D}	65.99^{E}	24.52^{A}	51.82^{C}	30.27^{B}	65.47^{DE}
BI	23.88^{A}	21.17^{A}	32.91^{B}	22.56^{A}	44.82^{C}	25.33^{A}
CI	29.15	36.14	26.41	28.18	47.40	56.71

C - control treatment, LCMP - treatment with liquid chicken manure preparation / *C - kontrolni tretman, LCMP - tretman pripravkom tekućega pilećega stajnjaka*

*Values marked with different letters in rows refer to statistically significant differences (P<0.05) depending on treatment / *Vrijednosti označene različitim slovima u redovima odnose se na statistički značajne razlike (P<0,05) ovisno o tretmanu*

MI is a measure of the soil maturity based on nematode fauna excluding plant parasitic nematodes used for assessment of the soil disturbance, the same as MI (2-5) which excludes c-p 1 groups caused by a recent disturbance (Bongers, 1990). Soil treated with LCMP has a higher stability shown as higher MI and MI (2-5) values and percentage share of omnivorous nematodes (Table 2 and 3). Similarly, ratio PPI/MI indicates higher stability of the ecosystem in treatments with LCMP compared to C treatments with statistical significance (LSD 0.01). Bongers et al. (1997) concluded that under certain conditions such as nutrient enrichment effect, MI and PPI are inversely related and plant parasitic nematodes are rarely dominant under semi-natural conditions. High reproductive potential such as efficiency of food uptake and conversion plays a direct role in establishing equilibrium between colonizers and persisters. Temporary decrease and slow recovery of MI after applications (Table 2 and 3) might be caused by nutrient uptake. On the other hand, increased nutrient uptake by plant causes a shift within the composition of the plant parasitic nematodes. Cp-2 group is replaced by cp-3 group of plant parasitic nematodes feeding on more nutrient-rich cells in deeper root zones. Further nutrient exhaustion leads to increased MI while making plants compete for nutrients leads to changes in cp groups of plant parasitic nematodes making epidermal cell feeders dominant again (cp 2) (Bongers et al. 1997).

Enrichment index (EI) is an indicator for the availability of nutrition in the soil showing increased activity of bacterivores and better organic decomposition (Table 2 and 3) while Structural index (SI) indicates the maturity of a certain ecosystem that has been exposed to some disturbance or is in a recovery period from disturbance. In this study a higher value of SI in the variant with LCMP compared to the control resulted in more stable ecosystem conditions with high biodiversity of nematode genera. Basal index (BI) includes abundance of general opportunists and shows the level reduction of their density in the soil due to various stress conditions. Channel index (CI) gives insight into the decomposition of organic matter in the soil. Low values indicate the high number of bacterivores participating in organic decomposition in the soil while high CI values indicate fungal dominated decomposition (Ferris et al., 2001; Minoshima et al., 2007).

Table 4. Agrochemical soil properties

Tablica 4. Agrokemijska svojstva tla

Treatments *Tretmani*	**C**	**LCMP**
Depth	0-30	0-30
pH (H_20)	8.09	7,98
pH (KCl)	7,62	7,30
AL-P_20_5 mg/100 g	38.90	68,36
AL-K_20 mg/100 g	27,96	58,82
organic matter	1,21	2,00
% $CaCo_3$	3,35	2,90

C - control treatment, LCMP - treatment with liquid chicken manure preparation

C - kontrolni tretman, LCMP - tretman pripravkom tekućega pilećega stajnjaka

Control data for soil-fertility in Croatia commonly include properties such as soil pH, organic matter content and plant-available phosphorus (P) and potassium (K) (Popović et al., 2010). The analysed agrochemical properties (Table 4) highlight the organic matter increase by 60% which changed organic matter content from poor (C) to well supplied (LCMP). This result is of great importance from the aspect of soil enrichment with organic matter considering the ongoing soil degradation in Croatia which in long-term leads to an aberration of soil fertility, productivity and lower biodiversity (Gao et al., 2011).

Also, the content of P and K increased in treatment with LCMP compared to C (57% P and 47% K) which indicates elevated supplies of these nutrients in the soil as a result of the higher concentrations of these elements in chicken manure compared to other organic fertilizers. According to Elamin (1991.) a large fraction of the phosphorus in manure is considered to be plant available immediately after application and availability of potassium (K) is usually near 100% with proper application.

Moreover, higher contents of P and K in the soil result in a longer period of accessibility of these elements to the plants, without the need for fertilizers application (mineral and organic), contributing to economic and ecological production sustainability (Scherer, 2004.).

CONCLUSION

Distribution of nematode genera varied between the first and second year, also seasonally within the same year. LCMP applications increased nematode genera diversity and enhanced nematological indices of disturbance. Reduction of plant parasitic nematodes and the multitude of beneficial nematodes were observed in the amended treatments. The analysed agrochemical soil properties showed that organic matter, P and K contents in LCMP treatment plots increased compared to the control. It can be concluded that the use of LCMP significantly increases the bio-diversity of the nematode in the soil and the stability of the ecosystem as well as a nutrient content in the soil which results in a reduced economic investment, primarily in the mineral fertilizers use.

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UTJECAJ PRIPRAVKA TEKUĆEG PILEĆEG STAJNJAKA NA ZDRAVLJE TLA I AGROKEMIJSKA SVOJSTVA TLA

SAŽETAK

Cilj ovog istraživanja bio je utvrditi utjecaj preparata na bazi tekućega pilećega stajnjaka na biološku raznolikost nematoda kao pokazatelja zdravlja tla i agrokemijska svojstva tla. Pokus na terenu proveden je kroz dvije godine u dva tretmana i tri uzorkovanja godišnje. Jedna parcela tretirana je preparatima na bazi tekućega pilećega stajnjaka (LCMP), dok je druga bila kontrolna (C). U prvoj godini na pokusnim parcelicama bila je zasijana pšenica, a u drugoj uljana repica. Rezultati pokazuju da je bioraznolikost rodova bila značajno brojnija u tretmanima s LCMP-om (18% pšenica, 28% uljana repica). Indeksi uznemirenja (MI, MI[2-5], PPI/MI) pokazuju statistički značajne razlike kroz dvije godine te upućuju na stabilnost ekosustava, smanjenje fitoparazitskih nematoda i mnoštvo korisnih nematoda u LCMP tretmanu usporedno s kontrolom. Analizirana agrokemijska svojstva ukazuju na povećanje organske tvari za 60%, kao i sadržaja P i K u tlu u tretmanu s LCMP-om usporedno s kontrolom. Može se zaključiti da upotreba LCMP-a značajno povećava biološku raznolikost nematoda u tlu te stabilnost ekosustava, kao sadržaj hranjivih tvari u tlu, što za posljedicu ima smanjena ekonomska ulaganja, prvenstveno u upotrebi mineralnih gnojiva.

Ključne riječi:* *bioraznolikost nematoda, pšenica, uljana repica, ekološki indeksi, agrokemijska svojstva

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