Effects of Foliar Application of Oil Palm Empty Fruit Bunch Ash Nanoparticles on Stomatal Anatomy of Potato Leaf Plants (Solanum tuberosum L.)

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ABSTRACT

The productivity of potatoes (Solanum tuberosum L.) in Indonesia is still low. Fertilization needs to be done to increase potato productivity. This study aimed to examine the effects of applying oil palm empty fruit bunch (OPEFB) ash nanoparticles on the anatomy of potato stomatal and leaf cells (Solanum tuberosum L.). The research was carried out from March to June 2021 in the Sumberejo Village, Ngablak District, Magelang Regency, Central Java, and at the Agrobiotechnology Laboratory, Faculty of Agriculture, University of Muhammadiyah Yogyakarta. The study used a single-factor treatment design with a Randomized Completely Block Design (RCBD). The treatments tested included the foliar application of OPEFB ash nanoparticles at several concentration, consisting of 0% (control), 0.1%, 0.2%, 0.3%, and 0.4%. The results showed that foliar application of nanoparticles OPEFB ash affected stomatal anatomy, namely guard cell width, stomatal aperture, and density. The application of OPEFB ash nanoparticles with a concentration of 0.3% was most effective in increasing the opening of stomata because it affects the activity of the photosynthetic process.

Keywords: Nano fertilizer, Oil palm empty fruit bunch ash, Potassium, Stomata

ABSTRAK

Tingkat produktivitas tanaman kentang (Solanum tuberosum L.) di Indonesia masih rendah. Pemupukan perlu dilakukan untuk meningkatkan produktivitas kentang. Penelitian ini bertujuan untuk menguji pengaruh penyemprotan nano partikel abu tandan kosong kelapa sawit (TKKS) terhadap anatomi stomatal dan sel daun tanaman kentang (Solanum Tuberosum L.). Penelitian dilaksanakan pada bulan Maret hingga Juni 2021 di lahan desa Sumberejo, kecamatan Ngablak, kabupaten Magelang, Jawa Tengah dan di Laboratorium Agrobioteknologi Fakultas Pertanian, Universitas Muhammadiyah Yogyakarta. Penelitian menggunakan Rancangan Acak Kelompok Lengkap (RAKL) faktor tungga terdiri dari 5 perlakuanl. Perlakuan vang diuji meliputi penvemprotan foliar partikel nano abu TKKS dengan konsentrasi 0% (kontrol); nano TKKS konsentrasi 0,1%; konsentrasi 0,2%; konsentrasi 0,3%; konsentrasi 0,4%. Hasil penelitian menunjukkan bahwa aplikasi foliar partikel abu TKKS berpengaruh terhadap anatomi stomata yaitu lebar sel penjaga, bukaan stomata dan kerapatan stomata. Aplikasi partikel nano abu TKKS dengan konsentrasi 0,3% paling efektif dalam pembukaan stomata yang mempengaruhi proses fotosintesis.

Kata kunci: Pupuk nano, Abu tandan kosong kelapa sawit, Kalium, Stomata

INTRODUCTION

important food ingredients for humans and the ing demand for potatoes. In contrast, potato promain vegetable crop, after rice, wheat, and corn. duction in Indonesia fluctuates from year to year. In Indonesia, potato plants have become one of National potato production in 2019 (1.31 million the priority foods to be developed as a source of tons/ha) has increased compared to 2018 (1.28 carbohydrates to support food diversification. The million tons/ha). However, potato production in demand for potatoes is increasing yearly, along with 2020 decreased by 1.28 million tons/ha (Badan changes in lifestyle and the development of the <u>Pusat Statistik, 2020</u>). The decrease in production potato processing industry (Isra, 2020). However, will impact potato productivity, which is also low. potato production in Indonesia has not been able One factor that leads to low potato production is

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Potato (Solanum tuberosum L.) is one of the to meet the demand for potatoes due to the increas-





the lack of nutrients that potato plants need.

activity, cation-anion homeostasis, and membrane 3450 ppm, Cu of 183 ppm, and Zn of 28 ppm, polarization. These are based on its osmotic nature, with pH ranging from 11.9 to 12.0. Based on regulation, and stomatal movement (Warnita, <u>2019</u>). One important role of K in the stomatal 0.4% increased the shallots' productivity. functions is stimulating enzyme to starch synthase for starch synthesis. Potassium also plays a role in through the soil or the leaves (foliar application). the the stomatal aperture to meet the needs of CO₂ Leaf fertilization will be effective if the particle and water vapor for photosynthesis and in the sto-size of the fertilizer material is smaller than the matal closing to prevent excessive water loss from leaf stomatal pore size. The effective absorption of plant tissues. Suppose the function of the stomatal nutrients in OPEFB ash through the leaves requires guard cells is not optimal. In that case, drought technological innovation by reducing particle size stress can occur in plants because K-deficient plants through nano-fication. can significantly reduce the net CO₂ assimilation rate (Naumann et al., 2020).

leaf surfaces, but stomata are mostly found on the rial, such as being easily absorbed by plants with underside of the leaves (abaxial). The anatomical slow-release fertilizers (Ratih et al., 2021). For this structure of stomata is closely related to the on- reason, nanoparticles of OPEFB ash are needed as togeny of the epidermis or the type of epidermis a source of potassium fertilizer that can be used to because stomata are from the modification of some meet the K needs in the stomata of potato plants. of the epidermal cells. There are several factors that In addition, research on the application of OPEFB affect the opening and closing of stomata, includ- ash nanoparticles on potato plants has never been and temperature (Driesen et al., 2020). If these the effects of foliar application of OPEFB ash components are met, it will affect plant physiologi- nanoparticles on the stomatal anatomy of potato cal processes such as transpiration, photosynthesis, plants. and respiration that occur in leaf stomata.

The need for potassium (K) in potato plants MATERIALS AND METHODS can be fulfilled by the application of inorganic (synthetic) fertilizers or organic fertilizers (fertilizers derived from organic waste). One of the organic wastes that can be used as a potassium (K) fertilizer source is oil palm empty fruit bunch ash. According

to the results of research by Efendi et al., (2020), Potato production is greatly affected by nitrogen, OPEFB ash contains nutrients such as total N of phosphorus, and potassium nutrients. The major 0.05%, P₂O of 54.79%, K₂O of 36.48%, MgO of functions of K in plants are controlling enzyme 2.63%, CaO of 5.46%, Mn of 1,230 ppm, Fe of which is why it is needed for cell extension, turgor the research by <u>Azizah (2019)</u>, the application of OPEFB ash nanoparticles with a concentration of

The fertilization for potato plants can be done

Nanotechnology is a technique for creating materials, functional structures, and devices at the The type of stomata of potato leaves belongs to nanometer scale. Fertilizers with -size has properthe Amphistomatic type, which is located on both ties and abilities far superior to the starting mateing sunlight, potassium, availability of CO₂, water carried out. Thus, this study aimed to determine

Study area

Field research was carried out in Sumberejo Village, Ngablak Sub-district, Magelang District, Central Java, with coordinates of -7.4018090 LS 110.3908880 east longitude starting from March

to June 2021. Observations were made in the field and in the Agrobiotechnology Laboratory, Faculty of Agriculture, University of Muhammadiyah Yogyakarta.

Experimental design

Experimental research was conducted with a single factor treatment arranged in a Randomized Completely Block Design (RCBD), consisting of five concentrations of OPEFB nanoparticles (0% (control), 0.1%, 0.2%, 0.3%, and 0.4%. Each treatment consisted of five replications, in which there were 25 plants in each unit. Thus, there were 125 potato plants. Each experimental unit contained a physiological plot consisting of three physiological plants. OPEFB ash nanoparticles were applied 20 days after planting, and the next application was carried out once every 10 days.

stomatal guard cell width, stomatal aperture, ity and cause guard cells to expand, resulting in stomatal density, cell wall thickness, and leaf cell the elongation of cellulose microfibrils outward. area. Observations were made at each phase of In the process of stomatal opening and closing, potato plant growth at 40, 65, and 75 days after stomatal elongation occurs only in the cellulose planting. Sampling was carried out directly in the microfibrils or cellulose fine fibers contained in sun without picking the leaves to keep the stomatal the guard cell walls in a radial shape; this arrangecells open using the replica method. As for the cross-section of the leaf, thin slices were made with a transverse direction in the thickness of the leaf, and the incisions were observed through an Olympus CX-22LEDRFS1 computer microscope with a magnification of 400x.

Statistical Analysis

The data obtained from this study were analyzed using Statistic Analysis System (SAS) 9.0 applications. Analytical method with Analysis of Variance (ANOVA) significance level of 5%. Means comparison between treatments was tested using Duncan Multiple Range Test (DMRT) at 5%.

RESULTS AND DISCUSSIONS

The results showed that foliar application concentration of OPEFB nanoparticles did not significantly affect the stomatal length during the vegetative phase and tuber ripening phase, but there was a significant difference observed in the tuber initiation phase (Table 1). Further test results showed that foliar application of OPEFB ash nanoparticles at a concentration of 0% produced the longest stomata of 50.78 µm. It was significantly different compared to that in the 0.4% concentration treatment.

Based on these results (table 1), the stomatal length is included in the very long category >25 μ m (Makin et al., 2022). Foliar application of OPEFB nanoparticles at a concentration of 0% resulted in the longest stomatal length compared to that at a concentration of 0.4%. OPEFB ash nanoparticles The data collected include stomatal length, contain potassium, which can maintain cell turgidment pattern is referred to as radial mycelation. The shape of the pattern allows only the long stretching of the cellulose microfibrils, but the two ends of the guard cells stick together so that when the cellulose microfibrils elongate, the thick abdominal wall limits the stretching. As a result, guard cells will bend and open, which affects the stomatal width instead of the stomatal length (Pautov et al., 2018). The stomatal length is in a fixed state when the stomatal aperture is based on the hardening of the stomatal poles, and polar clamping occurs (Carter et al., 2017). Thus, the stomatal length is related to the stomatal width, where the process becomes a single entity that affects the size of the stomatal porous. The longer cellulose microfibril

Foliar application - concentration	Stomatal Length (μm)			
	Vegetative Phase (40 day)	Bulb Initiation Phase (65 day)	Tubers Ripening Phase (75 day)	
0 %	44.66 ± 5.55 a	50.78 ± 8.80 a	47.03 ± 5.66 a	
0.1 %	40.40 ± 4.89 a	40.83 ± 3.97 b	42.85 ± 4.09 a	
0.2 %	40.34 ± 6.34 a	39.69 ± 3.56 b	42.44 ± 8.70 a	
0.3 %	38.76 ± 4.23 a	± 4.23 a 47.00 ± 7.15 ab 51.6		
0.4 %	41.40 ± 6.12 a	40.83 ± 4.20 b	50.08 ± 5.35 a	
CV	12.80	11.95	13.93	

Table 1. Stomatal length (µm) as affected by various concentrations of OPEFB ash nanoparticles under different phases

Remarks: Means followed by the same letters are not significantly different based on the DMRT test at the level of 5%

	Aperture Stomatal (μm)			
Foliar application concentration	Vegetative Phase (40 day)	Bulb Initiation Phase (65 day)	Tubers Ripening Phase (75 day)	
0 %	2.86 ± 0.61 a	2.76 ± 0.33 a	2.54 ± 0.41 ab	
0.1 %	2.76 ± 0.43 a	2.77 ± 0.59 a	2.23 ± 0.46 b	
0.2 %	2.50 ± 0.42 a	2.24 ± 0.59 a	2.37 ± 0.45 b	
0.3 %	3.12 ± 0.10 a	2.76 ± 0.48 a	3.03 ± 0.75 a	
0.4 %	2.71 ± 0.50 a	2.45 ± 0.65 a	2.82 ± 0.32 ab	
CV	15.70	21.58	16.60	

Table 2. Stomatal aperture (µm) as affected by various concentrations of OPEFB ash nanoparticles under different phases

Remarks: Means followed by the same letters are not significantly different based on the DMRT test at the level of 5%

will experience withdrawal due to the widening of that the foliar treatment of OPEFB ash nanoparthe guard cells outwards.

creased stomatal length.

tration of 0%.

Based on the results of the analysis, it was shown stomatal guard cells, where there are cell organelles

ticles had an effect on the stomatal aperture. This There was no significant effect of OPEFB ash was because the stomatal aperture occurred as a renanoparticle concentration on the stomatal length sult of activity in guard cells that require potassium because the higher the concentration of OPEFB to maintain turgor pressure so that the stomatal are nanoparticle ash, the smaller the stomatal length. open. According to <u>Barita et al., (2018)</u>, potassium Lu et al., (2017) reported that K deficiency de- plays a role in stimulating water absorption, thereby affecting the increase in cell turgor pressure; if the Based on Table 2, the concentration of OPEFB high cell turgor pressure is maintained, the stomata ash nanoparticles did not significantly affect the can be maximally open wider and longer. Potassium stomatal aperture in the vegetative and tuber initia- has a role in the process of opening and closing tion phase, but there was significant effect in the of stomata, which is influenced by several factors, tuber ripening phase. Further test results showed namely the mechanism of turgor, the presence of that foliar application of OPEFB ash nanoparticles osmotic pressure, accumulation of potassium ions, at a concentration of 0.3% had the largest stomatal accumulation of abscisic acid, and environmental aperture of 3.03 µm, but it was not significantly factors, such as sunlight, temperature, humidity different from the stomatal aperture at a concen- and CO₂ concentration (<u>Ratnasari et al., 2020</u>). The opening of stomata results from activity in the

Foliar application concentration	Guard Cell Width (μm)			
	Vegetative Phase (40 day)	Bulb Initiation Phase (65 day)	Tubers Ripening Phase (75 day)	
0 %	11.30 ± 1.54 a	10.75 ± 1.86 a	8.03 ± 1.74 b	
0.1 %	11.46 ± 1.40 a	9.51 ± 2.04 a	8.79 ± 1.80 b	
0.2 %	10.75 ± 2.33 a	9.65 ± 1.27 a	8.97 ± 2.12 b	
0.3 %	10.81 ± 1.80 a	10.66 ± 0.57 a	8.26 ± 1.24 b	
0.4 %	12.66 ± 1.88 a	9.47 ± 1.74 a	11.26 ± 1.51 a	
CV	15.81	15.57	14.65	

Table 3. Guard cell width (µm) as affected by concentrations of OPEFB ash nanoparticles under different phases

Remarks: Means followed by the same letters are not significantly different based on the DMRT test at the level of 5%

in these guard cells.

tal opening are vacuoles, which play a role in cell foliar application of OPEFB ash nanoparticles at turgidity and shape. Stomata will open if the two guard cells experience increased cell turgor pressure. Turgor pressure is a condition where the cell expands because water from the surrounding cells enters it. Water movement is influenced by water OPEFB ash nanoparticles affected the width of the potential, in which high water potential will go to cells with lower water potential. For stimulating water to enter the guard cell, the solute in the cell must be increased. According to <u>Abidin (2022</u>), the main solutes that mediate cell osmoregulation are K⁺ and sucrose because of their high mobility of K⁺ and solubility. The guard cells accumulate large amounts of K+ in the vacuole. Accumulating K+ in the vacuole against the electrochemical gradient (Lu et al., 2017) produces sufficient turgor for stomatal opening.

Foliar application of OPEFB ash nanoparticles at a concentration of 0.3% significantly affected the stomatal pore opening of potato plants. Likewise, according to Lu et al., (2017, an increase in potassium concentration to 0.12% showed a significant effect on the stomatal opening of Brassica napus leaves.

did not significantly affect the width of guard cells (Advinda, 2018). Guard cells will also increase the in the vegetative and tuber initiation phase, but a osmotic potential of their cells, thereby increasing

significant effect was observed in the tuber ripening Cell organelles that play an active role in stoma- phase (Table 3). Further test results showed that a concentration of 0.4% had the highest guard cell width of 11.2660 µm compared to other OPEFB ash nanoparticle concentrations.

The results showed that the foliar application of guard cells. This is because the content of OPEFB ash nanoparticles in the form of potassium can maintain the turgidity of the vacuole cells in the guard cells, where the guard cells can change shape and size, which is reversible. The mechanism of guard cell dilation occurs due to changes or regulation of turgor, which is influenced by the theory of K ion movement or pump. The leaves absorb potassium by diffusion through ion exchange. According to Jasmi (2018), the main function of K is to activate enzymes and maintain cell water. K⁺ ions support the activity of phosphorylase enzymes, which play a role in converting starch into glucose. Glucose plays a role in the osmotic potential of cells, which will move water to guard cells. As a result, the turgor pressure of the guard cells increases, and the stomata open by dilating the guard cells. Thus, when K⁺ ions increase in guard cells, the ac-Foliar application of OPEFB ash nanoparticles tivity of converting starch to glucose also increases

	Stoffiatal defisity (fiffit-)			
Foliar application concentration	Vegetative Phase (40 day)	Bulb Initiation Phase (65 day)	Tubers Ripening Phase (75 day)	
0 %	486.8 ± 97.31a	308.89 ± 44.02 b	411.11 ± 82.03 a	
0.1 %	442.2 ± 89.72a	331.11 ± 69.12 ab	468.89 ± 70.89 a	
0.2 %	431.2 ± 187.81a	428.89 ± 63.64 a	382.22 ± 94.80 a	
0.3 %	419.8 ± 136.40a	362.22 ± 64.12 ab	364.44 ± 101.95 a	
0.4 %	406.8 ± 150.43a	426.66 ± 90.13 a	326.66 ± 51.88 a	
CV	5.61	18.61	21.95	

Table 4. Stomatal density (mm⁻²) as affected by various concentrations of OPEFB ash nanoparticles under different phases

Stomatal density (mm⁻²)

Remarks: Means followed by the same letters are not significantly different based on the DMRT test at the level of 5%

	Leaf Cell Wall Thickness (µm)			
Foliar application concentration	Vegetative Phase (40 day)	Bulb Initiation Phase (65 day)	Tubers Ripening Phase (75 day)	
0 %	5.23 ± 0.38 a	5.54 ± 0.06 a	4.98 ± 0.51 a	
0.1 %	5.17 ± 0.21 a	5.33 ± 0.32 a	5.04 ± 0.32 a	
0.2 %	5.19 ± 0.31 a	5.47 ± 0.10 a	5.32 ± 0.15 a	
0.3 %	5.11 ± 0.12 a 5.37 ± 0.28 a		5.41 ± 0.16 a	
0.4 %	5.53 ± 0.62 a	5.63 ± 0.32 a	5.50 ± 0.13 a	
CV	7.54	4.82	5.77	

Table 5. Cell wall thickness (μ m) as affected by various concentrations of OPEFB ash nanoparticles under different phases

Remarks: Means followed by the same letters are not significantly different based on the DMRT test at the level of 5%

turgor pressure and forming guard cell dilation.

curs because the back cell wall is thin and elastic. protruding away from the opening, while the front cell wall will be straight or concave, the entire cell nanoparticles has not a significant effect on the will appear bent, and openings with an increased size are formed (Roux & Leonhardt, 2018). When the guard cells widen, the metabolic activity in the cell will also be easy with the accumulation of the foliar application of OPEFB ash nanoparticles ions or materials needed in the process. Thus, this at a concentration of 0.2% had the highest stomatal mechanism proves that it can affect cell activities density of 428.89 mm⁻², but was not significantly such as photosynthesis, respiration, transpiration, different compared to that at the concentrations and another cell metabolism.

at a concentration of 0.4% significantly affected concentration treatment. the width of the stomatal guard cells in potato

the application of potassium fertilizer significantly This change in the shape of the guard cells oc- affected the width of the stomatal guard cells on Brassica napus leaves.

In table 4, the foliar application of OPEFB ash stomatal density in vegetative and ripening phases of tubers, but a significant effect was found in tuber initiation phase. Further test results showed that of 0.4%, 0.3% and 0.1%. Meanwhile, the 0.4% Foliar application of OPEFB ash nanoparticles concentration differed significantly from the 0%

Based on the results, concentration of 0% and leaves. Research by Lu et al., (2017) showed that 0.4% had significantly different results, and the

	1	8	3

	Leaf Cell Area (µm²)			
Foliar application concentration	Vegetative Phase (40 day)	Bulb Initiation Phase (65 day)	Tubers Ripening Phase (75 day)	
0 %	3.05 ± 0.15 a	3.25 ± 0.08 a	3.22 ± 0.41 a	
0.1 %	3.32 ± 0.16 a	3.30 ± 0.18 a	3.18 ± 0.21 a	
0.2 %	3.32 ± 0.09 a	3.68 ± 0.82 a	3.27 ± 0.26 a	
0.3 %	3.36 ± 0.26 a	3.44 ± 0.14 a	3.05 ± 0.32 a	
0.4 %	3.15 ± 0.09 a	3.11 ± 0.23 a	3.29 ± 0.23 a	
CV	5.33	12.27	7.91	

Table 6. Leaf cell area (μ m²) as affected by various concentrations of OPEFB ash nanoparticles under different phases

Remarks: Means followed by the same letters are not significantly different based on the DMRT test at the level of 5%

average stomatal density was classified as moderate, which was in the range of 300 - 500/mm² (Claudia had no significant effect on cell wall thickness (Taet al., 2020). This is because in general, stomatal ble 5). Based on the analysis results, the responses density is related to stomatal size. High potassium of leaf cells to the foliar application of OPEFB ash concentrations can widen stomata and cause sto- nanoparticles were not significantly different. This matal density to be quite high. According to Siho- result is because potassium only affects the activity tang (2017), if the size of the stomatal is larger, the of the phosphorylase enzyme in stomatal guard distance between the stomatal gets further by 20 cells that are not related to leaf cells. However, times its diameter so that the evaporation process Widiyawati (2019) mentioned that thickening of can take place optimally. This is evidenced by the the epidermal tissue was a structural defense rewidth of the stomatal guard cells resulting from sponse of plants against pathogen attacks. Epiderthe foliar application of OPEFB ash nanoparticles mal cells are the outermost cell network as a place at a concentration of 0.4%, showing the highest of penetration of pathogens. Structural defenses value compared to other concentrations (Table 4). when attacked by pathogens include thickened Stomatal density affects two important processes in epidermal cell structures that affect the stomatal plants, namely transpiration and photosynthesis. surface and thickened cell walls to inhibit patho-Plants with high stomatal density have a higher gen penetration so that pathogens do not damage transpiration rate than plants with low density. deeper cell layers. However, in this case, the foliar Because more stomata per unit area mean more application of OPEFB ash nanoparticles did not CO₂ can be taken in and more water can be re- affect the cell walls' thickness, so pathogens would leased. (Mercyana et al., 2021). Foliar application easily attack potato plants due to lack of protection. of OPEFB ash nanoparticles at various concen- The results also showed that the foliar application trations of potassium significantly affected the of OPEFB ash nanoparticles on potato plants stomatal density of potato plants. Pratama et al., showed no significant effect on the thickness of (2020) reported that the application of potassium the leaf epidermal cells. Likewise, research of Lu fertilizer at a concentration of 0.3% significantly et al., (2016) reported that cell wall thickness was affected the stomatal density of oil palm plants not affected by K nutrition. experiencing drought stress.

Foliar application of OPEFB ash nanoparticles

Based on Table 6, foliar application of OPEFB

ash nanoparticles had no significant effect on potato leaf cell area. This is because the leaf cells had enough water when taking sample in the morning. According to Saragih dan Ardian (2017), the content of OPEFB in the form of potassium can affect the optimal leaf cell area if the condition of the plant lacks water. Cell enlargement will also be hampered due to a decrease in the rate of photosynthesis because in these conditions, there is a decrease in the availability of nutrients, inhibition of protein synthesis so that the leaf area also decreases. Potassium will play a role in regulating the availability of sufficient water for cell enlargement. Enlargement of leaf cells becomes inhibited if the water content is low due to the need for turgor pressure for cell enlargement. The results of photosynthesis support the work of plant tissue cells in differentiation so that it will accelerate the growth and development of the plant, forming parts such as leaves. But in this case, the high potassium treatment did not affect the leaf area of potato plants. Foliar application of OPEFB ash nanoparticles on potato plants did not significantly affect the leaf cell area. However, Lu et al., (2016) reported that the leaf area was significantly down-regulated under K deficiency conditions.

CONCLUSIONS

Based on this research, it can be concluded that foliar application of oil palm empty fruit bunch ash nanoparticles on potato plants can affect stomatal anatomy, including the width of stomatal guard cells, stomatal opening (aperture), and stomatal density. Meanwhile, the application did not affect the anatomy of potato plant leaf cells. Also, foliar application of oil palm empty fruit bunch ash nanoparticles at a concentration of 0.3% is the most effective in increasing the stomatal aperture because it affects the activity of the photosynthetic process.

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