

Effect of agro-chemicals on *in vitro* growth of the entomopathogenic fungi *Metarhizium anisopliae* (Metschnikoff) Sorokin and *Paecilomyces fumosoroseus* (Wize) Brown & Smith

MIGUEL ANGEL AYALA-ZERMEÑO, HORTENSIA NAVARRO-BARRANCO, TERESA MIER¹ AND CONCHITA TORIELLO*

Departamento de Microbiología y Parasitología, Facultad de Medicina, Universidad Nacional Autónoma de México, Ciudad Universitaria, 04510 Mexico D.F., Mexico.

Departamento El Hombre y Su Ambiente, División de Ciencias Biológicas y de la Salud, Universidad Autónoma Metropolitana-Xochimilco, 04960, Mexico D.F., Mexico.

*Corresponding author: Tel. (52 5) 623-24-61. Fax (52 5) 623-24-59. E mail toriello@servidor.unam.mx

ABSTRACT. Seven adherents/surfactants (Adhefix, Adhefix 12, Citowett Plus, Inex-A, Kaytar, Kinetic, Penetrator Plus), two foliar fertilizers (Arco iris, Musol), seven insecticides (Diazinon, Malathion, Methamidophos, Trichlorfon, Carbaryl, Endosulfan, Permethrin,) and two fungicides (Benomyl, Captan) were tested in vitro on Metarhizium anisopliae and Paecilomyces fumosoroseus growth. Assessments were made on solid medium with two concentrations of each agrochemical. The best compatibility with adherents/surfactants (A/S) was exhibited by Penetrator and Kinetic on M. anisopliae; and Adhefix 12, Kinetic and Penetrator on P. fumosoroseus. In general, fertilizers did not affect growth of fungi tested. Insecticides showed a significant deleterious effect (P<0.05) on fungal growth. The least growth inhibition percent was exhibited by both concentrations of trichlorfon on M. anisopliae; and 0.5% methamidophos, without significant inhibition, and both concentrations of endosulfan on P. fumosoroseus. Malathion fully inhibited growth of both fungi. The fungicides were particularly toxic on fungal growth of fungi tested. In field applications, care should be taken to test compatibilities when using A/S with entomopathogenic fungi, as well as to choose the best fungal compatible insecticide when required.

Key Words: Metarhizium anisopliae, Paecilomyces fumosoroseus, agro-chemicals, compatibility.

RESUMEN. Se ensayó el efecto de siete adherentes/surfactantes (Adhefix, Adhefix 12, Citowett Plus, Inex-A, Kaytar, Kinetic, Penetrator Plus), dos fertilizantes foliares (Arco iris, Musol), siete insecticidas (Diazinón, Malatión, Metamidofós, Triclorfón, Carbarilo, Endosulfán, Permetrina), y dos fungicidas (Benomil, Captán) sobre el crecimiento in vitro de Metarhizium anisopliae y Paecilomyces fumosoroseus. Los ensayos se llevaron a cabo en medio sólido con dos concentraciones de cada producto. La mejor compatibilidad de los adherentes/surfactantes (A/S) se observó con Penetrator y Kinetic para M. anisopliae; y con Adhefix 12, Kinetic y Penetrator para P. fumosoroseus. En general, el crecimiento de ambos hongos no se alteró con los fertilizantes. Los insecticidas mostraron un efecto dañino significativo (p<0.05) sobre el crecimiento fúngico. El menor porcentaje de inhibición del crecimiento en M. anisopliae se observó con ambas concentraciones de triclorfón, y en P. fumosoroseus, con metamidofós al 0.5%, sin inhibición significativa, y ambas concentraciones de endosulfán. El malation inhibió totalmente el crecimiento de ambos hongos. Los fungicidas fueron particularmente tóxicos para los dos hongos. En el campo, se debe tener especial cuidado en probar la compatibilidad de los A/S con el hongo entomopatógeno, y cuando necesario, escoger el insecticida más compatible con el entomopatógeno a aplicar.

Palabras clave: Metarhizium anisopliae, Paecilomyces fumosoroseus, productos agrícolas, compatibilidad.

INTRODUCTION

Spittlebugs (Homoptera: Cercopidae) are nuisance pests of sugarcane, rice and grasslands crops in tropical countries in Latin America. Riess and Flores listed Aeneolamia albofasciata (Walk), Aeneolamia postica (Walk.) and Prosapia simulans (Walk) among the most relevant species causing damage in Mexico. Whiteflies

(Homoptera: Aleyrodidae) are polyphagus pests of increasing economic importance throughout the world, not only on field crops but also on ornamentals in greenhouses. 6.8 Seven insect biotype strains have been identified in the Mexican State of Sinaloa¹³. Chemical control of the pests is becoming increasingly difficult due to the insects' acquired resistance, favored by repeated insecticide applications. Alternative control methods for these pests are ento-



mopathogenic fungi.4

According to ecologically-based pest management, not only biological control organisms and products are used, but also resistant plants and reduced spectrum chemical insecticides are introduced in the agroecosystem in order to increase the natural processes that maintain the plague population under control. 10 The compatibility of entomopathogenic fungi with chemical insecticides and other agrochemicals is critical for this field application strategy. Although sensitivity of pesticides has been tested in fungal entomopathogens elsewhere. 9,11,12,17 formulations assayed are those commonly used in other geographic regions. We present data on the effects in vitro of agro-chemicals commonly used in México and Guatemala on growth of Metarhizium anisopliae and Paecilomyces fumosoroseus, mycoinsecticides for the spittlebug and whitefly, respectively, which offer an alternative to chemical insecticides

in pest management programs.

MATERIAL AND METHODS

Fungi. M. anisopliae EH-350 isolated from a spittlebug and P. fumosoroseus EH-349 isolated from a whitefly were used in this study. Both isolates are maintained on culture slants of mycological agar (Bioxón, Mexico) at 28°C and in liquid nitrogen in the Laboratorio de Micología Básica, Departamento de Microbiología y Parasitología, Facultad de Medicina, Universidad Nacional Autónoma de México, México D.F., México.

Assay medium and product preparation. All agrochemicals tested, with their common and commercial names, type, concentrations used, and suppliers are listed in Table 1. Carbaryl and Permethrin insecticides are only

Table 1. Common and commercial names, type, concentration and supplier of agrochemicals tested.

Common and commercial names	Type Concentration (%) I ^a II		Supplier	
Adherents/surfactants				
Adhefix	A/S	0.075	0.15	Arco Iris, Fertilizantes foliares, Mex.
Adhefix	A/S	0.075	0.15	Guatemala
Citowett	A/S	0.125	0.25	BASF, Mex.
Inex-A	A/S	0.2	0.4	Cosmocel, Mex.
Kaytar	A/S	0.075	0.15	Guatemala
Kinetic	A/S	0.125	0.25	Cuproquim, Mex.
Penetrator	A/S	0.5	. 1	Ciba-Geigy, Mex.
Fertilizers				
Arco iris	Foliar	0.5	0.8	Fertilizantes foliares, Mex.
Musol	Foliar	0.75	2.5	Foliares líquidos mexicanos
Insecticides				
Diazinon (Diazinon-Bio)	Organophosphorous	0.5° 0.75 ^d	0.75°	AGM, Mex.
Malathion (Biothion)	Organophosphorous	1° 0.5d	1.25° 0.75 ^d	AGM, Mex.
Methamidophose (Biometa)	Organophosphorous	0.5	0.75	AGM, Mex.
Trichlorfon (Dipterex)	Organophosphorous	0.5	0.875	Bayer, Mex.
Carbaryl ^f (Sevin)	Methylcarbamate	0.75	1	Rhóne-Poulenc, Mex.
Endosulfan (Biosulfan)	Organochlorine	1	1.5	AGM, Mex.
Permethrin [†] (Corsair)	Pyrethroid	0.17	0.29	Rhóne-Poulenc, Mex.
Fungicides				
Benomyl (Benlate)	Benzimidazole	0.05	0.5	Dupont, Mex.
Captan (Biocaptan)	Phthalimide	0.25	2	AGM, Mex.

^aLower and ^bhigher concentrations recommended on the product label for field use. ^cConcentrations recommended for the spittlebug. ^d Concentrations recommended for the whitefly. ^cAssayed only with *P. fumosoroseus*. ^fAssayed only with *M. anisopliae* (see materials and methods).

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recommended for the spittlebug plague, and Methamidophos for whiteflies, therefore, the former two were tested only with *M. anisopliae*, and the latter only with *P. fumosoroseus*. Potato dextrose agar (PDA) medium [300 g potatoes, diced, boiled and filtered, 20 g dextrose (Droguería Cosmopolita, México) and 15 g agar (Bioxón) per liter] was used as solid medium for all experiments. After sterilization, the medium was cooled to 45°C, and all test products were added individually at the concentrations recommended by the supplier for use in the field.

Each product was prepared concentrated so that it could be diluted with the medium to the desired concentration before plating. The test products were thoroughly mixed with the molten PDA and poured into 50-mm Petri dishes.

Compatibility test. Each fungus was grown on PDA plates at 28°C for 10 days. Conidia were collected and diluted with 0.05% Tween 80 (Droguería Cosmopolita), and counted with a hemocytometer. Fungal test suspensions were diluted to 1 x 10⁶ conidia/ml.

A central 5 mm circular well was made in the PDA plate containing the test product, and filled with 50 μ l of each fungal suspension to attain 5 x 10⁴ conidia per well. Plates were then incubated at 28°C for 10 days. Both concentrations of all test products were replicated three to five times, and compared with a control consisting of only each fungal suspension in 0.05% Tween 80. All experiments were repeated separately at least three times.

Fungal colony measurement. Fungal growth was determined by measuring daily the colony diameter always along a same pre-marked line during 10 days. The growth inhibition percentage (GI%) for each treatment was calculated from the fungal colony diameter (mm) at day 10, with the following formula:

GI% = Control colony diameter - Treated colony diameter
x 100
Control colony diameter

Statistical Analysis. Data was submitted to ANOVA, P<0.05. Whenever significance was found, the Tukey test was applied at the same level of significance.

RESULTS

Adherents/surfactants (A/S) had a significant effect (P<0.05) on growth of *M. anisopliae* when compared to the control (Table 2). The least growth inhibition percentage (Gl%) occurred at both concentrations of Kinetic and Penetrator. Both concentrations of Citowett showed Gl% detrimental to *M. anisopliae*. The other A/S tested showed inhibition percentages intermediate between 28.6 and 52.6 Gl% (Table 2). Adherents/surfactants also had a significant effect (P < 0.05) on growth of *P. fumosoroseus* as compared to the control (Table 2). The best compatibilities occurred at both concentrations of Adhefix 12, Kinetic, Penetrator and Kaytar. Within this group of A/S, Citowett and Inex-A were the most inhibitory of fungal growth. The other products showed inhibition percentages intermediate between 24.3 to 35.2 (Table 2).

The morphology of M. anisopliae colonies grown on

Table 2. Fungal growth percent inhibition (GI%) of Metarhizium anisopliae and Paecilomyces fumosoroseus by two concentrations of adherents/surfactants.

Fungus	Concentration I (%)	GI% x ± SD ^a	Concentration II (%)	GI% x ± SD ^a
M. anisopliae	Citowett 0.125 Adhefix 0.075 Inex-A 0.2 Kaytar 0.075 Adhefix-12 0.075 Kinetic 0.125 Penetrator 0.5 Control	60.2 ± 4.08ab 47.3 ± 3.10b 45.8 ± 4.89b 35.9 ± 4.89c 28.6 ± 2.44d 22.6 ± 5.83de 18.3 ± 4.86e 0 ± 0f	Citowett 0.25 Adhefix 0.15 Inex-A 0.4 Kaytar 0.15 Adhefix-12 0.15 Penetrator 1 Kinetic 0.25 Control	$61.7 \pm 8.57a$ $52.6 \pm 2.45b$ $50.2 \pm 2.83b$ $42.9 \pm 3.11c$ $32.5 \pm 3.36d$ $27.7 \pm 7.27de$ $23.8 \pm 4.71e$ $0 \pm 0f$
P. fumosoroseus	Citowett 0.125 Inex-A 0.2 Adhefix 0.075 Kaytar 0.075 Penetrator 0.5 Kinetic 0.125 Adhefix-12 0.075 Control	$41.8 \pm 4.62a$ $37.1 \pm 7.92a$ $25.6 \pm 4.44b$ $24.3 \pm 5.82bc$ $17.4 \pm 4.30cd$ $15.3 \pm 5.89d$ $10.7 \pm 4.55d$ $0 \pm 0e$	Inex-A 0.4 Citowett 0.25 Kaytar 0.15 Adhefix 0.15 Penetrator 1 Kinetic 0.25 Adhefix-12 0.15 Control	45.2 ± 7.33a 44.8 ± 5.75a 35.2 ± 7.57b 28.7 ± 2.45b 19.4 ± 4.73c 16.4 ± 5.82cd 9.3 ± 8.20d 0 ± 0e

^{*}Mean GI% ± standard deviation, data from day 10 of growth. bMeans with the same letter are not significantly different (Tukey, P<0.05).



the A/S-treated medium was diverse. The colonies exposed to 0.125% Kinetic had a normal morphology and size with respect to the control. The most evident morphological change was observed with lnex-A at 0.4%, with a small, velvety and non-pigmented colony (Fig. 1). With 0.25% Citowett, the colony was small, with sinuous borders, and decreased pigmentation when compared with the control.

The morphology of P. fumosoroseus was not altered by the A/S except on Penetrator (0.5 and 1%) medium on which the colony had an aberrant cerebriform aspect (Fig. 2). The microscopic morphology of hyphae and conidia of M. anisopliae and P. fumosoroseus was the same as the control (data not shown) on all A/S.

Fragments of all colonies from A/S-treated medium were transferred after 14 days to PDA untreated medium. None of the A/S caused an irreversible effect in the colonial morphology of the two fungi, since both grew as the respective control when transferred to the untreated medium.

Growth of both fungi was usually not affected by fertilizers. Only Arco iris at 0.8 % had a significant effect (P<0.05) on M. anisopliae when compared to the control, inhibition percentages for both fungi were very low (Table 3). At day 10, the colonies of P. fumosoroseus exhibited an increase in growth on Musol; the colonial morphology did not show changes, except for a light yellowish color on this fungus. Both concentrations of Musol showed increased pigmentation on M. anisopliae as compared with the control (Fig. 3).

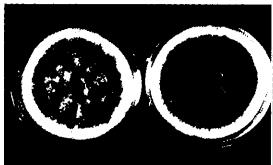
Inhibition of fungal growth by insecticides varied with



Fig. 1. Fungal colony morphology of Metarhizium anisopliae, 10 days of incubation at 28°C. Left: control colony on PDA medium. Right: 0.4% Inex-A surfactant-treated medium with a small velvety colony without pigmentation.



Fig. 2. Fungal colony morphology of Paecilomyces fumosoroseus, 10 days of incubation at 28°C. Left: control colony on PDA medium. Right: 0.5% Penetrator surfactanttreated medium with a cerebriform aberrant colony.



sopliae, 10 days of incubation at 28°C. Left: control colony on PDA medium. Right: 0.75% Musol fertilizer-treated medium showing an increase in pigmentation when compared treated medium showing a diminished, centrally comwith the control.

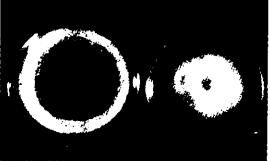


Fig. 3. Fungal colony morphology of Metarhizium ani- Fig 4. Fungal colony morphology of Metarhizium anisopliae, 10 days of incubation at 28°C. Left: control colony on PDA medium. Right: 0.875% trichlorfon insecticidepressed and extended colony without pigmentation.



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the product tested. All insecticides, in the assays with *M. anisopliae*, had a significant effect (P<0.05) on fungal growth when compared to the control (Table 4). The best compatibility was found at both concentrations of Trichlorfon, and the lowest concentration of Permethrin. The most detrimental insecticides to fungal growth were Malathion at both concentrations, and Diazinon at the highest concentration. The other tested products showed inhibition percentages intermediate between 52.4 and 71.7% (Table 4).

Insecticides also had a significant effect (P<0.05) on P. fumosoroseus growth when compared to the control (Table 4), with the exception of the lower concentration of Methamidophos. The least inhibition percentage was found at both concentrations of Methamidophos. Endosulfan and Trichlorfon showed intermediate growth inhibition. The most toxic products were the three organophosphorous insecticides, Malathion and Diazinon at both concentrations tested, and Trichlorfon at the highest concentration (Table

4).

Fungal colonies had diverse morphologies depending on the insecticide-treated media. When 0.17% Permethrin and 0.5% Trichlorfon were used, *M. anisopliae* had a different morphology from that of the control colony. With Permethrin, the colony was small and with abundant aerial mycelia; with Trichlorfon, the colony was centrally compressed with extended flat borders, and without pigmentation (Fig. 4). With 1% Carbaryl the fungus developed into a velvety, convex colony without pigmentation, and with 1% Endosulfan into a small irregular border colony with irregular pigmentation. *P. fumosoroseus* did not present major colony alterations, except that colonies were smaller than controls. The microscopic characteristics of both fungi were the same as the controls (data not shown).

After 14 days of incubation, fungal fragments from insecticide-treated media were transferred to PDA untreated medium. The colonial morphological changes reported

Table 3. Fungal growth percent inhibition (GI%) of Metarhizium anisopliae and Paecilomyces fumosoroseus by two concentrations of fertilizers

Fungus	Concentration I (%)	GI% x ± SD ^a	Concentration II (%)	GI% x ± SD ^a
M. anisopliae	Musol 0.75	$1.4 \pm 3.13a^{b}$	Arco iris 0.8	$3.7 \pm 4.13a$
	Arco iris 0.5	$0.2 \pm 0.36a$	Musol 2.5	$2.2 \pm 3.84b$
	Control	$0 \pm 0ab$	Control	$0 \pm 0b$
P. fumosoroseus	Arco iris 0.5	$0.3 \pm 0.9a$	Arco iris 0.8	$0.4 \pm 1a$
	Musol 0.75	$0 \pm 0a$	Musol 2.5	$0 \pm 0a$
	Control	$0 \pm 0a$	Control	$0 \pm 0a$

^aMean GI% ± standard deviation, data from day 10 of growth. ^bMeans with the same letter are not significantly different (Tukey, P<0.05).

Table 4. Fungal growth percent inhibition (GI%) of Metarhizium anisopliae and Paecilomyces fumosoroseus by two concentrations of chemical insecticides

Fungus	Concentration I (%)	GI% x ± SD*	Concentration II (%)	$GI\% x \pm SD^a$
M. anisopliae	Malation 1 Diazinon 0.5 Endosulfan 1 Carbaryl 0.75 Permethrin 0.17 Trichlorfon 0.5 Control	$94.7 \pm 7.85a^{b}$ $71.7 \pm 4.79b$ $66.1 \pm 1.68b$ $52.1 \pm 3.96c$ $42.1 \pm 2.63d$ $27.2 \pm 3.83e$ $0 \pm 0f$	Malation 1.25 Diazinon 0.75 Endosulfan 1.5 Carbaryl 1 Permethrin 0.29 Trichlorfon 0.875 Control	$100 \pm 0a$ $84.7 \pm 13.03b$ $67.5 \pm 2.57c$ $66.6 \pm 7.13c$ $52.4 \pm 2.81d$ $40.7 \pm 5.83e$ $0 \pm 0f$
P. fumosoroseus	Malation 0.5 Diazinon 0.75 Trichlorfon 0.5 Endosulfan 1 Methamidophos 0.5 Control	$100 \pm 0a$ $83.9 \pm 12.3b$ $38.0 \pm 6.27c$ $31.7 \pm 1.6c$ $10.5 \pm 6.56d$ $0 \pm 0d$	Malation 0.75 Diazinon 1 Trichlorfon 0.875 Endosulfan 1.5 Methamidophos 0.75 Control	$100 \pm 0a$ $100 \pm 0a$ $69.6 \pm 23.1b$ $37.2 \pm 3.6c$ $16.4 \pm 4.8d$ $0 \pm 0e$

^aMean GI% ± standard deviation, data from day 10 of growth. ^bMeans with the same letter are not significantly different (Tukey, P<0.05).





above disappeared, and the fungal colonies showed again a normal growth as the control. The only exception was observed with *M. anisopliae* from both concentrations of Trichlorfon-treated medium, which, when transferred to the untreated medium, although with normal growth, showed a less pigmented colony when compared to the control.

The two fungicides (Benomyl and Captan) at the two concentrations used (Table 1) were fully incompatible with both fungi, exhibiting 100 GI%.

DISCUSSION

Interactions of fungal entomopathogens with agrochemicals used in the field are of outmost importance for the optimal performance of mycoinsecticides. Hence, A/S and fertilizers should also be assayed for compatibility with fungal entomopathogens, in addition to pesticides.

M. anisopliae and P. fumosoroseus had different compatibilities with A/S. Citowett induced the highest growth inhibition for both tested fungi. The adherent Adhefix, with the same concentration and active ingredient (alkylphenol, polyoxyethylene ether), but manufactured in two different countries (Table 1) induced significant different inhibitions; Adhefix 12 from Guatemala had a low inhibitory effect on both fungi tested and was the most compatible with P. fumosoroseus of all adherents tested. From these facts it can be inferred that different additives are used for each product, which may explain the difference in compatibilities. Anderson and Roberts² with B. bassiana and Li and Holdom9 with M. anisopliae, suggested that fungal inhibition might be due to the formulation rather than to the products' active ingredient. Hence, the need to test the commercial product to be used in the field. Testing M. anisopliae, Alvest found moderate compatibility at the lowest and a medium concentration indicated on the product label of the A/S Extravon 200L (alkylphenol, polyglycol ether), and moderate compatibilities with the lowest concentration of other surfactants, considering a moderate compatibility as an inhibitory effect on fungal growth, such as the results obtained in this work with the same fungus.

Commercial fertilizers are another group of agricultural products of common use in the field in almost all type of crops. No inhibition was found when the lower concentrations on the product label of Arco iris and Musol were used. When evaluating the effects of nitrogen-containing fertilizers on the persistence of *B. bassiana* in soil, Rosin et al.¹⁵ observed that high rates of composted manure can increase the efficacy of *B. bassiana* as a biological control agent. Although, in our study, different fertilizers were used, we observed a stronger pigmentation (enhanced sporulation) with Musol on *M. anisopliae* (Fig. 3), and increase of growth on *P. fumosoroseus*. This would suggest that joint applications of fertilizers and mycoinsecticides would be compatible.

In ecologically-based pest management, the compati-

bility of entomopathogens with chemical insecticides should be known to choose among the least toxic compounds for humans, mammals, and fungus-based bioinsecticides. The insecticides used in this study (samples of commercial products) were some of those listed for the control of spittlebugs and whiteflies in Mexico.^{7,16}

The variability of response (compatibility or noncompatibility) among the insecticide classes does not suggest one class as more detrimental than another. The organophosphorous insecticides, however, produced the higher inhibition of growth in both fungi.

Hernández and Berlanga⁵ observed no negative effect on germination and development of five isolates of *Paecilomyces* sp. exposed to Endosulfan and Metamidophos. These insecticides also were compatible with *P. fumosoroseus* in the present study. Another entomopathogen, *B. bassiana* strain GHA, is highly compatible (can be tank mixed) with many commonly used chemical adulticides, including the synthetic pyrethroids; however, it is not recommended to tank mix *B. bassiana* with Endosulfan although they are fully compatible when applied separately.¹⁸

Dose response interactions have been demonstrated in compatibility experiments with pesticides and different entomopathogenic fungi. 3,9,12 In the present study, almost all insecticides showed a higher GI% at the highest concentration tested, although without significant differences between concentrations.

Interactions between fungicides and fungal entomopathogens have been widely documented, 9,11 due to the frequent use of fungicides against phytopathogenic fungi. Our results confirm the non-compatibility of both fungi assayed with Benomyl (benzimidazole) and Captan (phthalimide) at both concentrations recommended for field use. Li and Holdom⁹ reported on the incompatibility of *M. anisopliae* with Propiconazole, Prochloraz, and Flusilazole (conazole fungicides) and Carbendazim (benzimidazole fungicide) even at concentrations lower than recommended on the labels. Therefore, careful precaution should be taken when fungicide applications are performed on a field where mycoinsecticides are or will be used.

From a practical point of view, those moderate and lightly toxic insecticides for mammals with acceptable fungal compatibility should be sought for ecologically-based pest management programs. Based on this concept and on our results, Trichlorfon and Permethrin (moderately toxic to mammals) should be tested in the field with *M. anisopliae* and Methamidophos and Endosulfan (moderately toxic to mammals) with *P. fumosoroseus*.

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