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The effect of two compost soil amendments, based on municipal green and penicillin production wastes, on plant parasitic nematodes

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Summary

Suppressive effect of two composts, applied at five doses (0 %, 1 %, 2.5 %, 5 % and 10 % w/w), was studied on spring barley to control different genera of plant parasitic nematodes in potting mixtures. Amendment of soil with these materials resulted in a significant decreasing effect of plant parasitic nematode populations. The best reduction of number of plant parasitic nematodes was found for the nematode genera *Bitylenchus*, *Helicotylenchus*, *Heterodera, Paratylenchus* and *Rotylenchulus* by a municipal green compost (C_1) and nematode genera *Bitylenchus, Geocenamus, Helicotylenchus*, and *Rotylenchulus* by a compost derived from penicillin production residues (C_2). The compost C_1 with a lower C:N ratio was more effective in the nematode control than the compost C_2. The analysis of variance showed a significant interaction among all factors involved in the experiment: type of compost, different doses and nematode genera. Relationship between applied doses and number of nematodes showed a significantly high negative correlation.

Keywords: plant parasitic nematode; compost; suppression; compost amendment

Introduction

Phytoparasitic nematodes are a serious threat to numerous crop plants of agricultural interest (Lišková & Renčo, 2007; Kumari & Decreamer, 2007, Renčo & Čerevková 2008), can cause their yield losses (Seinhorst, 1986; Sasanelli, 1994). Control of these phytoparasitic nematodes, as for soil pathogens and weeds, has been generally based on chemical treatments (Basile *et al.*, 2003).
In every case the increasing attention to the environment safety and to the human health, require to find new alternative control strategies, environmentally sound and economically convenient at the same time (Bridge, 1996; Ciccarese *et al.*, 2008; Sasanelli *et al.*, 2008).
Among these alternative strategies use of organic amendments may be interesting because of their low cost and the more general positive agronomical effects related to the incorporation of organic matter into the soil (Sasanelli *et al.*, 2002). Use of organic amendments is known to suppress soil phytoparasitic nematode populations since many years (Linford *et al.*, 1938), although research was improved in the past decade by the above reported environmental concerns (D'Addabbo, 1995).
Organic matter is known to affect physical, chemical and biological properties of the soil (Davey, 1996). Incorporating organic amendments have a direct impact on plant health and crop productivity (Rodríguez-Kábana, 1986; Akthar & Malik, 2000). There are more than sufficient data to indicate that organic materials reduce disease incidence caused by a wide range of plant pathogens and plant pests including bacteria, fungi and nematode species (Abawi & Widmer, 2000; Bailey & Lazarovits, 2003). Disease suppressive conditions have been obtained in soil following addition of composts from different origin (Hadar *et al.*, 1992; Hoitink & Fahy, 1986).
D´Addabbo (1995) has reviewed the literature on organic amendments dating from 1982 to 1994 and found 224 papers concerning the use of different animal and plant wastes as nematode suppressant.
Incorporation into the soil of olive and grape pomace soil amendments significantly reduced the density population of the root-knot nematode *Meloidogyne incognita* in the soil (D'Addabbo & Sasanelli, 1998; Sasanelli & D'Addabbo, 2002).
Other organic soil amendments significantly reduced the populations of root-knot nematodes, *Meloidogyne* spp. (Oka & Yermiyahu, 2002; Pérez *et al.*, 2003). Similarly, Ismail *et al.*, (2006) and Renčo *et al.* (2007) observed the suppression of nematode populations of *Rotylenchulus reniformis* and *Globodera rostochiensis* in compost amended soils. Soil treated by vermicomposts contains

a lower number of plant parasitic nematodes than non-amended soil (Ribeiro *et al.*, 1998; Arancon *et al.*, 2003).
The plant parasitic nematodes were also suppressed by application of poultry manure (Khan *et al.*, 2001b; Conn & Lazarovitz, 1999), pigeon manure (Khan *et al.*, 2001b), chicken manure (Gonzalez & Canto-Sanenz, 1993; D'Addabbo *et al.*, 2003), sawdust (Khan *et al.*, 2001b) as well as by many others organic matters.
Mechanisms of this suppressive action seem to be related to the release of nemato-toxic compounds and/or ammoniacal nitrogen liberation during the degradation of organic matter and to the increased activity of naturally occurring antagonists (predators and parasites) of nematode pests (Stirling, 1991).
However, in other cases, no inhibition or increase in populations of plant parasitic nematodes in soil amended with organic materials was observed (Kimpinski *et al.*, 2003; McSorley & Gallaher, 1995, 1996). On the other hand, Akhtar & Mahmood (1994) reported an increase in numbers of predatory and free-living nematodes after soil amendments with composted manures and urea. There are several other studies which confirm that soil organic amendments improved the beneficial free living nematode (Griffits *et al.*, 1994; Arancon *et al.*, 2003; Nahar *et al.*, 2006).
Therefore, an experiment was carried out in outdoor condition, considering that: i) the use of composts is a nematode control strategy at low enviromental impact, ii) the composting process must be considered as a part of an integrated waste solution and iii) the large amount of the organic materials produced every year are at low cost. In particular, the aim of this study was to test the ability of two different composts produced in Slovak Republic, from municipal wastes and agroindustrial byproducts, with a different C/N ratio, to suppress various genera of plant parasitic nematodes in natural infested soils and to investigate the relationship between the dynamic of nematode population density and the applied compost rates.

Material and methods

Two different composts, C_1 and C_2, suitable for soil application were used in the experiment. The C_1 compost (municipal green compost) consisted of grass (60 %), leaves (25 %), tree branches (5 %) and soil (10 %) with a pH of 7.7 and the C/N ratio of 4.2:1. The C_2 compost (commercially produced) was a by-product of penicillin production, mycelium (90 %), straw (5%) and sawdust (5 %) with a pH of 7.7 and the C/N ratio of 20:1.
Composts were added and thoroughly mixed with nematode naturally infested soil at 1.0, 2.5, 5.0, 10.0 % w/w rates of dry weight. The sandy-loam soil used was collected from a cultivated field situated near the city of Košice. Each mixture (400g) was then poured into a 350 ml plastic pot, with eight replicates per treatment. Non-amended soil was used as a control. Spring barley seeds were then sown into each pot. Pot experiment was arranged under outdoor conditions according to a randomized block design and focused only to study the effect of compost applications on plant parasitic nematode population in the amended soil and to investigate the relationship between applied compost rates and the dynamic of nematode population density. Differences in growth of barley plants in the different treatments were not studied.
At the end of the experiment, after four months, the soil was taken from each pot and nematodes were isolated by the Cobb flotation-sieving method (1918), fixed in FAA and identified microscopically to genera level.
Data from the experiment were statistically analysed by the factorial analysis of variance (ANOVA) for a 2 x 5 x 7 factorial design, and means compared by the Least Significant Difference's Test and Student's *t* test.
Suppression of nematode genera by treatments was investigated through regression analyses conducted by program for simple equations models (linear, power, exponential and logarithmic) of Table-Curve Windows v1.0 (Jandel Scientific).

Results

Seven genera of plant-parasitic nematodes were identified in the control and treated pots (Table 1). In general comparison, the compost C_1 significantly reduced the number of *Helicotylenchus, Heterodera, Paratylenchus* and *Rotylenchulus* nematodes in comparison to compost C_2 (Table 1). No statistical difference was observed between the two composts for *Bitylenchus*, *Geocenamus* and *Pratylenchus* genera.
All tested rates (1, 2.5, 5 and 10 % w/w) of municipal green compost C_1 significantly reduced the number of *Bitylenchus*, *Helicotylenchus*, *Heterodera*, *Paratylenchus* and *Rotylenchulus* compared with no amended soil (control) (P = 0.01) (Table 2). However, a reduction of population of *Geocenamus* and *Pratylenchus* genera was only

Table 1 General comparison of the effect of the tested composts on different genera of plant parasitic nematodes

Nematode genera	Nematodes number		Student's *t* test
	C_1	C_2	
Bitylenchus	5.1	6.5	---
Geocenamus	14.8	10.8	---
Helicotylenchus	10.4	16.9	*
Heterodera	0.8	1.5	*
Paratylenchus	10.7	15.2	*
Pratylenchus	3.3	3.6	---
Rotylenchulus	30.5	51.5	**

* Significant for P = 0.05; ** for P = 0.01

Table 2. Effect of composts C1 and C2, applied at five rates, on different genera of plant parasitic nematodes

Treatment	Doses (% w/w)	Nematode genera																				
		Bitylenchus			***Geocenamus***			***Helicotylenchus***			***Heterodera***			***Paratylenchus***			***Pratylenchus***			***Rotylenchulus***		
Compost 1	0.0	18.5[1] (±3.5)[2]	a[3]	A	28.3 (±5.8)	a	A	39.6 (±3.3)	a	A	2.8 (±1.1)	a	A	24.0 (±6.1)	a	A	5.0 (±1.8)	a	A	92.3 (±9.1)	a	A
	1.0	1.0 (±0.8)	b	B	26.1 (±7.1)	a	A	2.6 (±1.7)	bc	B	0.3 (±0.7)	b	B	17.0 (±3.3)	b	B	4.9 (±1.9)	a	A	21.0 (±4.7)	b	B
	2.5	1.1 (±0.6)	b	B	15.5 (±3.7)	b	B	1.0 (±1.5)	c	B	0.6 (±1.1)	b	B	5.4 (±1.8)	c	C	4.4 (±1.9)	a	A	18.5 (±3.4)	b	BC
	5.0	2.9 (±2.1)	b	B	1.8 (±2.5)	c	C	5.1 (±4.9)	B	B	0.4 (±0.5)	b	B	4.5 (±2.2)	c	C	0.4 (±0.7)	b	B	11.5 (±3.1)	c	C
	10.0	1.8 (±1.9)	b	B	2.0 (±1.0)	c	C	3.5 (±1.6)	bc	B	0.0 (±0.0)	b	B	2.4 (±1.4)	c	C	0.5 (±0.7)	b	B	9.1 (±2.1)	c	C
Compost 2	0.0	18.5 (±3.5)	a	A	28.3 (±5.8)	a	A	39.6 (±3.3)	a	A	2.8 (±1.1)	a	A	24.0 (±6.1)	a	A	5.0 (±1.8)	a	A	92.3 (±9.1)	a	A
	1.0	2.8 (±1.2)	c	BC	4.1 (±2.6)	b	B	11.6 (±3.2)	c	C	2.1 (±1.4)	ab	A	19.5 (±3.8)	b	AB	4.6 (±1.1)	a	AB	46.9 (±6.2)	b	B
	2.5	1.9 (±1.1)	c	C	7.3 (±2.6)	b	B	17.4 (±4.1)	b	B	1.6 (±0.7)	b	AB	15.0 (±3.8)	c	BC	3.8 (±1.8)	ab	AB	52.6 (±12.4)	b	B
	5.0	5.5 (±1.6)	b	B	7.6 (±1.6)	b	B	8.9 (±1.1)	cd	C	0.4 (±0.5)	c	B	12.5 (±2.3)	c	C	2.6 (±1.2)	bc	BC	43.1 (±12.4)	b	B
	10.0	4.0 (±1.3)	bc	BC	6.5 (±1.0)	b	B	7.0 (±2.6)	d	C	0.5 (±0.5)	c	B	4.8 (±2.1)	d	D	2.0 (±0.8)	c	C	22.8 (±4.3)	c	C

ANOVA F values

Factor A - Treatments	**129.1**	**
Factor B – Dose	**526.5**	**
Factor C – Nematodes genera	**679.3**	**
A x B	**14.5**	**
A x C	**61.7**	**
B x C	**78.6**	**
A x B x C	**10.1**	**

(1) Values are means of eight replications;
(2) ± Standard deviation;
(3) For each compost, data followed by the same letters in any column are not significantly different according to Least Significance Difference (LSD) Test (small letters for P = 0.05; capital letters for P = 0.01);
** = F values significant at P = 0.01.

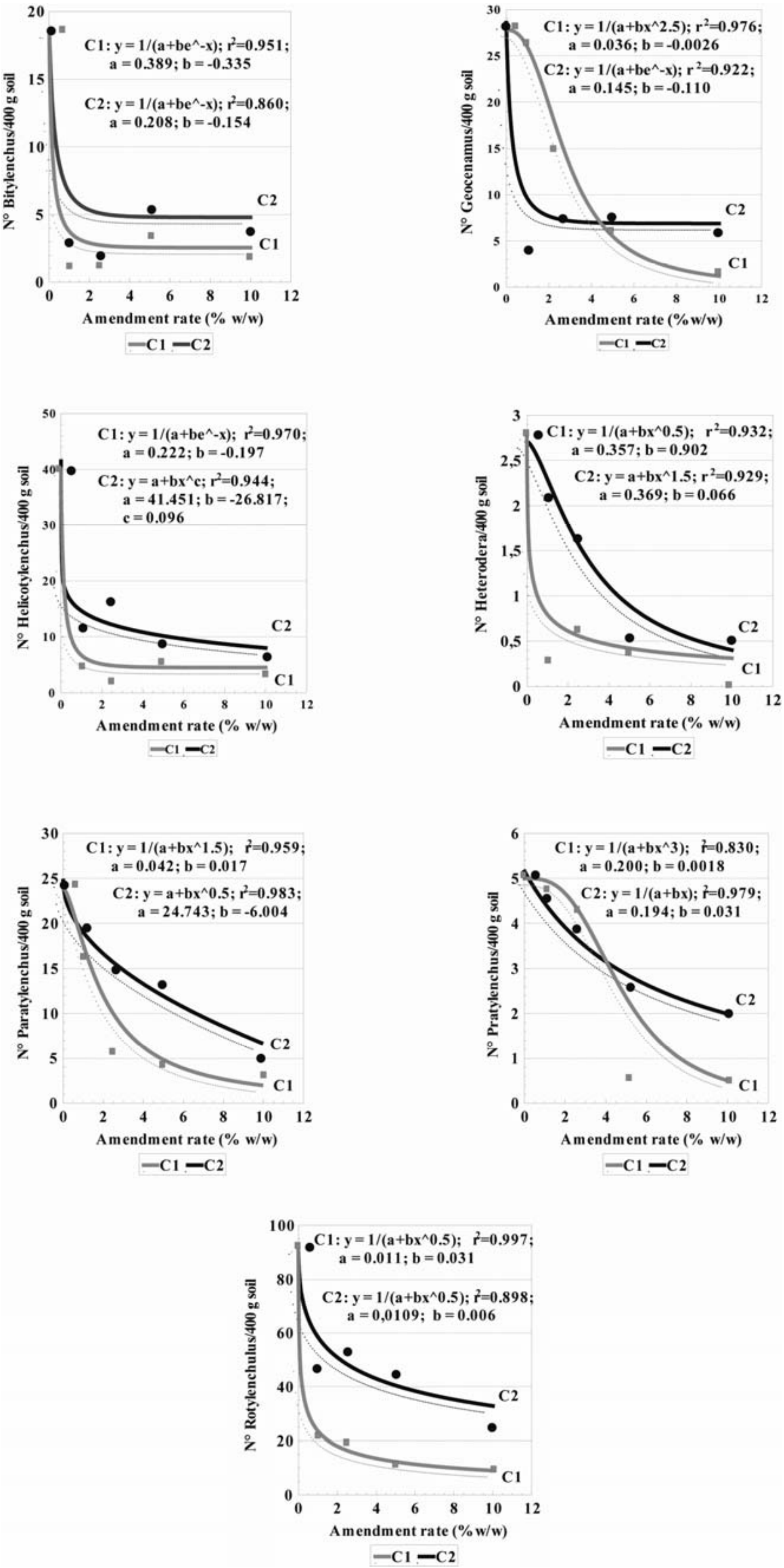

Fig. 1. Relationship between rates of composts C1 and C2 amending the pot mixtures (0%, 1%, 2.5%, 5%, 10%) and number of nematodes in each genera. Each point represents the average of eight replications. Lines represent the predicted function calculated by fitting experimental data to different equations.

observed at 2.5 % and 5 % C_1 rates, respectively (Table 2). In compost C_1, all tested doses were not statistically different each other to reduce *Bitylenchus*, *Helicotylenchus* and *Heterodera* populations (P = 0.01). In every case, no statistical differences were observed between the two highest rates for all nematode genera (P=0.01) (Table 2).
In the potting mixtures, with compost C_2 a similar significant reduction of plant parasitic nematodes in the soil was observed at all tested rates compared with untreated control, except the genus *Heterodera*, *Paratylenchus* and *Pratylenchus* (P = 0.01) (Table 2). To obtain a significant reduction (P = 0.01) of number of *Heterodera* and *Pratylenchus* it was necessary a minimum of 5 % (w/w) rate, whereas a 2.5 % dose of compost C_2 was useful to reduce *Paratylenchus* populations. No significant difference was found between the two highest rates in *Bitylenchus*, *Geocenamus*, *Helicotylenchus*, *Heterodera* and *Pratylenchus* genera (Table 2).
To study the interactions among the different factors involved in the experiment the analysis of variance was performed on three factors: i) factor A, the type of compost (two levels), ii) factor B, the applied rates (5 levels) and iii) factor C, the nematode genera (seven levels). The analysis of variance showed for factor A a high significant interaction with the factors B and C at P = 0.01 (A x B and A x C). Also the interaction between the factor B and factor C (B x C) was highly significant (P = 0.01). So all factors involeved in the experiment were significanly correlated each other (A x B x C) as reported in Table 2.
The relationship between amendment rates and number of nematodes showed a high significant negative correlation in each nematode genus (Fig. 1). In each fitting curve the values observed in C_1 compost were lower than that observed with compost C_2. Only in *Geocenamus* and *Pratylenchus* genera, the values recorded in C_2 were higher than that of C_1 for 1 % and 2.5 % rates (Fig.1).

Discussion

Results of our experiment demonstrated that soil treatments with municipal green compost and compost derived from the penicillin production residues significantly suppressed the number of plant parasitic nematodes compared to non-amended soil.
Numerous research studies worldwide also showed that organic amendments of soil are a means of the nematode control (Arancon *et al.*, 2003; Nahar *et al.*, 2006; Renčo *et al.*, 2007). However, in plant parasitic nematodes, efficiency of suppression in organic amendment varies depending upon nematode species and type of organic matter (Akthar & Alam, 1993). This influence is often variable and conflicting. Gallaher and McSorley (1996) reported that the number of plant parasitic nematodes *Criconemella* spp. and *Pratylenchus* spp. was lower in plots amended with yard waste compost compared to untreated plots. Suppression of *Pratylenchus penetrans* population was observed on potato by applying poultry manure (Conn & Lazarovits, 1999) or, in microplot condition, adding to the soil mushroom composts (LaMondia *et al.*, 1999). However, no reduction in populations of *Pratylenchus* and *Meloidogyne* nematodes, after compost amendment, was observed by McSorley and Gallaher (1995), Kimpinski *et al.*, (2003) and Yao *et al.* (2006). In our test, the two lower doses of municipal green compost and compost from penicillin residues significantly no reduced the number of *Pratylenchus* nematodes compared to untreated control, whereas the two highest doses were effective in the control (Table 2).
The level of reduction of plant parasitic nematodes increased with higher doses and over longer periods of treatments (Gutpa & Kumar, 1997). Similar findings were reported by Akhtar (1999), in which increasing applied doses of compost treatments caused a progressive reduction of the number of plant parasitic nematodes. In our experiment, a significant reduction of nematode number associated with an increasing dose of compost addition was observed in nematodes of the genera *Bitylenchus, Helicotylenchus, Paratylenchus* and *Rotylenchulus* in C_1 and C_2 compost, *Heterodera* in C_1 and *Geocenamus* in C_2 compost. Khan *et al.*, (2001a, b) reported the reduction of population densities of *Tylenchorhynchus curvus*, *Helicotylenchus indicus* and *Merlinius brevidens* by organic amendments of pigeon manure, poultry, horse and donkey manure and saw dust, at all applied doses. A reduction of number of *Heterodera*, *Paratylenchus* and *Tylenchorhynchus* nematodes by compost application was also obtained by Yao *et al.*, (2006). As reported by Kimpinski *et al.*, (2003), the addition of compost amendments in barley plots increased *Heterodera trifolli* populations, although, this species parasitized the preceding red clover crop, but not barley. They stated that compost treatments may have stimulated egg hatching. A similar, vermicompost application had no effect on *Heterodera schachtii* population (Szczech *et al*., 1993). On the contrary, soil from the vermicompost treated plots contained lower populations of plant parasitic nematodes than soil from inorganic fertilizer treated plots (Arancon *et al.*, 2003).
In our study, *Rotylenchulus* along with other nematode genera were significantly suppressed by the addition of composts to soil, as also found by Ismail *et al.* (2006). They found a positive correlation between applied doses of seven compost treatments and reduction of population of *Rotylenchulus reniformis.*
In general, the efficacy of nematode suppression by organic amendments depends on the C:N ratio and a time of microbial decomposition of organic matter (Rodriguez-Kabana *et al.*, 1995; D'Addabbo & Sasanelli, 1997; Akhtar & Malik, 2000). Kirmani *et al.*, (1975) changed the C:N ratio in organic treatments and observed that when more nitrogen sources was available, nematode control was enhanced. This also confirms our results because C_1 compost with the lower C:N ratio (4.2:1) had the best effect on reduction of number of plant parasitic nematodes in comparison to compost C_2 (Fig. 1). Similarly, Ismail *et al.*, (2006) found the best reduction in the number of *Rotylenchulus reniformis* after applying the composts with lower

C:N ratio (C:N=6.7:1). The application of slurry (C:N=8:1) and fruit or garden waste compost (C:N=10:1) reduced the population of *Pratylenchus* spp. and *Tylenchidae* in maize monoculture (Leroy *et al.*, 2007). Reversal, yard waste compost (leaves, branches, grass clippings) with the C:N ratio from 35:1 to 46:1 had no effect on population densities of *Pratylenchus* spp. and *Meloidogyne incognita* (McSorley & Gallaher*, 1996).*

On the other hand, the organic amendments supported the beneficial free-living nematodes, mainly bacterial feeding nematodes (Griffits *et al.*, 1994; Nahar *et al.*, 2006). Arancon *et al.* (2003) found that vermicomposts applications increase population of bacterial and fungal feeding nematodes in field crops. Edwards (1988) stated that vermicomposts improved the microbial activity by providing resources for the growth of soil bacteria and some fungi. Griffiths *et al.* (1994) also reported that number of bacterial and fungal feeding nematodes in organic management systems tend to increase their numbers, as the organic matter provides a food base for them.

In our experiment, significant nematode suppression was found at all tested concentration of compost including the low doses of 1 % and 2.5 %, with the exception of *Pratylenchus* for both composts, which is important in field applications. The higher application doses would be more difficult to achieve on a practical basis. Although both the tested composts significantly reduced the number of plant parasitic nematodes in comparison to untreated control, our results confirm that the compost with the low C:N (4.2:1) ratio is more effective in suppression of plant parasitic nematodes than the compost by-product of penicillin production with a C:N ratio of 20:1. The composts release compounds toxic to plant nematodes like phenols, tannins, terpens, (Mian & Rodriguez-Kabana, 1982) or derived from decomposition processes in the soil, like ammonia, nityrites, hydrogen sulphide (Rodriguez- Kabana, 1986). So, the higher nitrogen content in C_1 might explain the more effective nematicidal activity observed in this compost in comparison to C_2 (Fig. 1). Moreover, considering the relatively low dosages needed and the large availability at low or zero cost of municipal green and penicillin production wastes make the use of these organic residues easy, immediate and promising in economical convenient composting processes. The main aim of compost use should be a progressive reduction of infestation level under thew tolerance limit of the target nematode species (Sasanelli, 1994; McSorley & Duncan, 1995).

Therefore, our results confirm the potential of using different locally available composted agro-industrial or municipal wastes as an alternative plant parasitic nematode control method, at low environmental impact, to chemicals.

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