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MYCOBIOTA OF SOLARIZED AND UNSOLARIZED CUCUMBER SOILS AND ROLE OF SOLARIZATION IN CONTROLLING OF *SCLEROTINIA SCLEROTIORUM* (LIB.) de BARY UNDER GREENHOUSE CONDITIONS

[42]

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ABSTRACT

Soil mycoflora play an important role in agricultural economy of a country. The current study was made to have the knowledge about soilborne fungi associated with cucumber crop in solarized and unsolarized soils. Solarization exerted various effects, some of which are biological, others are chemical and still others are physical. All together these changes affected directly or indirectly the mycoflora of the soil, especially the soilborne pathogenic ones. Forty-nine fungal species belong to thirty genera have been isolated from solarized and unsolarized soils. The diversity as well as the count was greatly affected by solarization. By comparison of the species lists of the fungal flora of solarized and unsolarized soils it was evident that soil fungi behave differently toward soil solarization, while some new species developed e.g. *Absidia, Acrophialophora, Talaromyces, Gliocladium*, some remained unaffected e. g. *Aspergillus, Penicillium, Chaetomium, Botryotrichum*, still others disappeared e. g. *Acremonium, Cephaliophora, Eurotium* and others. Regarding solarization for controlling white cucumber rot caused by *Sclerotinia sclerotiorum*, the obtained data clearly show that solarization had led to a marked increase in the number of healthy plants up to 72.5%.

INTRODUCTION

Soil solarization is a mulching process that occurs in moist soil which is covered by polyethylene sheets and exposed to sunlight, especially during summer months. Heat is trapped in the soil and rising soil temperature up to levels which are lethal to many plant pathogens and pests. This process causes also complex changes in the biological, physical and chemical properties of the soil in a way or another that improve growth and development of plants (**Pullman *et al* 1981**). These changes include also sharp decreases in the populations of soilborne pathogens with increased populations of beneficial fungi and bacteria (**De Vay, 1995**).

Changes in populations of soilborne microorganisms, associated with the sharp decline of most plant pathogens during soil solarization, are changes in saprophytic fungi and bacterial species. After soil solarization, populations of soil fungi were reduced (**Stapleton and De Vay, 1982, 1984; El-Zayat *et al* 1990; El-Shanawany *et al* 2004**). However, population's densities of thermophilic fungi remained relatively high and increased to levels higher than those present in unsolarized soil (**De Vay, 1995; Stapleton and De Vay, 1982**). The effect of solarization on soil microbiota has been the target of many investigators in Egypt and different countries (**Stapleton and De Vay, 1982, 1984, 1995; El-Zayat *et al* 1990; Gamliel and Katan, 1991; De Vay, 1995; Ibrahim, 1999; Botross *et al* 2000 and E-Shanawany *et al* 2004**).

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In Egypt the total vegetable-growing area in 2003 was about 464997 Feddan (18%) of the total cultivated area. It reflects the high domestic consumption rate which is one of the highest all over the world. Cucumber-growing area in 2003 was 11881 Feddan (about 3%) of the total vegetable-growing area with a production rate represented by 88575 Ton **(Annual Report–Ministry of Agriculture, 2003)**. As an important vegetable, cucumber (*Cucumis sativus*) has been attracted the attention of many scientists. The data concerning mycobiota of cucumber soils is either fragmented or mystery. The aim of this study is to throw some light on the structure, diversity of mycobiota of solarized and unsolarized soils of cucumber growing under green house conditions and role of solarization in the reduction or preventing of cucumber white rot caused by *Sclerotinia sclerotiorum*.

MATERIALS AND METHODS

Soil solarization

Mulching, with 1mm thick polyethylene clear sheets in single layers, was applied to soils moistened by irrigation for the purpose of increasing soil temperature. The mulch (8 m wide x 10 m lengths) was applied manually to plots and remained in place for 6 weeks. The experiments were carried out during summer (July through August) and were repeated twice during the two consecutive seasons 2005 & 2006. Soil temperature was measured daily at the depth of 5 and 10 cm in solarized and unsolarized plots.

Sampling:

Soil samples were collected from the upper soil layer (5–10cm deep) from solarized and unsolarized plots. Thirty soil samples (500 g each) were collected from solarized and unsolarized plots (15 samples each). Samples were transferred to the laboratory in tight sterilized polyethylene bags and kept at low temperature until plating.

Isolation and identification

Fungi were isolated from subsurface layer (ca. 5-10 cm) by using dilution plate method (**Johnson *et al* 1960**) in which six plates was used for isolation/sample. Czapek's agar supplemented with 0.5 % yeast extract (CYA) and potato dextrose agar (PDA), amended with rose bengal (1/15000) and chloramphenicol (50 ppm) was used for primary isolation. Plates were incubated at 28 °C for 10 days and developing fungi were counted. For maintaining cultures and for proper identification, pure cultures of isolated fungi were grown on standard media such as Vegetable Agar (V8), Oatmeal Agar (OA), Malt Extract Agar (MEA) Potato Dextrose Agar (PDA) and Potato Carrot Agar (PCA).

Taxonomic identification by morphology of fungal isolates was mainly based on the following identification keys: **Raper & Thom (1949)**, **Pitt (1980)** for *Penicillium*; **Raper & Fennell (1965)** for *Aspergillus*; **Ellis (1971 and 1976)** for dematiaceous hyphomycetes; **Booth (1971)** for *Fusarium*; **Arx (1981)**, **Domsch *et al* (1980)** for miscellaneous fungi; **Arx *et al* (1986)** for *Chaetomium*. The systematic arrangement follows the latest system of classification appearing in the 9th edition of Anisworth & Bisby's Dictionary of the fungi (**Kirk *et al* 2001**).

Field experiment

This experiment has been conducted in naturally infested soil (solarized and unsolarized). Solarized and unsolarized plots have been divided into beds where seeds of cucumber (Hasham cultivar) were planted 50 cm apart from each other. After a growth period of 70 days of sowing, disease incidence was determined.

RESULTS

Microbial characterization of the investigated soils

During this study, a total number of 49 species belong to 30 genera, has been isolated from solarized and unsolarized soils. Taxonomically, isolated species were assigned to eleven families, eight orders, five subclasses, five classes and two phyla. Taxa with uncertain position were distributed among families, orders, subclasses and phyla **(Table 1)**.

While order Eurotiales accommodates the greatest range of species (19 species), the order Pleosporales and Capnodiales accommodated the lowest range (one species each). Family Trichocomaceae had the highest contribution to the mycobiota (19 species out of 49) followed by Mucoraceae & Chaetomiaceae (5 species each) while, the remaining families were represented only by three to one species each.

Table 1. Taxonomic position of the isolated taxa according to **Kirk *et al* (2001).**

Phylum	Class	Subclass	Order	Family
Zygomycota	Zygomycetes	Incertae sedis	Mucorales	Mucoraceae
				Syncephalastraceae
Ascomycota	Dothideomycetes	Dothidiomycetadae	Capnodiales	Mycosphaerellaceae
		Pleosporomycetidae	Pleosporales	Pleosporaceae
	Eurotiomycetes	Eurotiomycetidae	Eurotiales	Trichocomaceae
			Onygenales	Gymnoascaceae
				Onygenaceae
	Sordariomycetes	Hypocreomycetidae	Hypocreales	Hypocreaceae
				Nectriaceae
				Incertae sedis
			Microascales	Microascaceae
		Sordariomycetidae	Sordariales	Chaetomiaceae
	Ascomycetes	Incertae sedis	Incertae sedis	Incertae sedis
Mitosporic Fungi	Incertae sedis	Incertae sedis	Incertae sedis	Incertae sedis

Number of species isolated was affected by solarization, while new taxa were developed, some remained unaffected, and few others disappeared **(Table 2).** The genera isolated have been arranged in decreasing order of species richness **(Table 3).** From the table, the prevailing genera were *Aspergillus* (10 species including anamorph stages of one *Emericella* and one *Eurotium* species; 20.40%), *Penicillium* (6 species including anamorph stage of one *Talaromyces* species; 12.24 %). They are followed by *Chaetomium* and *Fusarium* by showing a spectrum of 4 and 3 species respectively. The remainders are represented by only by 1 or 2 species.

Table 2. Number of isolated species in unmulched and mulched soil plots.

Classes \ Soil	Unsolarized No. of spp. isolated	Solarized No. of spp isolated	Total	%
Mitosporic fungi	1	2	2	4.08
Ascomycota (teleomorphic)	8	10	11	22.44
Ascomycota (anamorphic)*	27	19	29	59.20
Zygomycota	6	4	7	14.28
Total No. of species	42	35	49	100.00

Table 3. Genera and species richness of isolated fungi.

Genera	Unsolarized soil No. of species	Solarized soil No. of species	Total No. of species
Absidia	0	1	1
Acremonium	1	0	1
Acrophialophora	0	1	1
Agonomycete	1	1	1
Alternaria	1	1	1
Aspergillus	7	7	8
Botryotrichum	1	1	1
Cephaliophora	1	0	1
Chaetomium	4	4	4
Chrysosporium	1	1	1
Circinella	1	0	1
Cladosporium	1	1	1
Emericella	1	1	1
Eurotium	1	0	1
Fusarium	3	2	3
Gliocladium	1	1	1
Gymnascella	0	1	1
Gymnoascus	0	1	1
Humicola	1	1	1
Lophotrichus	1	1	1
Microascus	1	1	1
Mucor	2	1	2
Mycocladus	1	1	1
Myrothecium	1	0	1
Paecilomyces	1	0	1
Penicillium	5	2	5
Rhizopus	1	0	1
Scopulariopsis	1	2	2
Syncephalastrum	1	0	1
Talaromyces	0	1	1
Trichoderma	1	1	1
Total	42	35	49

Concerning the total counts of fungi isolated from solarized and unsolarized soils of cucumber plants the counts ranged between 4080-14120 cfu with a mean colony count of 9108.67 cfu/g in solarized soil and 2520-9400 with a mean colony count of 5132.67 cfu/g in unsolarized soil **(Table 4)**. The difference between the total counts of solarized and unsolarized soils has been proved to be highly significant.

Table 4. Total counts of isolated fungi (cfu/g) from solarized and unsolarized soils

Treatment \ Counts	Counts range (cfu/g)*	Mean
Solarized soil	4060-14120	9108.67
Unsolarized soil	2520-9400	5132.67

• cfu/g: colony forming units per gram dry soil

In view of species density, a total number of 49 species were isolated from solarized and unsolarized soils **(Table 5).** The following species are the most dominant in decreasing order: *Botryotrichum piluliferum* > *Scopulariopsis brevicaulis* > *Aspergillus versicolor* > *Aspergillus terreus* > *Aspergillus flavus* > *Fusarium oxysporum*. Regarding the range of species isolated, unsolarized soils revealed a spectrum of 42 species while solarized soils obtained only 35 species.

According to the frequency values, recorded species have been given in **(Table 6)** where they are arranged in decreasing order of frequency. Four ecological classes of occurrence are recognized: a high occurrence group (H), include species recorded in50 % or more; moderate occurrence (M), from 25 %-49%; low occurrence (L), from 12%-24 %; and rare occurrence (R), less than 12 %.

Impact of solarization on white rot disease

The results histogramed in **Figure (1)** clearly indicate that solarization, by covering soil with transparent polyethylene sheets for 7 weeks during the hottest summer months, had led to a marked increase in the healthy plants up to 72.5 %. Such a figure is highly significant by comparison to unsolarized soil which revealed only a mean of 20 % healthy plants.

DISCUSSION

Soil solarization induces various effects, some of which are considered physical, others are chemical, and still others are biological. All together these changes affect directly or indirectly the mycobiota of the soil especially the soilborne pathogenic ones.

Forty-nine species belonging to thirty genera of filamentous fungi were recorded from solarized and unsolarized soils during the present investigation. Ascomycota (anamorphic) accounted for the major part 59.20 %, followed by Ascomycota (teleomorphic) and Zygomycetes where represented by 22.44 % and 14.28 % respectively, while Mitosporic by comparison is less frequent.

Regarding fungal counts, solarized soils held the higher counts, while unsolarized soil held the lowest counts. While solarized soils revealed a mean colony count of 9108.67 cfu/g, unsolarized plots showed a mean colony counts of 5132.67 cfu/g. This result is in line with those reported in Egypt and elsewhere **(Gamliel & Stapleton, 1993; Ibrahim, 1999 and Stapleton & De Vay, 1982, 1984)**

According to the species density (number of colony forming unit per dry gram soil) the data revealed that solarization effected on the population density of isolated fungi. While the population density of some species increased by solarization e.g. *Aspergillus versicolor*, *A. terreus*, *Scopulariopsis brevicaulis*, *Fusarium* spp., *Emericella nidulans* and *Penicillium cyclopium*; others decreased (in comparison with unsolarized soils) like: *Botryotrichum piluliferum*, *Aspergillus flavus*, *Alternaria alternate* and *Chrysosporum xerophilum*. Similar observation on the survival and/or increase of some fungi following solarization has been noticed by some investigators. Increasing in the number of heat-resistant *Aspergillus terreus* was recorded by **Tjamos & Paplomatas (1987 & 1988) and Tjamos *et al* (1990). Triolo *et al* (1988)** recorded the prevalence of heat-tolerant species belonging to the genera of *Aspergillus*, *Penicillium*, *Fusarium* and *Trichoderma*.

In addition to species density, species frequency was also used to assure reasonable and fair characterization of the mycobiota of solarized and unsolarized soils. Species frequency calculated as percentage number of cases of isolation of each species regardless of its count. Based on the frequency value, fungal isolates were classified into four ecological groups: High, Moderate, Low, and Rare.

Table 5. Frequency and frequency classes of isolated fungi from solarized and unsolarized soils

Species	Unsolarized	Solarized	NCI	F.%	FC.
Zygomycota					
Absidia glauca Hagem	0	1	1	3.3	R
Circinella mucoroides Saito	1	•	1	3.3	R
Mucor circinelloides Tiegh.	1	•	1	3.3	R
M. racemosus Fresen.	2	3	5	16.7	L
Mycocladus corymbiferus (Cohn) J.H. Mirza	5	8	13	43.3	M
Rhizopus stolonifer var. *stolonifer* (Ehrenb.) Vuill.	1	•	1	3.3	R
Syncephalastrum racemosum Cohn ex J. Schröt.	1	•	1	3.3	R
Ascomycota (teleomorphic)					
Chaetomium globosum Kunze	6	8	14	46.7	M
Ch. madrasense Natarajan	1	1	2	6.7	R
Ch. nigricolor L.M. Ames	3	3	6	20.0	L
Ch. piluliferum J. Daniels	1	1	2	6.7	R
Emericella nidulans (Eidam) Vuill.	5	11	16	53.3	H
Eurotium chevalieri Mangin	3	•	3	10.0	R
Gymnascella dankaliensis (Castell.) Currah	•	5	5	16.7	L
Gymnoascus sp.	•	1	1	3.3	R
Lophotrichus plumbescens Morin., Min. & Udag.	3	8	11	36.7	M
Microascus cinereus Curzi	1	1	2	6.7	R
Talaromyces flavus var. *flavus* (Klocker) Stolk & Samson	•	1	1	3.3	R
Ascomycota (anamorphic)					
Acremonium implicatum (Gilman & Abbott) Gams	2	•	2	6.7	R
Alternaria alternata (Fr.) Keissl.	1	1	2	6.7	R
Aspergillus flavus Link	13	14	27	90.0	H
A. fumigatus Fresen.	6	•	6	20.0	L
A. niger var. *niger* Tiegh.	4	5	9	30.0	M
A. ochraceous G. Wilh.	19	21	31	23.3	L
A. sydowii (Bain. & Sart.) Thom & Church	4	1	5	16.7	L
A. terreus Thom	5	13	18	60.0	H
A. versicolor (Vuill.) Tirab.	12	13	25	83.3	H
A. wentii Wehmer	•	1	1	3.3	R
Botryotrichum piluliferum Sacc. & Marchal	15	13	28	93.3	H
Cephaliophora irregularis Thaxt.	4	•	4	13.3	L
Chrysosporium xerophilum Link	5	2	7	23.3	L
Cladosporium cladosporioides (Fresen.) de Vries	1	4	5	16.7	L
Fusarium oxysporum Schltdl.	12	10	22	73.3	H
Fusarium solani (Mart.) Sacc.	9	9	18	60.0	H
Fusarium sp.	1	•	1	3.3	R
Gliocladium sp	1	1	2	6.7	R
Humicola fuscoatra Traaen	1	1	2	6.7	R
Myrothecium verrucaria (Alb. & Schwein.) Ditmar	2	•	2	6.7	R
Paecilomyces variotii Bainier	2	•	2	6.7	R
Penicillium aurantiogriseum Dierckx	2	9	11	36.7	M
P. chrysogenum var. *chrysogenum* Thom	4	2	6	20.0	L
P. roqueforti Thom	2	•	2	6.7	R
Penicillium sp. (1)	2	•	2	6.7	R
Penicillium sp.(2)	1	•	1	3.3	R
Scopulariopsis brevicaulis (Sacc.) Bainier	12	13	25	83.3	H
S. candida (Guég.) Vuill.	0	3	3	10.0	R
Trichoderma pseudokoningii Rifai	4	2	6	20.0	L
Mitosporic fungi					
Acrophialophora levis Samson & T. Mahmood	•	1	1	3.3	R
Agonomycete	1	3	4	13.3	L

NCI: number of cases of isolation, F%: frequency percentage, FC: frequency class

Table 6. Population density (cfu/g) of isolated fungi from solarized and unsolarized soils

Fungal species	Unsolarized	Solarized
Absidia glauca	0	29±8.8
Acremonium implicatum	10±3.0	0
Acrophialophora levis	·	15±3.1
Agonomycete	2±0.0	4±0.5
Alternaria alternata	18±0.0	9±0.8
Aspergillus flavus	161±12.2	78±4.9
Aspergillus fumigatus	29±2.0	0
Aspergillus niger var. *niger*	16±3.0	10±0.5
Aspergillus ochraceous	19±3.4	21±3.7
Aspergillus sydowii	32±4.7	27±3.6
Aspergillus terreus	15±3.7	399±61.4
Aspergillus versicolor	222±16.5	637±56.4
Aspergillus wentii	0	92.5
Botryotrichum piluliferum	755±52.7	265±28.0
Cephaliophora irregularis	13±2.3	0
Chaetomium globosum	22±2.1	22±4.6
Chaetomium madrasense	16±4.1	8±1.2
Chaetomium nigricolor	11±3.8	19±4.5
Chaetomium piluliferum	33±0.0	32±1.7
Chrysosporium xerophilum	26±3.1	6±0.0
Circinella mucoroides	3±0.0	·
Cladosporium cladosporioides	7±0.0	6±1.6
Emericella nidulans	21±1.7	81±8.2
Eurotium chevalieri	4±1.0	0
Fusarium oxysporum	26±0.0	133±7.6
Fusarium solani	20±0.6	58±3.8
Fusarium sp.	3±0.0	0
Gliocladium sp.	6±2.0	14±1.1
Gymnascella dankaliensis	·	4±1.0
Gymnoascus sp.	·	18±1.5
Humicola fuscoatra	11±3.5	17±1.7
Lophotrichus plumbescens	20±7.6	51±7.3
Microascus cinereus	10±3.0	33±2.5
Mucor circinelloides	5±1.5	2±0.0
Mucor racemosus	13±2.5	·
Mycocladus corymbiferus	23±1.7	45±4.8
Myrothecium verrucaria	38±11.0	0
Paecilomyces variotii	4±01.3	0
Penicillium aurantiogriseum	22±0.8	80±2.0
Penicillium chrysogenum var. *chrysogenum*	23±2.6	8±1.0
Penicillium roqueforti	2±0.0	0
Penicillium sp. (1)	17±2.7	·
Penicillium sp.(2)	3±0.5	0
Rhizopus stolonifer var. *stolonifer*	3±0.0	0
Scopulariopsis brevicaulis	237±16.5	655±28.3
Scopulariopsis candida	·	13±2.5
Syncephalastrum racemosum	9±0.0	·
Talaromyces flavus var. *flavus*	·	7±1.2
Trichoderma pseudokoningii	18±2.9	5±0.5

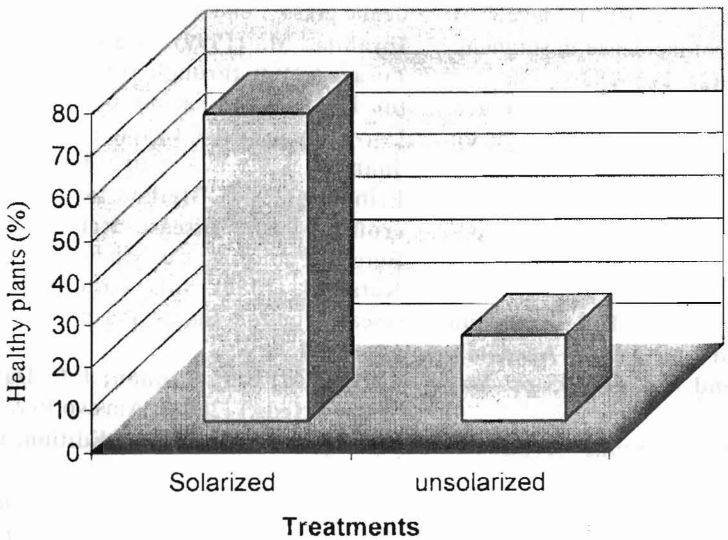

Fig. 1. Disease severity in solarized and unsolarized soils

High frequency group, contained species showing frequency values of 50 % or more. This group contained 8 species among which *Botryotrichum piluliferum*, *Aspergillus flavus*, *A. versicolor* and *Scopulariopsis brevicaulis* came first by revealing high frequency values of 93.3 %, 90.0 %, 83.3 %, and 83.3 % respectively. Moderate frequency group, consisting of species showing frequency values between 25 % and 49 %. Assigned to this group: *Chaetomium globosum*, *Absidia croymbifera*, *Lophotrichum sp.*, *Penicillium cyclopium*, *Aspergillus niger*

Low frequency group containing species showing frequency values between 12 % and 24 %. This group consists of 11 species among which species known by having good antagonistic potentiality like *Trichoderma psuedokoningii* and *Chaetomium nigricolor*. Rare frequency group was isolated: accommodates species showing frequency values less than 12 %. This group includes species of heat-tolerant genera such as *Aspergillus* and *Penicillium*, as well as the newly developed taxa after solarization like *Talaromyces*, *Acrophialophora*, and *Gymnoascus*.

Concerning the impact of solarization on the cucumber white rot, our data clearly indicated that this approach, apart from being feasible is more effective. The number of healthy plants significantly increased from 20 % in unsolarized soils up to 72.5 % in solarized soils. A very similar level of increase has also been reported by many investigators in Egypt and elsewhere (**Abdel-Rahim *et al* 1987; El-Shami *et al* 1990; Sarahan, 1990; Katan, 1980; Greenberger *et al* 1985; Tamietti *et al* 1987 and Torres *et al* 1987**). However, by using the same approach very much acceptable results were obtained by several authors (**Grinsten *et al* 1979; Ristaino *et al* 1991; Stevens *et al* 1992; Chellemi *et al* 1994 and Swaminathan *et al* 1999**).

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الفلورا الفطرية لتربة نبات الخيار المشمسة والغير مشمسة و دور التشميس فى مقاومة عفن الخيار الأبيض تحت ظروف الحقل

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يعتبر التشميس عملية غير كيميائية و تتماشى مع المبادئ الرئيسية للمكافحة المتكاملة، فهى بديل كفء للتعقيم الكيميائى. فى خلال هذه الدراسة تم عزل ٤٩ نوع فطرى (من التربة المشمسة والغيـــر مشمـــسة) تنتمى الى ٣٠ جنس موزعة على خمس طوائف. وقد أثر التشميس على فطريات التربة حيث ظهرت أنواع وهى تلك المتحملة لدرجة الحرارة العاليـــة وأختفـت أخرى. كما أدى التشميس الى زيـــادة العـــدد الكلـــى لبعض الفطرياتالمحبة للحرارة وأيـــضا ذات القـــدرة العالية على الترمم.

كمـــا أظهـــرت النتائـــج الكفـاءة العاليـــة للتشميس فى الحد ومقاومة مرض العفن الأبيض فـــى الخيـــار المتـــسبب عـــن فطـــر اســـكليروتونيا اسكليروشيورم.

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