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Pestalotiopsis sp. infection causes leaf fall disease of new arrivals in several clones of rubber plants

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Abstract. *Damiri N, Pratama Y, Febbiyanti TP, Rahim SE, Astuti DT, Purwanti Y. 2022.* Pestalotiopsis *sp. infection causes leaf fall disease of new arrivals in several clones of rubber plants. Biodiversitas 23: 3943-3949.* This study aims to investigate the response of rubber clones commonly cultivated by farming communities to infection with the new leaf fall pathogen of *Pestalotiopsis* in rubber plantations. The study was conducted in smallholder rubber plantations, laboratories and greenhouses Department of Pests and Plant Diseases, Faculty of Agriculture, Sriwijaya University. The study consists of two stages. The first stage was a survey of the leaf fall disease of *Pestalotiopsis* in smallholder rubber plantations in Banyuasin Regency. The second stage was testing the isolates of *Pestalotiopsis* sp. against several rubber clones in the greenhouse using the Randomized Block Design (RBD) method, with six treatment clones and six replications. Each replication consisted of three polybags. The treatments tested were rubber plant clones aged four months, namely rubber clones PB 260, RRIC 100, IRR 112, IRR 118, BPM 24 and PB 340. The inoculated leaves were rubber leaves aged 10 days after opening. The results showed that Pestalotiopsis leaf fall disease in 10 smallholder rubber plantation areas in the field showed that new leaf fall attacks caused by the *Pestalotiopsis* fungus on smallholder rubber plants ranged from 80 to 100 percent with a decrease in production between 20 to 50 percent. All clones planted were susceptible to this new disease, which resulted in a very significant reduction in yield. The treatment of *Pestalotiopsis* sp. inoculation on six rubber clones significantly affected the severity of *Pestalotiopsis* new leaf fall disease. Effect of *Pestalotiopsis* sp. the number of rubber leaves that fall is very significant. The rubber clone that experienced the most leaf fall due to disease was PB 340 although the percentage of leaf fall was not significantly different from the treatment of PB 260 but significantly different from the treatment of clones RRIC 100, IRR 112, IRR 118 and BPM 24. The percentage of *Pestalotiopsis* leaf fall experienced by clones PB 340 reached 47.19% while the RRIC 100 clone was only 4.98%.

Keywords: Infection, leaf fall disease, *Pestaloptiopsis* sp., rubber clones

INTRODUCTION

Rubber (*Hevea brasiliensis* Muell. Arg) is an important plantation commodity for Indonesia because it makes a significant contribution to the local and national economy and is a source of income for millions of farmers (Lahjie et al. 2018). Rubber plants are the largest contributor to foreign exchange for Indonesia. Indonesia is the second world rubber producer after Thailand, with production reaching 3,100,000 tons. However, world rubber production from year to year shows a decline. The decline in rubber production from year to year is thought to be caused by several factors, one of which is disease. Lately, leaf fall disease in rubber plants has become a big problem for rubber producing countries in the world (International Rubber Study Group 2021; Association of Natural Rubber Producing Country 2021).

Diseases are one of the limiting factors for rubber plant production because they can reduce the quality of rubber and cause significant losses in the rubber plantation industry (Oktavia et al. 2021). Every year losses caused by disease can reach billions of rupiah. Many types of pathogens can attack rubber plants either on root, stems or leaves. Pathogen attacks can also occur in rubber plantation in nurseries, immature plants and mature plants in the field (Kusdiana et al. 2020).

Leaf fall disease in rubber plantations is one of the diseases that is quite important in both rubber plants in Indonesia and in rubber-producing countries. This leaf fall disease is believed to be caused by many pathogens that can attack rubber plants either individually or together and cause considerable losses. Pathogen of leaf fall disease in rubber plants in Indonesia include *Colletotrichum gloeosporioides* (Penz.) Sacc, *Oidium heveae* B.A. Steinm, *Corynespora cassiicola* (Berk. & Curt) Wei. and *Pestalotiopsis* sp. (Speg.) G.S Zhao N.Li. The four pathogens cause leaf fall of rubber plants, but the symptoms of attack are different from each other. Symptoms of *Colletotrichum* attack on rubber leaves cause the leaves to shrivel, curl and die at the tips of leaves. *Corynespora cassiicola* infection on rubber leaves will show symptoms in the form of distinctive spots such as fish fins. The leaves change color from green to yellow or brown and then fall. *Oidium heveae* attack on rubber leaves

is indicated by the presence of a white powdery layer consisting of conidia of *Oidium* fungus on the infected leaf surface. In severe attack, the leaves dry up and fall (Kusdiana et al. 2020).

Rubber leaf fall disease caused by *Pestalotiopsis* sp. is a new disease in rubber plantations in Indonesia. This disease was first reported as an outbreak in rubber plantations in Malaysia in 1987 and 2003. Apart from Malaysia, this new disease has been identified as attacking rubber plantation in Indonesia, Sri Lanka, India and Thailand. In Indonesia, the first outbreak of leaf fall in 2016 spread from rubber plantations in North Sumatra, followed by an outbreak in South Sumatra in 2017 until mid-2018. This disease spread to the provinces of Lampung, Central Java, East Java, South Kalimantan, and Central Sulawesi. The total area of this disease is more than 22,000 ha, causing loss of plant canopy >50% and loss of latex production >25%. (Cahyo 2018; Aliya et al. 2022). The latest data in 2021, as many as 30,328.84 ha of rubber plantations were infected with this fungus which resulted in a decrease in latex production by up to 30% (Directorate General of Plantations 2022). This disease causes continuous defoliation up to 75-90%, the canopy becomes thinner and production drops to 25-45%. The disease causes the leaves to fall prematurely so that rubber plants appear to be withering in the provinces of South Sumatra and North Sumatra. Symptoms of this disease are known to be different from the symptoms of leaf disease that have existed before. Symptoms of attack on the leaves are brown spots, then develop into dark brown spots and there is a clear boundary between the spots and the healthy parts. The spots continue to widen to a size of 1-2 cm, then the tissue around the spots undergoes necrosis. In severe attacks it will cause leaf fall to continue until the plant crown is bare (Maryani and Astuti 2019; Febbiyanti and Fairuzah 2019). *Pestalotiopsis* sp. is an airborne pathogen so that it spreads very quickly and can result in the fall of rubber leaves so that the plant withers throughout the year and decreases rubber production (Febbiyanti 2019). *Pestalotiopsis* spp. also attack coconut, pine and bayberry and cause huge losses to farmers (Dung et al. 2016; Li et al. 2020; Bhuiyan et al. 2021).

This pathogen causes plants to die throughout the year so that the canopy looks thin and even does not exist at all (Panusunan 2019). Rubber disease causes enormous losses in production economically with the number of billions of rupiah and very expensive costs in controlling the rubber disease. *Pestalotiopsis* leaf fall disease attacks rubber plants at all stages, starting from nurseries, entry gardens, immature plants and also mature plants. The disease is also known to infect all cultivated clones (Febbiyanti and Fairuzah 2019). Rubber clones in Indonesia generally come from parent trees with no different properties, so their resistance to disease is pseudo which will eventually appear sensitive to leaf fall disease (Rivano et al. 2013). This study tried to study the response of six rubber clones that are commonly cultivated by farming communities to the infection of *Pestalotiopsis* new leaf fall pathogen in rubber plantations.

MATERIALS AND METHODS

This research was carried out in the area of smallholder rubber plantations, laboratories and greenhouses of Phytopathology Department of Pests and Plant Diseases, Faculty of Agriculture, Sriwijaya University, Indonesia. The research consisted of two stages. The first stage is a survey on the attack of *Pestaloptiopsis* leaf fall disease in smallholder rubber plantations in Banyuasin district. The second stage was testing the isolates of *Pestaloptiopsis* sp. against several rubber clones in the greenhouse using the Randomized Block Design (RBD) method, with six treatment clones and six replications. Each replication consisted of three polybags. The treatments tested were rubber plant clones aged four months, namely rubber clones PB 260, RRIC 100, IRR 112, IRR 118, BPM 24 and PB 340. The leaves that were inoculated were rubber leaves aged 10 days after opening.

Leaves infected with *Pestalotiopsis* sp. obtained from the field were brought to the laboratory for identification and propagation. The infected leaves are cut 0.5 cm healthy x 0.5 cm sick, then soaked in 70% alcohol for ±2 minutes, then drained on a tissue, then rinsed with sterile water, then air-dried. Isolation of *Pestalotiopsis* sp. was carried out by the method of planting tissue in Potato Dextrose Agar (PDA) media in separate petri dishes to be used as pure cultures. Pure cultures obtained were then identified using the book Illustrated genera of imperfect fungi (Barnett and Hunter 1986). The pure isolates obtained were then propagated in sterile PDA media that had been given the antibiotic amoxcyllin in a petri dish for use in the study. Inoculum preparation was carried out by sweeping the pure culture that had formed conidia and had been given sterile water, then filtered using nylon gauze to separate the lumps that had occurred and put into an Erlenmeyer flask. Conidia concentration was calculated by hemocytometer to obtain a concentration of 6 x 104 conidia.

Spraying of conidia was carried out using a jet sprayer on the surface of the leaves that were 10 days after opening or the leaves were brown. Rubber leaves that were still brown were inoculated by spraying *Pestalotiopsis* sp. with a concentration of 6x104 until wet. Furthermore, the leaves are covered with transparent plastic measuring 10 x 15 cm, to retain moisture. After 3 days the plastic cover was removed. Observations were made on the incubation period, severity and number of fallen leaves. The incubation period was observed every day after inoculation. Observation of disease severity was carried out by counting the number of leaf spots on rubber plants in each inoculated treatment. The calculation of the spots was carried out using the attack scale on the leaves as follows: 0: no attack at all, 1: spot appears but does not develop, 2: spot 1mm to 3 mm in diameter 3: spot 3,1 mm to 5 mm in diameter, 4: spots 5,5 mm to 7 mm in diameter, 5: spots >7,1 mm in diameter or leaves starting to turn orange, 6: Leaves turn orange, 7: leaves fall (Kusdiana et al. 2020).

Furthermore, the results of the assessment of the attack scale are entered in the formula:

$$IP = \frac{\sum (n \times v)}{Z \times N} \times 100\%$$

Where:
IP : Disease intensity
n : Leaf amount from each attack category
v : the scale value for each attack category
Z : the scale value of the highest attack category
N : leaf amount that was observed

The classification of disease attack intensity is divided into four categories, namely resistant (attack intensity 0-20%), moderately resistant (attack intensity 21-40%), moderate (attack intensity 41-60%), moderately susceptible (attack intensity 61-80%) and vulnerable (attack intensity 81-100%) (Febbiyanti 2019). The data obtained in this study were arranged and analyzed using variance fingerprints and if the results were significantly different, it was continued with Duncan's test.

RESULTS AND DISCUSSION

Pestalotiopsis leaf fall disease infection in rubber plantations

The results of observations in the field showed that the symptoms of attack on the leaves were brown spots, then developed into dark brown spots and there was a clear boundary between the spots and the healthy parts. Rubber plants on rubber plantations look withered, leaves turn yellow and fall. Pathogen isolates appear white with conidia that are wider at the apical and slenderer at the base, having brown to black septa (Figure 1).

The results of observations of *Pestalotiopsis* leaf fall disease in 10 areas of smallholder rubber plantations in the field showed that new leaf fall attacks caused by the fungus *Pestalotiopsis* on smallholder rubber plantations ranged from 80 to 100 percent with a decrease in production between 20 to 50 percent. All clones planted were susceptible to this new disease, resulting in a very significant reduction in yield (Table 1).

Incubation period and symptoms

On average, symptoms were first detected in the six rubber clones tested, four to 16 days after inoculation. The results showed that the initial symptom was brown spots on the leaf surface. Small spots will turn dark brown. Then these spots will coalesce to form larger necrotic areas on the leaf. Symptoms of attack on the six rubber clones can be seen in Figure 2.

Table 1. The extent of leaf fall of *Pestalotiopsis* on rubber plantations in the field and its impact on decline in rubber production

Location/ farmer	Rubber clones that were planted	Attack area (%)	Production decline (%)
A	BPM 24	95	35
B	RRIC 100	80	20
C	PB 260	98	40
D	GT1	100	50
E	BPM24	90	35
F	IRR118	87	30
G	PB260	95	35
H	GT1	100	30
I	BPM 1	86	40
J	PB260	95	35

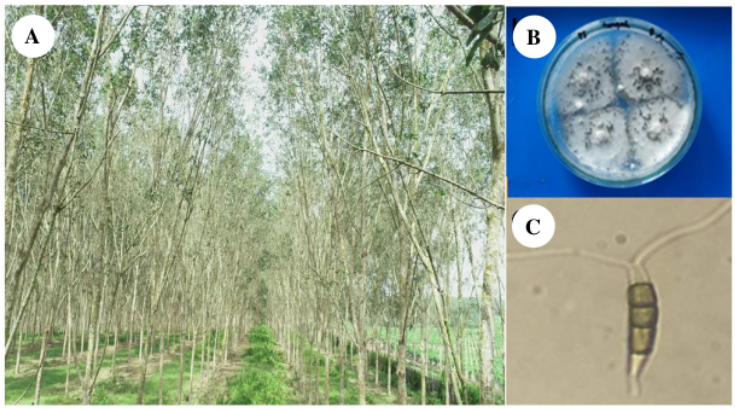

Figure 1. Leaf fall disease in rubber plantations. A. Rubber plantation infected by Pestalotia leaf fall disease, B. Isolat of *Pestalotiopsis* sp., C. Conidia of *Pestalotiopsis* sp.

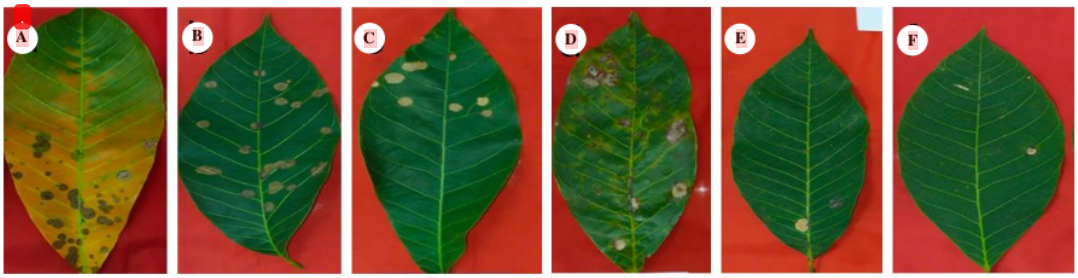

Figure 2. Symptoms of infection with *Pestolotiopsis* sp. on six rubber clones. A. PB340, B. PB 260, C. BPM 24, D. IRR 118, E. IRR 112, F. RRIC 100

Symptoms of this disease attack are marked by the formation of patches that continue to widen so that the surrounding tissue undergoes necrosis. There is a change in the color of the leaves and the leaves turn yellowish. In heavy attacks it can cause leaf fall (Malik 2018). The typical symptom of *Pestalotiopsis* sp. infection on old leaves is that the attack spots will appear as brownish margins and areas that are concentric with conidia on the leaf surface. Severely infected leaves After leaf fall due to this disease, the new leaves formed are smaller than normal leaf size, some branches die and the plant crown withers and decreases by up to 50% (Ngobisa et al. 2012; Directorate General of Plantation 2019). The occurrence of abortion due to *Pestalotiopsis* sp. infection can cause the rubber plant canopy to wither which will affect rubber productivity. Productivity of rubber plants is closely related to canopy conditions, this is because the raw material of assimilated rubber particles is very dependent on the photosynthesis process that occurs in the leaves (Chow et al. 2012).

Disease severity

The results of the variance of *Pestalotiopsis* sp. inoculation treatment on six rubber clones had a significant effect on the severity of Pestalotiopsis new leaf fall disease. Further tests of the effect of *Pestalotiopsis* sp. infection on six rubber clones are presented in Table 2 and Figure 3.

Table 1 shows that *Pestalotiopsis* sp. was able to infect the six clones tested. Severe infection was indicated by clone PB 340 which was the most severely affected clone with a percentage of disease severity reaching 67.39% at the fourth week after inoculation, followed by clones PB 260, BPM 24, IRR 118 with disease severity of 61.47% consecutively, 41.73% and 38.80%. PB 340 and PB 260 were not significantly different from each other but significantly different in severity in rubber clones BPM 24, IRR 118, IRR 112 and RRIC 100. Based on the classification of disease intensity, rubber clones that had the classification was susceptible, namely PB 340 and PB 260, moderate clones were BPM 24, moderately resistant clones were IRR 118 while resistant clones were clones RRIC 100 and IRR 112. Resistance of rubber to leaf fall attacks was highly dependent on the genetic characteristics of the clones. The mechanism of leaf resistance to pathogens is also determined by the ability of plants to overcome the spread of toxins produced by pathogens. This is also indicated by damage to epidermal cells and other organelles resulting in severe leaf damage (Daslin 2013; Dalimunthe et al. 2015).

Table 2. Effect of *Pestalotiopsis* sp. infection causing new leaf fall disease on six rubber clones

Rubber clones	Average severity of *Pestalotiopsis* new leaf fall disease (%)			
	1 week after inoculation	1 week after inoculation	1 week after inoculation	1 week after inoculation
RRIC100	1.17 a	1.17 a	9.33 a	12.61 a
IRR 112	1.17 a	8.79 ab	14.59 ab	19.39 a
IRR 118	13.46 b	17.28 bc	24.72 bc	38.80 b
BPM 24	13.71 b	22.60 cd	27.87 c	41.73 b
PB 260	25.82 c	32.66 de	34.85 c	61.47 c
PB 340	31.68 c	38.36 e	53.56 d	67.39 c

Note: The numbers followed by the same letter in the same column are not significantly different in 5% level according to the Least Significant Difference Test

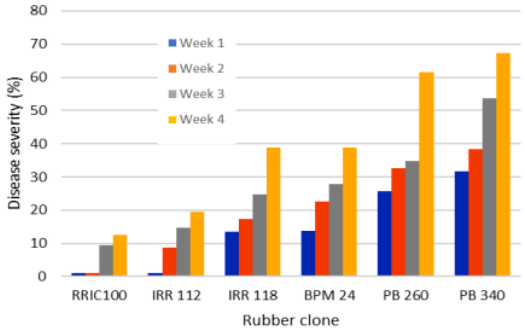

Figure 3. Development of *Pestalotiopsis* leaf fall disease in rubber clones

Clones RRIC 100 and IRR 112 showed resistance to *Pestalotiopsis* infection, it was suspected that the presence of this pathogen could not develop properly. It is also known that this clone is also resistant to pathogens that cause other deciduous diseases such as *Corynespora* Leaf Fall disease. RRIC clones are resistant to this leaf fall pathogen, possibly because the clones are genetically resistant (Kusdiana et al. 2017). The high resistance of the two clones to *Pestalotiopsis* leaf fall infection was also suspected because this pathogen was relatively new so it had not been able to adapt and break the resistance of this clone. Genetically, resistance can be inherited. Rubber clones in Indonesia and even in Asia come from parent trees with similar characteristics, therefore their resistance to disease is also not much different. Clones PB 340 and PB 260 were clones that were very susceptible to *Pestalotiopsis* leaf fall infection. It was suspected that these clones were unable to suppress or limit the development of *Pestalotiopsis* sp. These pathogenic fungi are able to produce toxins that can accelerate the damage to the infected rubber leaf tissue. There was a different response shown by each clone to *Pestalotiopsis* sp. infection, due to differences in the level of phenotypic variability, genetic variability and the interaction of the two. Plant resistance to leaf fall disease is a complex resistance that is regulated as polygenic or monogenic so that there are variations in plant resistance that are regulated by additive genes or regulated by recessive resistance genes (Andersen et al. 2018; Kusdiana et al. 2018).

Number of fall leaves

The results of the analysis of the effect of *Pestalotiopsis* sp. The number of rubber leaf fall is very significant. The rubber clone that experienced the most leaf fall due to disease was PB 340 although the percentage of leaf fall was not significantly different from the treatment of PB 260 but significantly different from the treatment of clones RRIC 100, IRR 112, IRR 118 and BPM 24. The percentage of *Pestalotiopsis* leaf fall experienced by clone PB 340 reached 47.19% while in clone RRIC 100 it was only 4.98%, as shown in Table 3 and Figure 4.

Interaction between fungi can occur in two ways, including beneficial interaction and adverse interaction. Pathogenic fungi can produce several compounds that can interfere with physiological processes in plants (Dean et al. 2012; Gust et al. 2012). Leaves infected with this fungus experience leaf fall at a more severe level of attack. Deciduous leaves show a yellowish or orange discoloration. The discoloration of the leaves is thought to be due to the pathogen releasing toxic compounds that affect the leaves. Disease-causing fungi can produce phytotoxin compounds that can interfere with host cell function or even kill host cells during the infection process. Compounds produced when phytopathogenic fungi come into contact with plants are a factor in fungal pathogens. The metabolism of the compound begins in the fungus through physiological and biochemical reactions that last during the process of molecular interaction of the surface and the transmission of signals, and these factors act as pathogens for plants (Felix et al. 2019; Vincent et al. 2020). *Pestalotiopsis* sp. can produce bioactive secondary metabolic compounds that can support interactions between host plants and pathogens. It was reported that many types of secondary metabolic compounds that can be produced by various *Petalotiopsis* species such as *P. theae* cause gray leaf blight in plants and can produce phytotoxins known as oxysporone, PT-toxin and epiepoxydon. *P. oenotherae* pathogenic leaf spot is capable of producing partylopyrenone compounds, and *P. guepini* pathogenic blight can produce secondary compounds called Pestalopyrone. *P. neglecta* can produce pestalopyrone A-D (Feng et al. 2020). Toxins produced by pathogens at the onset of infection can spread rapidly through leaf vessels, resulting in leakage of the plasma membrane of plant cells. A further effect of the leak is the appearance of necrotic symptoms due to the death of leaf cells. Generally, each type of host will react differently to the toxin (Tran et al. 2016; Deon et al. 2012).

Table 3. Percentage of leaf fall due to *Pestalotiopsis* sp. on six rubber clones

Rubber clones	Percentage of fallen leaves (%)
RRIC 100	4.98 a
IRR 112	12.61 a
IRR 118	14.47 a
BPM 24	16.34 a
PB 260	36.63 b
PB 340	47.19 b

Note: The numbers followed by the same letter in the same column are not significantly different in 5% level according to the Least Significant Difference test

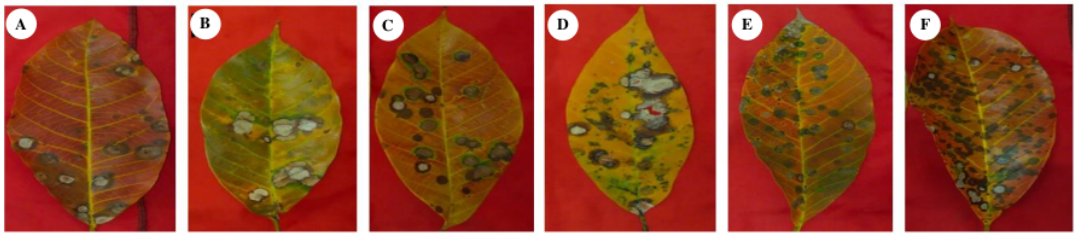

Figure 4. Leaf fall on six rubber clones infected with Pestaloptiopsis leaf fall. A. RRIC 100, B. IRR 112, C. IRR 118, D. BPM 24, E. PB 260, F. PB 340

The high number of fallen leaves is thought to be due to the presence of pathogens in plants that can produce enzymes that resemble IAA oxidase which can also be produced by plants. Generally, plant pathogenic fungi produce plant growth regulator to disturb the levels of the endogenous hormone in plants and weaken the plants defenses. The abscisic acid, gibberellic acid and ethylene produced by pathogenic fungi participate in their pathogenicity, while auxin and cytokinins could be positive regulators required for pathogen virulence. The presence of this enzyme accelerates the rate of degradation of IAA in the host-pathogen complex and disrupts the flow of auxin from the leaf blade to the petiole, causing the leaves to fall prematurely (Chancloud and Morel 2016; Jaroszuk-Ściseł et al. 2019). The loss of leaves infected with fungi is a consequence of the presence of hyphae from pathogens that grow in stomata holes, injuring cells or interfering with the activity of closing and opening stomata so as to prevent or inhibit the exchange of CO_2, O_2 and water vapor so that pathogens inhibit the process of high photosynthesis. Stomata are the accessible entrance to several pathogens. The pathogen can reopen the stomata, locking the open-stomata or killing guard cells to prevent their closure so they can enter the plant tissue (Molotto et al. 2017; Ye et al. 2020).

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