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Analysing the characteristics of soil nematode communities at pepper (*Piper nigrum* L.) cultivation area in Loc Hung commune, Loc Ninh district, Binh Phuoc province

Phân tích đặc trưng quần xã tuyến trùng đất tại vùng trồng hồ tiêu (piper nigrum L.) thuộc xã Lộc Hưng, huyện Lộc Ninh, tỉnh Bình Phước

Research article

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Pepper (*Piper nigrum* L.) is a high economic value plant species that brings the main income to the people at Loc Hung commune. So the pepper's yield plays a significant role in people's life in this region. To assess the influence of environmental factors on the growth and development of pepper, we need to analyse the structural characteristics of soil nematode communities in order to promptly detect the levels of parasitic nematodes infection as well as assessment of soil environment status based on nematode communities. Study results at five sampling stations are analyses of 30 genera of nematodes belonging to 6 major trophic groups (bacterial feeders, fungal feeders, algal feeders, omnivores, carnivores and plant parasites). Calculation results of indices such as ΣMI, ΣMI2-5, MI and PPI showed that soil nematodes community is very little affected by the bacteria feeder group and the rate of parasitic nematode pepper quite severe. Analysis of correlation between trophic groups showed that nematodes are sensitive to environmental factors. This means the potential use of nematodes as a biological indicator for soil quality is possible.

*Hồ tiêu (*Piper nigrum *L.) là cây trồng có giá trị kinh tế cao và là nguồn thu nhập chính của người dân tại xã Lộc Hưng. Do đó, năng suất cây tiêu có ý nghĩa rất lớn đối với đời sống người dân trong vùng. Để đánh giá ảnh hưởng của các yếu tố môi trường lên sự sinh trưởng và phát triển của cây tiêu, cần phải phân tích đặc trưng cấu trúc quần xã tuyến trùng đất của vùng nhằm phát hiện kịp thời mức độ nhiễm tuyến trùng ký sinh trên hồ tiêu cũng như đánh giá nhanh hiện trạng môi trường đất dựa trên quần xã tuyến trùng. Kết quả nghiên cứu tại 5 điểm thu mẫu phân tích được 30 giống tuyến trùng thuộc 6 nhóm dinh dưỡng chính (ăn vi khuẩn, ăn nấm, ăn tảo, ăn tạp, ăn thịt và ký sinh thực vật). Kết quả tính toán các chỉ số ∑MI, ∑MI2-5, MI và PPI cho thấy quần xã tuyến trùng đất ở đây rất ít chịu ảnh hưởng của nhóm ăn vi khuẩn và tỷ lệ hồ tiêu nhiễm ký sinh khá nặng. Phân tích tương quan giữa các nhóm dinh dưỡng cho thấy tuyến trùng khá nhạy cảm với các yếu tố môi trường, điều này cho thấy tiềm năng sử dụng tuyến trùng như một sinh vật chỉ thị cho chất lượng môi trường đất.*

Keywords: correlation, biological indicators, nematodes, pepper, Loc Hung, Binh Phuoc

1. Introduction

Nematodes are an invertebrate group that has great potential in the use as indicator organisms for soil quality. Nematodes possess the most important attributes of any prospective bio-indicator (Cairns et al., 1993): abundance in virtually all environments, diversity of life strategies and feeding habits (Freckman, 1988; Yeates et al., 1993a),

short life cycles, and relatively well-defined sampling procedures. Therefore, nematode community structure will reflect the differences of environmental quality. The environmental factors will impact on the nematode groups in soil and change the proportion of these groups to respond with disturbances. Conventional farming systems have been associated with many environmental risks, so the ability to monitor and assess the quality of soil agro-ecosystem would be of significant importance. The idea that changes in the soil environment imposed by agricultural management practices could be revealed by measures of nematode community patterns has been investigated in recent years (Ferris et al., 1996; Freckman and Ettema, 1993; Yeates et al., 1997; Nguyen Ngoc Chau and Nguyen Vu Thanh, 2005; Duong Duc Hieu et al., 2009).

For these reasons, several researchers have attempted to develop relationships between nematode community structure and succession of natural ecosystems or environmental disturbance (Ettema and Bongers, 1993; Freckman and Ettema, 1993; Freckman and Virginia, 1997; Wasilewska, 1994; Yeates and Bird, 1994). Thus, it is important to find trends illustrating the soil condition, but more important to find their explanation.

For pepper cultivation area in Loc Hung – Binh Phuoc province, the soil nematode community structure will reflect characterized farming and environmental status of the land. Besides, pepper parasitic nematodes cause loss of economic. Therefore, analysing community structure to assess parasitic level and environmental status of land in the study area is important to give methods to minimize the effects of parasitic nematodes and to give appropriate cultivation methods to improve plant productivity without causing degradation of land environment.

2. Location, materials and methods

2.1 Location
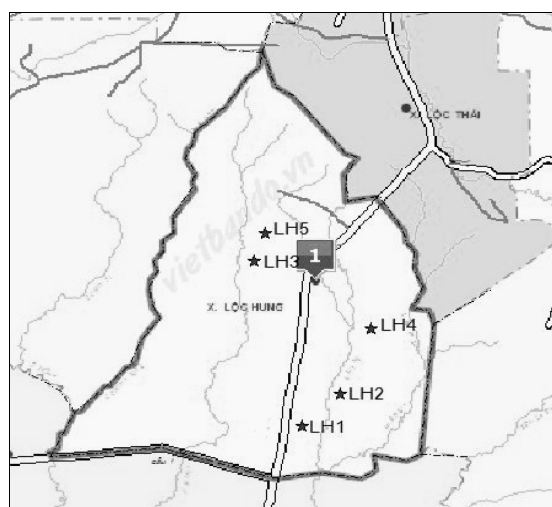
Figure 1. The sampling stations map in Loc Hung (Binh Phuoc province)

Loc Hung commune (Loc Ninh district, Binh Phuoc province) locates in the south of Vietnam and near Ho Chi Minh city. We used GPS to determine the coordinates of

sampling points (Table 1) and used MapInfo 9.0 software to build the diagram sampling position (Figure 1).

Table 1. The coordinates of stations in Loc Hung

Stations	Sample coordinates	
	North latitude	East longitude
LH1	$12^028'53,20''$	$106^039'37,90''$
LH2	$12^029'15,63''$	$106^047'18,08''$
LH3	$11^001'00,64''$	$106^021'19,48''$
LH4	$12^045'01,06''$	$106^051'49,41''$
LH5	$12^006'06,08''$	$106^022'46,60''$

2.2 Materials and methods

Surveying and sampling at 5 pepper farms on January, 2012. We randomly selected 3 pepper stumps from each farms, in each stump we collected 3 soil samples by using the method of Nguyen Ngoc Chau (2003): 30cm out of stump, depth from 10 - 15cm, about 500 gram soil, then the samples were preserved in plastic bags. The total number of samples includes 15 soil samples that were respectively denoted LH1, LH2, LH3, LH4, and LH5. Sampling temperature was about $30\text{-}33^0C$.

Nematodes were extracted from 100 gram soil each sample by decanting and centrifugation with sugar media (Nic Smol, 2007). After counting on the square counting dish, nematodes were picked up and mounted by Seinhorst's method (1959), then 200 individuals per site were randomly selected and identified to taxonomic genus according to *Fauna in Vietnam* (Nguyen Ngoc Chau and Nguyen Vu Thanh 2000; Nguyen Vu Thanh, 2007); *Freshwater nematode: Ecology and Taxonomy* (Abebe, E., et al., 2007)*; Aphelenchida, Longidoridae and Trichodoridae* (Hunt, D.J., 1993).

Nematodes were classified as six trophic groups: bacterial feeders, fungal feeders, algal feeders, omnivores, carnivores and plant parasites. The classification of feeding groups mainly followed Yeates et al. (1993).

The determination of nematode types along the colonization-persistence gradient followed Bongers (1988; 1990), c-p index shows the sustainability level of ecosystems, it has value from 1 to 5 corresponding to the level from unstable (colonizers) to stable (persisters) of ecosystems. Nematode group with cp = 1 is high colonizable level, easy to change and consistent with an unsustainable environment; while nematode group with cp = 5 is a group of high settlement and indicates a sustainable environment.

The calculation of the maturity index MI and PPI followed Bongers (1990) and Tom Bongers and Howard Ferris (1999). MI or PPI = $\sum_{i=1}^{n} v(i) \times f(i)$, where v(i) is the c-p value of taxon i, f(i) is the frequency of taxon i in a sample. Maturity Indices of nematode community calculated by formula include: ∑MI is total maturity index (including free-living nematodes and parasitic nematodes), ∑MI2-5 is maturity index (except nematodes with

c-p = 1), MI is maturity index of free-living nematodes (not including plant parasitic nematode groups).

3. Results and discussion

3.1 Nematode community structure in Loc Hung, Binh Phuoc province

3.1.1 Component trophic groups

The analysis result of grouped nematode in 5 sampling points is shown in Table 2, c-p value for each genus is determined according to Bongers et al (1990).

At sampling point LH1, the result of analysis gained 19 genera. This was the sampling point having the highest number of genera (occurring 54.29% of total number of analysed genera). Therein, the dominant genera were *Meloidogyne* sp. (32.93%) and *Cephalobus* sp. (14.63%). Others occurred in a very low proportion (less than 6.5%).

At sampling point LH2, the result of analysis gained 13 genera (occurring 37.14% of total number of analysed genera). The dominant genera were *Helicotylenchus* sp. (31.97%), *Filenchus* sp. (23.13%) và *Meloidogyne* sp. (13.86%). Others were less than 9.0%.

Another sampling point having high number of genera was LH3 with 17 genera (occurring 48.57% of total number of analysed genera). Therein, *Meloidogyne* sp. was the most dominant genus with 38.27% in total individuals. Others were less than 9.5%.

The result of analysis at LH4 gained 15 genera (occurring 42.86% of total number of analysed genera). Specially, in this point, the extremely dominant genus was *Meloidogyne* sp. with 74.67% in total individuals, while the second dominant genus was only 8.44%. Others were less than 6%.

Table 2. Grouping of nematodes at the sampling points in Loc Hung, Binh Phuoc province

Genus	Trophic groups[1]	The sampling points				
		LH1	LH2	LH3	LH4	LH5
Cephalobus sp.	Ba_2	**	*	*	*	*
Cryptonchus sp.	Ba_3	*				
Eucephalobus sp.	Ba_2	*	*	*	*	
Geomonhystera sp.	Ba_2			*		
Heterocephalobus sp.	Ba_2	*	*	*	*	*
Panagrolaimus sp.	Ba_1	*	*	*	*	*
Prismatolaimus sp.	Ba_3	*	*	*	*	*
Protorhabditis sp.	Ba_1					*
Rhabditis sp.	Ba_1	*				*
Terschellingia sp.	Ba_3				*	
Aphelenchoides sp.	Fu_2	*	*		*	*
Aphelenchus sp.	Fu_2	*		*		
Achromadora sp.	Al_3			*	*	*
Actus sp.	Ca_4	*	*			
Iotonchus sp.	Ca_4	*				
Mylonchulus sp.	Ca_4	*			*	
Aporcelaimus sp.	Om_5			*		
Aporcelaimellus sp.	Om_5	*	*	*	*	
Crocodorylaimus sp.	Om_4	*		**	*	*
Dorylaimoides sp.	Om_3	*		*		*
Ischiodorylaimus sp.	Om_4				*	
Mesodorylaimus sp.	Om_4			*		
Prodorylaimus sp.	Om_4			*		
Aglenchus sp.	H_2		*			
Filenchus sp.	H_2	*	**	*		*
Helicotylenchus sp.	H_3		**		*	
Hoplolaimus sp.	H_3	*				
Meloidogyne sp.	H_3	**	**	**	***	***

Rotylenchulus sp.	H_3	*	*		*
Xiphinema sp.	H_5			*	*

*: < 10%;   **: 10 – 50%;   ***: > 50%

[1]*Nematodes were divided into different groups depending on trophic characteristics and their life cycle. Symbol: A_x (with A: trophic group, x: c-p value); Ba: Bacterivores; Fu: Fungivores; Al: Algivores; Ca: Carnivores; Om: Omnivores; H: Herbivores.*

The last sampling point was LH5 having 13 genera (occurring 37.14% of total number of analysed genera). The same to LH4, the extremely dominant genus at LH5 was *Meloidogyne* sp. with 76.22% in total individuals, while the second dominant genus was only 8.39%. Others were less than 6%.

The above results showed that *Meloidogyne* sp. – nematode genus was parasitic on pepper roots and was pestiferous for pepper – was the genus presenting in all of the sampling points with a very high percentage. Thus, the plant parasitic group of nematode was dominant in nematode community and they reflected the feature of region.

3.1.2 Proportion of trophic groups in Loc Hung

Analysis result of nematode community structure in Loc Hung gained 30 genera of six major trophic groups including: 7 genera of plant parasitic group (accounting for 55.19% of individuals), 10 genera of bacterivorous group (accounting for 29.67% of individuals), 7 genera of omnivorous group (accounting for 10.20% of total individuals), 2 genera of fungivorous group (accounting for 2.96% of total individuals), 3 genera of carnivorous group (accounting for 1.65% of total individuals) and 1 genus of algivorous group (accounting for 0.32% of total individual) as shown in Figure 2.

We can see that bacterivorous group and plant parasitic group are the dominant groups at most of sampling points (especially plant parasitic group), followed by omnivorous group, other groups occupy a negligible proportion. This rate reflects characteristics of the agricultural area. In this ecosystem, the use of fertilizers always leads to the significant increase of bacterivorous group and plant parasitic group. The rate of parasitic nematodes in the studied area is very high, this show that peppers in Loc Hung are infected parasitic nematodes seriously.
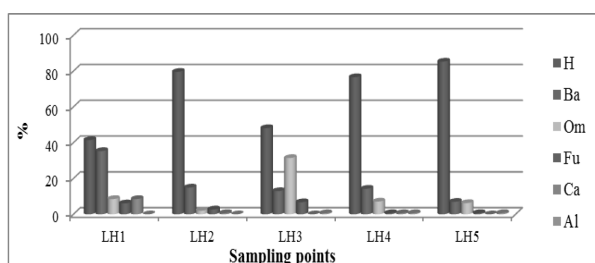

Figure 2. Percentage of nematode groups in the sampling points

Percentage of the nematode groups also shows that the carnivorous group in Loc Hung is very low; it is similar with the study result of Arantzazu Urzelai (2000), he showed that the carnivorous group was the nematode group less abundant in ecosystem types. This stems from

ecological characteristics of the carnivorous group, they have a long lifecycle, low metabolic activity, slow fertility, so they have small number and low frequency.

3.1.3 Gender structure

Gender percentage at the sampling points on Figure 3 shows that the number of juvenile individuals presents with very high rates in comparison to females and males. The general trend on farms is the dominance of juveniles, and the rate of females is almost higher than males but both of them are lower than juveniles.
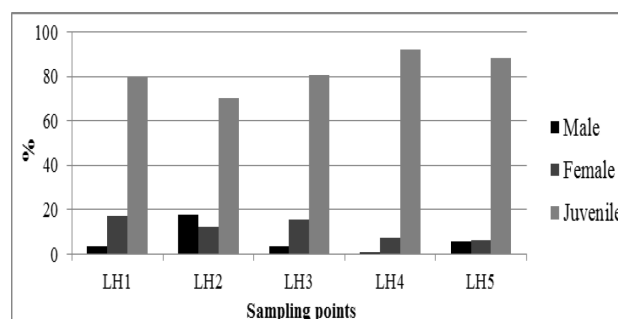

Figure 3. Gender structure at the sampling points

The presence of many juveniles shows that nematode community in the studied area is in the reproductive period and increases the number strongly.

For some of genera such as *Itonchus* sp*., Hoplolaimus* sp*., Ischiodorylaimus* sp*., Mylonchulus* sp*., Prodorylaimus* sp. **we** didn't detect the occurrence of juveniles. May be this isn't their reproductive stage, and from the dominant presence of females in some genera can predict nematode density in these genera will increase when environmental conditions are appropriate.

For nematodes, the asexual and sexual reproductive forms may occur on a genus. Therefore, in asexual reproduction genera, males rarely appear. This can explain for the low frequency of males, even not found (Randy and Anwar, 2004). However, the distribution process and structure formation are affected by many environmental factors and living conditions. In general, gender structure in the studied area with great frequency of juveniles in most of genera showed that soil environmental condition is favorable for the growth and development of nematode community.

## 3.2 The relationship between ecological indices

To assess infested level of pepper as well as assess status of soil environment in Loc Hung through their relationship, we used the ecological indices such as PPI and vari-

ous maturity indices (ΣMI, ΣMI2-5 and MI). Calculated results of ecological indices are shown on table 3.

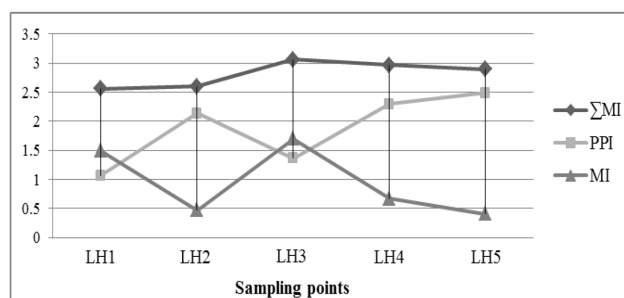**Table 3. Ecological indices of nematode community in Loc Hung**

Index	LH1	LH2	LH3	LH4	LH5
ΣMI	2.56	2.60	3.06	2.97	2.90
PPI	1.07	2.14	1.36	2.30	2.49
PPI/ΣMI	0.40	0.82	0.45	0.77	0.86
ΣMI2-5	2.49	2.58	3.05	2.96	2.87
MI	1.49	0.47	1.70	0.67	0.41

The relationship between ecological indicators calculated in Table 3 is shown on Figure 4 and Figure 5.
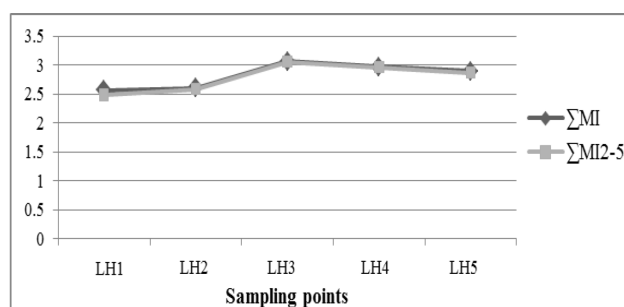

Figure 4. The relationship between ΣMI and ΣMI2-5

Figure 5. The relationship between ΣMI, PPI and MI

Curve that performs the relationship between ΣMI and ΣMI2-5 shows effect of bacterivorous group with cp = 1 negligible. The maturity index of nematode community in the sampling points was hardly influenced from the nematode group with cp = 1. Due to the ecological characteristic of nematodes group with cp = 1 is present with a high number in eutrophic environment, their presence indicates a less stable environment. Thus, the relationship between ΣMI and ΣMI2-5 on Figure 4 shows that the environment in the studied area is relatively stable.

The relationship among ΣMI, PPI and MI on Figure 5 shows that LH2, LH4 and LH5 are three sampling points having PPI higher than MI very much. This proves that the maturity of the nematode community at these points were influenced by plant parasitic nematodes rather than free-living nematodes. The rates of PPI/ΣMI at these points are very high (respectively 0.82, 0.77 and 0.86), this show that peppers in these points are infected parasitic nematodes seriously. The higher value of PPI compare

to MI value demonstrates that the growth of nematode community in LH1 and LH3 is less influenced by plant parasitic nematode. Therefore, pepers at LH1 and LH3 are less infected parasitic nematodes than others. Ratio of PPI / ΣMI reflects that infected parasitic level on target plants in study area was very high. It was an important ratio on assessing the impact of plant parasitic nematodes on pepper in particular and on other agricultural crops in general. From that the harmful effects of these parasites could be minimized.

3.3 The relationship between trophic groups
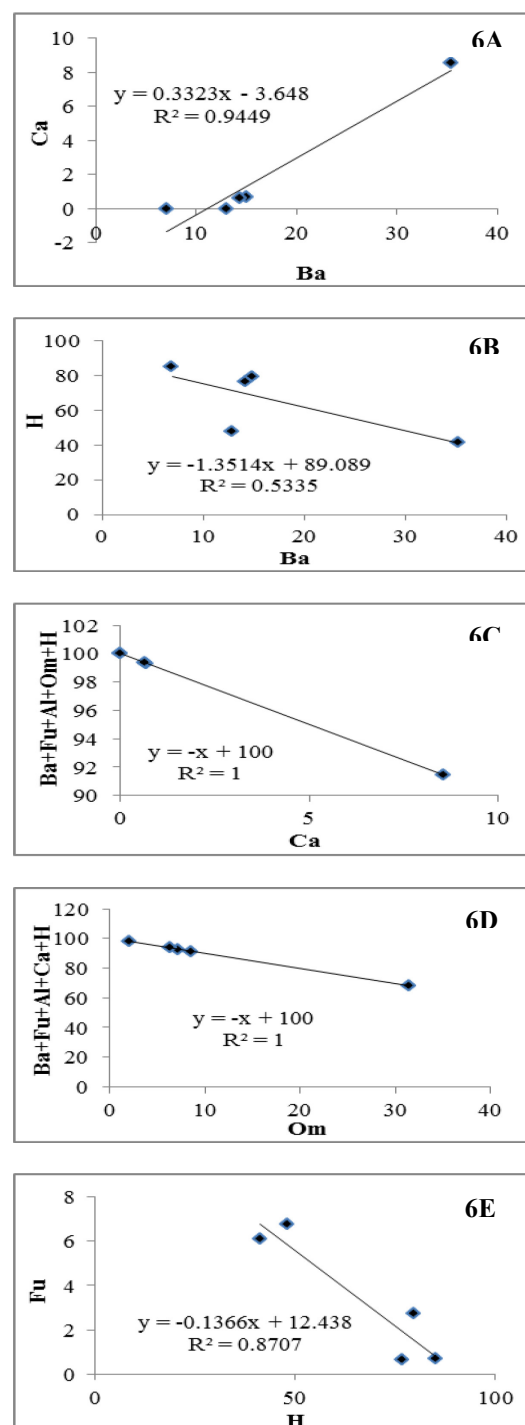
Figure 6. Relationship between trophic groups

Negative correlation was found between bacterivorous group and plant parasitic group ($R^2 = 0.5335$) (Figure

6B), plant parasitic group and fungivorous group (R^2 = 0.8707) (Figure 6E), carnivorous group and others (R^2 = 1) (Figure 6C), omnivorous group and others (R^2 = 1) (Figure 6D). The relationship between remaining trophic groups was negligible.

Carnivores and omnivores are two groups that had the most powerful influence on the rate of other groups. It could be seen in Figure 2, the sample that had high rate of carnivores and omnivores had rate of remaining groups lower than other points. Because they used other nematode groups as a food source, their presence would reduce rate of other groups. The negative correlation between omnivores and other groups is in agreement with the statement of Yeates et al. (1993) and Yeates (1999) that omnivore nematodes utilize a combination of hyphae, bacteria, microfaunal prey, diatoms and algae. Thus, an increase in the omnivore population will adversely affect the populations of other groups. These correlation results showed that in soil nematode community, trophic groups also influenced each other. So any external changes that made a certain nematode group increase or decrease leaded to the transformation of nematode related groups and changed nematode community structure. These changes reflected the current status of soil environment there.

4. Conclusion

This study gave a general background of the environmental status of studied area through the analysis of structural characteristics of nematode community. The result of study identified 30 genera belonging to six major trophic groups. Dominant trophic groups reflected the characteristics of pepper cultivation area. Research results also showed that pepper in the study was infected seriously with parasitic nematodes through assessing PPI and rate of PPI/ΣMI. Correlation analysis between trophic groups showed that trophic groups interacted closely and were sensitive to environmental factors. Therefore, nematodes are invertebrate animal groups having great potential in the use as biomarkers in evaluation of the soil environment quality.

5. Acknowledgement

6. References

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