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Composts from agricultural waste and the *Trichoderma asperellum* strain T-34 suppress *Rhizoctonia solani* in cucumber seedlings

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Abstract

Using composts in agriculture to minimize organic wastes and to reduce the addition of fertilizers and fungicides in crop production is highly effective. Our results show that among those tested composts aged 0.5–1 year, cork compost reduced diseases caused by *Rhizoctonia solani* in cucumber plants (53% of diseased plants) in comparison to peat (up to 89%). However, all composts aged 1.5–3 years (comprised of cork, grape marc, olive marc and spent mushroom) highly suppressed *Rhizoctonia* disease, measuring 3, 11, 27 and 29% of diseased plants, respectively. Plant growth media enriched with the biological control agent *Trichoderma asperellum* (strain T-34) reduced the incidence of *R. solani* disease when amended at 10^3 cfu ml^{-1}. In composts aged 0.5–1 year, T-34 was only efficient when added to spent mushroom and cork compost, although it remained well established in all of them. The fact that T-34 rendered all composts aged 1.5–3 years highly suppressive is attributed to the low levels of easily biodegradable substances. *Rhizoctonia* damping-off in cucumber plants can be reduced by using composts and/or the biological control agent *T. asperellum* strain T-34. In addition, the extent to which composts suppress this disease depends on the nature of the composted materials, increasing with the composts' maturity level.

Keywords: Biological control agents; Cork compost; *Cucumis sativus*; Grape marc compost; Olive marc compost; Peat; *Rhizoctonia solani*; Spent mushroom compost; *Trichoderma asperellum*

1. Introduction

Increasing concern regarding food safety and environmental pollution, as well as legislative pressures in European countries to reduce the number of approved active pesticide ingredients, has generated an interest in compost and other biological control agents to prevent and control plant diseases (http://europa.eu.int/comm/food/plant/ protection/evaluation/framework_en.htm; UNEP, 2002).

The use of compost as a peat substitute to control root pathogens was first suggested by Hoitink et al. (1975). Since then, several soil-borne plant pathogens have been reduced by using composts made of different raw materials (Borrero et al., 2004; Cotxarrera et al., 2002; Hoitink and Boehm, 1999; Hoitink and Fahy, 1986; Litterick et al., 2004). However, the capacity of composts to suppress *Rhizoctonia solani*, a pathogen that affects both seedlings and adult plants of many species, remains limited (Hoitink and Boehm, 1999; Scheuerell et al., 2005). Composts that reportedly reduce *Rhizoctonia* damping-off are detailed in Table 1. The capacity of certain composts to repress *R. solani* may be due to the presence and activity of specific antagonists (Kuter et al., 1983; Scheuerell et al., 2005; Tuitert et al., 1998) and depends on the degree of compost decomposition (Hoitink and Boehm, 1999). Moreover, matured composts can sustain biological control agents, whereas immature composts do not support them, negatively affecting the growth of crop plants and possibly containing pathogen populations (De Ceuster and Hoitink, 1999; Litterick et al., 2004).

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Table 1
Composts reported to be suppressive to *Rhizoctonia solani*

Compost raw materials	Plants	References
Hardwood bark	*Euphorbia pulcherrima* cv. Annette Hegg Dark Red	Daft et al. (1979).
	Celosia argentea cv. Red Fox	Nelson and Hoitink (1982), Stephens et al. (1981).
	Raphanus sativus cv. Early Scarlet Globe	Kwok et al. (1987), Chung et al. (1988), Chung and Hoitink (1990), Kuter et al. (1983), Nelson and Hoitink (1982).
	Impatiens wallerana cv. Dwarf Baby Mix	Stephens and Stebbins (1985).
	Cucumis sativus cv. Straight Eight	Tunlid et al. (1989).
Municipal sewage sludge	*Phaseolus vulgaris* cv. Blue lake	Lumsden et al. (1983).
	Gossypium hirsutum cv. Stoneville	
	Raphanus sativus cv. Scarlet Globe	
Grape marc, cattle manure	*Epipremnum aureum*	Gorodecki and Hadar (1990).
	Raphanus sativus radicula alpha	
Organic household and garden waste	*Cucumis sativus* cv. Lange Groene	Tuitert et al. (1998).
Spent forest mushroom, fish meal and blood waste	*Brassica oleracae* cv. K-Y Cross	Shiau et al. (1999).
Cork	*Cucumis sativus* cv. Negrito	Trillas et al. (2002).
Swine wastes and woodchips	*Impatiens balsamina* cv. Super Elfin	Diab et al. (2003).
Bark, mushroom, nursery regrind	*Brassica oleracae* cv. Cheers	Scheuerell et al. (2005).

The antagonistic activity (parasitism) of the genus *Trichoderma* and *Gliocladium* to *R. solani* has been widely demonstrated (Elad et al., 1980; Harman et al., 2004; Koch, 1999; Krause et al., 2001; Lewis et al., 1998; Nelson et al., 1983). Other antagonists to this pathogen are also well documented: *Bacillus* spp. (Pleban et al., 1995; Yu et al., 2002), *Pseudomonas* spp. (Kwok et al., 1987; Pal et al., 2000; Thrane et al., 2001), *Streptomyces* spp. (Sabaratnam and Traquair, 2002), as well as binucleate *Rhizoctonia* strains (Harris, 2000; Hwang and Benson, 2003).

The purpose of this study was to examine the degree of suppression of *R. solani* in cucumber seedlings by using several composts (cork, grape marc, olive marc and spent mushroom), obtained from agricultural waste, as plant growth media. *Trichoderma asperellum* (strain T-34), an efficient biological control agent against *Fusarium oxysporum* (Cotxarrera et al., 2002), was tested against *R. solani* when added to these composts at different stages of maturity.

2. Materials and methods

2.1. Composts

The suppressiveness of composts to damping-off, as generated by *R. solani*, was evaluated using Sphagnum light peat (Klasmann or Floratorf, Germany) as a conducive plant growth medium. The composts used were derived from turned piles as previously described (Trillas et al., 2002). Cork compost (CC) was made from cork industry residues (Carmona et al., 2003). Coarse CC, with 48% of particles > 1 mm, had the appropriate physical properties (water and air availability) for use as a stand-alone plant growth medium (Table 2). Fine CC, with 68% of particles < 1 mm, had low air capacity. Consequently, after the composting process, the fine CC was mixed with rice hulls (CC:rice hulls, 2:1, v/v), yielding a compound referred to as CCr (Table 2). The physical properties of cork compost including water release curve were assessed by the standard method described in De Boodt et al. (1974). The grape marc compost (GMC) consisted of grape skins, seeds and stalks from the alcohol industry. Oil residues, which consisted of olive cake (marc) and olive mill wastewaters from the oil industry, were always composted with a bulk agent (olive marc and cotton gin trash, 2:3, v/v) and mixed with rice hulls (1:1, v/v), referred to as OMC. We also evaluated composts obtained commercially (RECOMSA, Cuenca, Spain) made from spent mushrooms composted alone and mixed with peat (1:1, v/v) (SMC).

Table 2
Physical properties of the cork composts after the 4-month composting process

Cork compost[a]	Air capacity (%v)	Easy available water (%v)	Water buffer content (%v)
CC (coarse)	49.77	11.03	2.41
CC (fine)	3.69	24.27	4.91
CCr	32.55	14.05	3.32

[a] Cork compost (CC), fine cork compost formulated with rice hulls (CCr) (2:1, v/v).

Composts were chemically characterized 4–5.5 months after the start of the composting process. At that time, they were all stable and none proved phytotoxic when used as the plant growth medium. Peat and rice hulls were also characterized (Table 3). Chemical analyses were carried out using aqueous extracts (1:2, v/v). Ca, Mg, Fe, Cu, Mn and Zn were determined by atomic absorption spectroscopy and Na and K were analyzed by atomic emission spectrometry (Wright and Stuczynski, 1996). P was measured with colorimetry (Murphy and Riley, 1962).

Composts were tested for disease suppressiveness 0.5–1 year after the start of the composting process (composts aged 0.5–1 year), then again after 1.5–2 years, except for old CC, which was evaluated after 2.5–3 years (composts aged 1.5–3 years).

Table 3
Chemical composition of substrates

Substrates[a]	P	Ca	Mg	K	Na	Fe	Cu	Mn	Zn
Peat	0.1	8	2	0.08	4	0.07	0.01	0.01	0.01
CC	1.3	93	31	216	12	0.87	0.03	0.03	0.05
CCr	0.0	54	11	91	28	0.58	0.02	0.08	0.00
GMC	4.1	78	28	544	8	0.08	0.03	0.03	0.09
OMC	0.0	40	13	417	25	3.38	0.07	0.17	0.04
SMC	0.1	263	88	1602	159	0.87	0.03	0.02	0.03
Rice hulls	0.0	31	4	35	10	0.51	0.03	0.17	0.01

Data of composts corresponded to materials 0.5–1 year after the start of the composting process. Elements were extracted from the plant growth media with water (1:2, v/v) (mg l^{-1} of extract).

[a] Klasmann peat; cork compost (CC); cork compost formulated with rice hulls (CCr) (2:1, v/v); grape marc compost (GMC); olive marc composted with cotton gin trash (2:3, v/v) and formulated with rice hulls (1:1, v/v) (OMC) and spent mushroom compost formulated with peat, (1:1, v/v) (SMC). Values represent the means of three replicates.

2.2. Fungal strains and inoculum preparations

R. solani (isolate AG-4) soil inoculum was prepared as a potato-soil mixture (Ko and Hora, 1971). After 14 days, cultures were sieved, with those pieces remaining on the 1 mm sieve used to infest the container media. The quantity of inoculum used for the different bioassays varied between 1.0–2.0 g l^{-1} of container medium. The soil inoculum concentration of *R. solani* was adjusted to induce around 80–100% damping-off when the cucumber was grown in peat.

The *T. asperellum* [Spanish collection of type culture, C.E.C.T. 20417, European patent application EP 1 400 586 A1 (Trillas and Cotxarrera, 2002)] strain T-34 was derived from a suppressive compost. The T-34 strain consistently reduced Fusarium wilt disease in tomato plants (Cotxarrera et al., 2002). T-34 was grown in petri dishes on Malt Agar (Scharlau, Barcelona, Spain) for 7 days. Sterile water was added to the culture and the surface was scraped to obtain a conidial suspension. The concentration was determined by measuring with a haemocytometer. T-34 was added to the peat, as well as to different composts at 10^3, 10^4, or 10^5 cfu ml^{-1}, following the experimental design. Populations of T-34 were counted at 1, 2 and 3 weeks as total populations of *Trichoderma* spp. by serial dilution on semi-selective *Trichoderma* medium (Chung and Hoitink, 1990). Peat and the different composts amended with T-34 were incubated for 2 weeks at a water tension of 1 KPa (adjusted on a weight basis) and an incubation temperature of 25 ± 2 °C prior to use in the bioassay described below.

2.3. Bioassays and assessment of disease incidence and severity

The suppression of *Rhizoctonia* damping-off in cucumber seedlings was assessed by bioassays adapted from that described by Nelson et al. (1983). For each treatment, we used five pots (330 ml) with 15 cucumber (*Cucumis sativus*) cv. Negrito seeds per pot. Pots were placed in a growth chamber (25 ± 2 °C, 16 h light and 150–210 μE $m^{-2}s^{-1}$). Seedlings were fertirrigated twice a day with 50 ml of Peter's foliar feed 27-15-12 at 0.5 g l^{-1} (Scotts, Heerlen, The Netherlands), complemented with $CaCl_2$ at 0.6 g l^{-1} and $MgSO_4\ 7H_2O$ (pH 5.68) at 0.7 g l^{-1}. For each bioassay, and for peat and each of the composts, the treatments were as follows: (1) control pots, with no *R. solani* or T-34, (2) pots infested with *R. solani*, and (3) pots infested with *R. solani* and amended with *T. asperellum* strain T-34 at one (10^3 cfu ml^{-1}) or several concentrations (10^3, 10^4 and 10^5 cfu ml^{-1}).

Disease incidence was the percentage of diseased plants over the total number of plants and was evaluated after 7 days. Disease severity was evaluated as follows: 1, healthy plants, 2, small lesions, 3, large lesions, 4, post-emergence damping-off and 5, pre-emergence damping-off. At least three bioassays were performed for each experiment.

2.4. Statistical analysis

Differences in the disease incidence of *R. solani* were assessed with one-way ANOVA at the end of each bioassay between treatments (plant growth media and/or T-34 concentration). Duncan's multiple range test was applied when one-way ANOVA revealed significant differences ($P < 0.05$). All statistical analyses were performed with SPSS 12.0 (SPSS Inc., Chicago, IL).

3. Results

3.1. Colonisation of T-34 in different plant growth media

When T-34 was added at 10^3 cfu ml^{-1} to peat or various composts, the total *Trichoderma* spp. stabilised between 0.5 and 3.6×10^3 cfu ml^{-1} for composts and at higher levels for peat (4.1 and 9.6×10^3 cfu ml^{-1}) (Table 4). When T-34 was added at 10^5 cfu ml^{-1}, the *Trichoderma* spp. populations stabilised between 2.1×10^4 and 1.3×10^5 cfu ml^{-1} both for peat and composts (Table 4). *Trichoderma* spp. populations were similar after 1, 2 and even 3 weeks of incubation (Table 4).

3.2. Effects of composts aged 0.5–1 year and T-34 on suppressiveness to R. solani

Between 0.5–1 year after the start of the composting process, the only compost that significantly reduced *R. solani* disease in cucumber seedlings was the CC (53% diseased seedlings) (Table 5), which produced a disease severity of 1.4 compared with 2.8 obtained in the peat. At the same level of maturity, OMC also significantly reduced this disease, with diseased seedlings totalling 76% (Table 5) and a disease severity of 1.9. Cucumber seedlings grown in GMC, SMC and CCr (with rice hulls) had the same disease incidence (80–90%) and severity as that of Floratorf peat. The chemical composition of plant growth media at that age of maturity (Table 3) did not correlate with their level of suppressiveness to *R. solani*.

Table 4
Total *Trichoderma* spp. population for peat and composts after several weeks (1–3) of incubation of *Trichoderma asperellum*, strain T-34

Plant growth medium[a]	T-34 (cfu ml^{-1} of plant growth medium)	*Trichoderma* spp.(cfu ml^{-1})[b]		
		T-34[c] incubation time		
		1 week	2 weeks	3 weeks
Peat	10^3	8.7×10^3	4.1×10^3	9.6×10^3
	10^5	4.7×10^4	2.2×10^4	4.0×10^4
CC	10^3	2.5×10^3	2.0×10^3	0.6×10^3
	10^5	2.2×10^4	3.9×10^4	2.1×10^4
GMC	10^3	1.2×10^3	3.6×10^3	1.6×10^3
	10^5	—	8.0×10^4	6.4×10^4
OMC	10^3	2.7×10^3	3.4×10^3	0.5×10^3
	10^5	8.5×10^4	1.3×10^5	2.4×10^4
SMC	10^3	1.6×10^3	1.7×10^3	1.3×10^3
	10^5	8.6×10^4	7.5×10^4	2.8×10^4

All the analysed composts were 1.5–2 years old, except for the CC, which was 2.5–3 years old.

[a] Klasmann peat; cork compost (CC); grape marc compost (GMC); olive marc composted with cotton gin trash (2:3, v/v) and formulated with rice hulls (1:1, v/v) (OMC) and spent mushroom compost formulated with peat, (1:1, v/v) (SMC).

[b] Values represent the means of two samples incubated in the light and two samples incubated in the dark, and three replicates each.

[c] The incubation conditions for T-34 in the plant growth medium was at a water tension of 1 KPa, and 25 ± 2 °C.

Table 5
Disease incidence of *Rhizoctonia solani* in cucumber cv. Negrito grown in peat or composts (0.5–1 year after the start of the composting process), natural or amended with the *Trichoderma asperellum* strain T-34 (10^3 cfu ml^{-1})

Plant growth medium[a]	T34[b] (10^3 cfu ml^{-1})	Disease incidence (%)[c]	
		Experiment 1[d]	Experiment 2[d]
Peat	–	81.1 c	89.3 c
	+	80.7 c	76.6 b
CC	–	53.1 b	—
	+	26.1 a	—
CCr	–	—	80.7 bc
	+	—	75.9 b
GMC	–	84.3 c	—
	+	87.0 c	—
OMC	–	—	75.6 b
	+	—	83.9 bc
SMC	–	—	84.0 bc
	+	—	55.1 a

[a] Floratorf peat; cork compost (CC), cork compost with rice hulls (CCr), grape marc compost (GMC), olive marc composted with cotton gin trash (2:3, v/v) and formulated with rice hulls (1:1, v/v) (OMC) and spent mushroom compost formulated with peat, (1:1, v/v) (SMC).

[b] Plant growth media amended with T-34 were incubated for 2 weeks at a water tension of 1 KPa prior to use in bioassays.

[c] For each experiment, values represent the means of at least three bioassays. For each bioassay, we used five pots per treatment and 15 seeds per pot.

[d] For each experiment, different letters represent significant differences between treatments (one-way ANOVA, $P < 0.05$) according to Duncan's multiple range test.

In one of two experiments, cucumber grown in Floratorf peat medium amended with T-34 at 10^3 cfu ml^{-1} exhibited significantly less disease (77%) than the unamended medium (Table 5). When T-34 was added to CC and SMC, the disease incidence decreased to 26% and 55%, respectively. When used to enrich CCr, GMC, and OMC (Table 5), T-34 at 10^3 cfu ml^{-1} had no effect on *Rhizoctonia* damping-off.

3.3. Effects of composts aged 1.5–3 years and T-34 on suppressiveness to R. solani

Between 1.5–2 years after the start of the composting process, all studied composts became suppressive to *Rhizoctonia* damping-off (3–29% diseased seedlings), with CC (aged 2.5–3 years) proving to have the highest level of suppression (Table 6). It is worth noting that 8% of seedlings grown in Klassman peat (95% of diseased seedlings) presented pre-emergence damping-off symptoms, whereas none of those grown in composts showed pre-emergence damping-off, only post-emergence damping-off. Disease severity decreased from 3.1 in peat, to 1.0–1.5 in composts. Peat became moderately suppressive when T-34 was added at 10^3 or 10^4 cfu ml^{-1}. When T-34 was added at 10^5 cfu ml^{-1}, only 21% of seedlings showed disease symptoms (Table 6). T-34 added to OMC significantly reduced *Rhizoctonia* disease incidence in cucumber seedlings from 27% to 5–9%. Analogously, SMC enriched with T-34 significantly reduced disease incidence in cucumber seedlings from 29% to 3–15% (Table 6). The effects of CC (3% of disease incidence) and GMC (11% of disease incidence) composts, as well as of T-34 enrichment, were not additive, since no improvement in disease suppression was found at the various concentrations of T-34 tested.

4. Discussion

Among the composts aged 0.5–1 year, CC was the most highly suppressive plant growth medium studied in controlling *R. solani* disease in cucumber seedlings, although there was no suppression when rice hulls were needed to improve the air content of this plant medium. This reduction in suppressiveness may be due not only to the dilution effect caused by the addition of the rice hulls, but may also be considered that the composition of fine cork particles can differ from that of coarse cork particles. GMC from the same batches, which was conducive to disease as caused by *R. solani*, induced significant disease reduction to *Fusarium oxysporum* in tomato plants (Borrero et al., 2004). This GMC (aged 0.5–1 year), which proved conducive to disease produced by *R. solani*, contains high populations of cellulolytic and oligotrophic actinomycetes and cellulolytic bacteria (Borrero et al., 2004), despite the fact that these microorganisms have been associated with *Rhizoctonia* suppression (Diab et al., 2003; Tuitert et al., 1998). Natural compost suppressiveness to *Rhizoctonia* damping-off is not widespread, and only some 20% of composts are suppressive to this pathogen (Hoitink and Boehm, 1999). In contrast, most composts naturally suppress diseases caused by *Pythium* spp. and *Phytophthora* spp. (Hoitink and Boehm, 1999; Litterick et al., 2004; Noble and Coventry, 2005).

Table 6
Effect of T-34 population density (10^3, 10^4 and 10^5 cfu ml^{-1}) on disease incidence of *Rhizoctonia solani* in cucumber seedlings cv. Negrito grown in peat and composts

Plant Growth media[a]	Disease incidence (%)[b]				
	Control	*R. solani*[c]	*R. solani* + T-34[d] (10^3 cfu ml^{-1})	*R. solani* + T-34[d] (10^4 cfu ml^{-1})	*R. solani* + T-34[d] (10^5 cfu ml^{-1})
Peat[e]	1.3 a	94.6 d C	48.0 bc	63.0 cd	21.0 ab
CC[e]	0.0 a	2.7 a A	1.3 a	2.7 a	—
GMC[e]	0.0 a	10.7 a AB	12.0 a	26.7 a	—
OMC[e]	0.0 a	27.0 b B	5.3 a	9.3 a	5.3 a
SMC[e]	1.3 a	29.3 b B	14.7 ab	2.7 a	10.7 ab

[a] Klasmann peat; cork compost (CC); grape marc compost (GMC); olive marc composted with cotton gin trash (2:3, v/v) and formulated with rice hulls (1:1, v/v) (OMC) and spent mushroom compost formulated with peat, (1:1, v/v) (SMC). All the analysed composts were 1.5–2 years old; except for the CC that was 2.5–3 years old.
[b] Values represent the means of at least three bioassays. For each bioassay, we used five pots per treatment and 15 seeds per pot.
[c] For the *Rhizoctonia* treatment, different capital letters represent significant differences between treatments in that column (one-way ANOVA, $P < 0.05$) according to Duncan's multiple range test.
[d] T-34 was incubated for 2 weeks at a water tension of 1 KPa.
[e] For each plant growth medium, different lower case letters represent significant differences between treatments in the same line (one-way ANOVA, $P < 0.05$) according to Duncan's multiple range test.

All studied composts aged 1.5–3 years showed a significant decrease in *Rhizoctonia* damping-off in cucumber seedlings compared with composts aged 0.5–1 year. Accordingly, it has been reported that the degree of maturity is especially important in reducing disease caused by this pathogen (Diab et al., 2003; Hoitink and Boehm, 1999; Kuter et al., 1988; Nelson et al., 1983). This phenomenon might be due to the low levels of easily biodegradable substances that enhance the competitiveness of autochthonous microflora in long-term matured composts (Hoitink and Boehm, 1999; Litterick et al., 2004). Thus, in our study with *R. solani* the most suppressive composts, cork composts, liberate cellulose slowly, since the major compound in cork, suberine, is recalcitrant to degradation. Consequently, natural microorganisms might remain in a competitive state in both CC aged 0.5–1 year and 2.5–3 years. Natural *Trichoderma* spp. in cork composts (unpublished data), the main mycoparasite described for *Rhizoctonia* (Harman et al., 2004), were particularly scarce. However, this compost contains high populations of fluorescent *Pseudomonas* (Borrero et al., 2004), which have also been associated with suppression of *Rhizoctonia* damping-off (Kwok et al., 1987).

The biological control agent *T. asperellum* strain T-34 was able not only to establish itself in all composts aged 1.5–3 years, but also to reduce the disease caused by *R. solani* in cucumber seedlings. However, in composts aged 0.5–1 year, even though T-34 was established in all composts (data not shown), it improved suppressiveness only when added to CC and SMC. The chitinase activity of T-34 might be enhanced in the low cellulose environment of composts aged 1.5–3 years and of CC and SMC aged 0.5–1 year (high in suberine and chitin, respectively), since cellulose-enriched composts repress the chitin-degrading enzymes of *Trichoderma* spp. (Harman et al., 1993; Hoitink and Boehm, 1999; Lorito et al., 1996). CC aged 2.5–3 years and GMC aged 1.5–2 years were so suppressive to *R. solani* that the action of T-34 was underestimated vis-à-vis the other suppression mechanisms involved in such composts. Compost manufacturers should evaluate the economic viability of each compost in relation to long-term maturation and/or the addition of a biological control agent.

The role of T-34 in disease suppression to *Rhizoctonia* was evident when it was added to the conducive peat, since suppressiveness was clearly dose-dependent. The low suppression observed, in some cases, in peat enriched with T-34 is consistent with results obtained for peat enriched with another *Trichoderma* strain combined with *Chryseobacterium* (Krause et al., 2001).

In conclusion, our results indicate that *R. solani* disease in cucumber seedlings can be reduced by using composts and the biological control agent *T. asperellum* strain T-34. The suppressiveness of composts to *Rhizoctonia* damping-off depends on the nature of the composted materials, increasing with the maturity level of the composts.

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