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# Testing Two *Trichoderma* Biofertilizer Formulas on Soybean Plants Using Saline Soil as a Growing Medium

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**Abstract.** This study aims to examine the effect of Trichoderma biofertilizer formulated with husks or biochar applied to the soil as fertilizer and biofertilizer sprayed on the canopy surface on the vegetative growth of soybean plants in saline soil. The study was designed as a factorial design with a randomized block design (RBD). The first factor was the application of biofertilizer as soil fertilization, consisting of: no biofertilizer (NPK fertilization) and biofertilizer with husk and biochar as carriers. The second factor was the spraying of biofertilizer on the canopy, consisting of: no spraying and spraying. Data on height, stem diameter, number of leaves, and time of flower emergence were analyzed using 5% ANOVA followed by 5% BNJ test. There was a significant interaction effect on plant height, stem diameter, and number of leaves at 42 and 56 days after planting. The combination of Trichoderma biofertilizer with husk carrier and canopy spraying produced the highest plant response in terms of plant height (53.19±3.51 cm), stem diameter (0.53±0.02 cm), and number of leaves (9.50±0.65 leaves), as well as earlier flowering (37.50±0.33 days). The application of Trichoderma biofertilizer formulated with rice husk as a carrier has the potential to be used in fertilizing soybean plants grown in saline soil.

**Keywords:** biochar, soybean, rice husk, trichoderma

## 1 Introduction

The agricultural sector in Indonesia is an important sector in national economic growth. Agriculture contributes to the products produced as its contribution to the Gross Domestic Product (GDP) and market contribution. In addition, agriculture also provides for human food needs amid the current increase in population. In this agrarian country, the agricultural sector is certainly one of the livelihoods of the community, so there needs to be significant development in this field.However, agricultural products are often rejected or undervalued by the agricultural processing industry due to high pesticide content or even poor quality. In fact, pesticide content is also a result of the upstream sector (factories) promoting their products for use by farmers to combat pests and diseases.

Soybeans (*Glycine max* L. Merril) are a source of vegetable protein, the supply of which still depends on imports. According to the Central Statistics Agency, in the last five years, soybean production in Indonesia has tended to increase by 2.49% (BPS, 2015) [1], but this increase in production has not been able to keep up with the rate of consumption. In the last five years, Indonesia has imported at least two million tons of soybeans per year from abroad to meet domestic consumption needs.

To meet domestic demand in an effort to achieve soybean self-sufficiency, not only improvements in cultivation technology are needed, but also the expansion of soybean production areas, including the utilization of marginal dry land, such as the extensive saline land available in Indonesia, particularly in East Java, specifically in the districts of Lamongan and Tuban [2].

In general, soybeans cannot grow and produce well in saline soils. Therefore, in order to optimize the availability of marginal dry land to meet the need for increased soybean production, several studies have been conducted to find soybean varieties that are tolerant to salinity stress. The Anjasmoro variety is expected to have better resistance than other varieties in overcoming soil salinity stress [Directorate General of Food Crops, 2011) [3].

The use of varieties that are expected to be able to overcome salinity stress and be resistant to pests and diseases is not sufficient for the successful utilization of marginal land for soybean cultivation; soil fertility engineering is also required, including the use of effective microbes that can help plants overcome salinity stress while also helping to provide nutrients for the plants.

Certain isolates *of Trichoderma* sp. are a type of biofertilizer agent fungus that is believed to be able to help

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plants overcome salinity stress. This fungus is able to live optimally even in high salinity levels; with its ability to supply nutritional and secondary metabolic needs that can be utilized by plants as growth-regulating compounds [4], *Trichoderma* sp. has the potential to help soybean plants overcome salinity stress.

In testing the response of plant varieties to environmental stress treatments, including salinity, it is estimated that there are varieties that cannot survive to the final phase or harvest. Therefore, testing is only required until the end of the vegetative phase. Successful survival until the end of the vegetative phase ensures that soybean plants will successfully reach the final production phase [5].

Advances in science and technology, particularly in the field of biology, have led to the discovery of fungal species that can be used as biological control agents, one example being Trichoderma sp. This fungus is known to attack other fungi, interact both parasitically and symbiotically, produce antibiotics that can affect other microbes, and act as a biocontrol agent [6].*Trichoderma* consists of a large genus with a number of rhizocompetent filamentous fungal strains found in a wide variety of ecosystems. This fungus is mostly isolated from forests or agricultural soils at all latitudes and can be easily cultivated *in vitro* [7].

*Trichoderma* is a soil saprophytic fungus that naturally attacks pathogenic fungi and is beneficial to plants. The mechanisms of *Trichoderma* activity in the soil are (1) competition for space and nutrients, (2) antibiosis, where it releases ethanol that is toxic to pathogens, and (3) acting as a parasitic fungus that can inhibit the activity of pathogenic fungi [8].

The effectiveness of *Trichoderma* sp. as a biological agent has been widely reported, such as in the results of a study [9] which found that it can suppress the activity of the *Fusarium oxysporum* pathogen, which causes plant wilting, in the soil. In addition to being a biological agent, *Trichoderma* species can act as decomposing organisms and plant growth stimulators. Trichoderma can also be formulated into natural fertilizers and pesticides [10] that are beneficial for plant protection and increasing plant resistance to pests and pathogens.

Efforts to increase high soybean production are now being intensified by the government and all stakeholders, which of course must utilize various technologies in cultivation, including the use of *Trichoderma* sp biofertilizer, which can also act as a biological agent. However, a common obstacle is the limited budget to provide the media for mass propagation. Therefore, a new alternative medium is needed that can be used as a culture medium that is low in economic value, sufficiently nutritious, effective, easily obtainable, abundant in raw materials, and can be utilized by *Trichoderma* sp. to grow and develop.

From the brief review above, researchers used several alternative media, namely biochar and husks, to evaluate the media with the best effectiveness in propagating *Trichoderma* sp. In addition to determining how much *Trichoderma* sp population was produced from the media used.

Based on the above background, the following problems can be identified:

a. How effective are biochar and husk media in propagating *Trichoderma* sp., as indicated by the growth of the conidiospore population?
b. To what extent does the application of Trichoderma biofertilizer formulated from different propagation sources in different application methods affect the growth and production of Anjasmoro soybean varieties grown in saline soil media?

This study focuses on how efficient saline soil media formulated with biochar and husks are as a support for the propagation of *Trichoderma sp.*, which can affect soybean plant growth, where this study is intended to optimize soybean plant growth in media with high salinity stress.

The objectives of this study are to:

a. To determine the effectiveness of biochar and husk media as carriers of *Trichoderma* sp. biofertilizer on the vegetative growth of Anjasmaoro soybean plants grown in saline soil media.
b. To determine the effect of Trichoderma biofertilizer spraying on the vegetative growth of Anjasmaoro soybean varieties grown in saline soil media;
c. To determine the effect of the interaction between the carrier material and Trichoderma biofertilizer spraying on the vegetative growth of Anjasmaoro soybean varieties grown in saline soil.

# 2 Method

## 2.1 Time and Place of Implementation

This study was conducted in Wonokasian Village, Wonoayu District, Sidoarjo Regency, from June 2024 to August 2024.

## 2.2 Equipment and Materials

The equipment needed for this study included an analytical balance, autoclave, oven, Neubauer improved hemocytometer, 1000 ml Erlenmeyer flask, 500 ml Erlenmeyer flask, 500 ml beaker, 500 ml measuring cup, Petri dish, spatula, volumetric pipette, test tube, reaction tube, laminar flow, Bunsen burner, alcohol, aluminum foil, cotton, distilled water, plastic wrap, 1 ml syringe, magnetic stirrer, vortex, cover glass, and L-glass. For

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propagation, an 8-liter plastic container, plastic tubing, plastic bottles, and an aerator with a capacity of 5 watts per unit were used.

For the Trichoderma biofertilizer application experiment, Anjasmoro soybean seeds, saline soil from the Jabon pond embankment, and Trichoderma formula were used.

## 2.3 Experiment Implementation

After completing the Trichoderma propagation stage and mixing the growing medium, the application experiment was carried out. For this purpose, the application method was prepared.

There are two types of Trichoderma application, namely:

a. Soil fertilization. Propagated *Trichoderma* is mixed with husks at a ratio of 1 liter to 5 kg. The mixture is stirred evenly and incubated for one week. Similarly, for the formula using biochar, propagated *Trichoderma* is mixed with biochar that has been previously ground in a ratio of 1 l : 5 kg. Next, from the two types of formulas, 50 grams of husk or biochar containing propagated Trichoderma is applied to each 5 kg polybag or per plant.
b. Spraying the canopy or treatment in the form of spraying the canopy with 50 ml of husk or biochar containing Trichoderma propagated for each plant was carried out four times, namely at 2, 4, 6, and 8 weeks after planting. The husks or biochar containing Trichoderma were first soaked for six hours and stirred evenly, then filtered and ready to be sprayed using a hand sprayer;

Each polybag was filled with 5 kg of soil and each was given 180 g of cow manure per polybag, which was mixed evenly before being put into the polybag. Except for the planting media in polybags that were treated with Trichoderma biological fertilizer using either biochar or husk as a carrier, each polybag was given chemical fertilizer with a total of 0.45 g/plant of urea (N), 0.90/plant of SP-36 (P), and 0.67 g/plant of KCl (K).

A total of five Anjasmoro soybean seeds were placed on the surface of the growing medium in each polybag and covered lightly with soil. From the five seedlings per polybag that grew, one seedling was selected by cutting the other four seedlings; then they were maintained until the end of the study. For polybags treated with biofertilizer in the planting medium soil, Trichoderma was added with husk carrier and biochar at a rate of 50 g per polybag. The active spore content of Trichoderma sp. fungi as the active ingredient was $10^5$ CFU/g. For plants treated with biofertilizer spraying, a liquid biofertilizer formula was prepared with the following steps: (i) soaking 100 g of Trichoderma biofertilizer ($^{10^6}$CFU/g) formulated in rice husks in 10 liters of neutral water for at least two hours, (ii) stirring evenly for 10 minutes, (iii) pour the mixture into a hand sprayer equipped with a filter at the mouth to prevent the suspension from clogging the hand sprayer nozzle, and (iv) spray the suspension onto the plant canopy surface until wet, starting at two weeks of age and repeating every two weeks.

## 2.4 Experimental Design

This factorial experiment was arranged in a Completely Randomized Design (CRD). The first factor was: the type of fertilizer in the growing medium, consisting of: (i) Trichoderma biofertilizer with husk as a carrier, (ii) Trichoderma biofertilizer with biochar as a carrier, and (iii) no biofertilizer but with NPK base fertilizer. The second factor was: application of biofertilizer to the canopy, consisting of: (i) spraying biofertilizer on the canopy, and (ii) no spraying of biofertilizer on the canopy.

The combination of these two factors resulted in the following treatments:

a. Without Trichoderma biofertilizer, either as soil fertilizer or foliar spray, but with complete NPK fertilizer (P0A0) applied;
b. Trichoderma biofertilizer with husk as a carrier and without biofertilizer spraying (P1A0);
c. Trichoderma biofertilizer with biochar as a carrier and without biofertilizer spraying (P2A0);
d. Without Trichoderma biofertilizer as soil fertilizer but with complete NPK fertilizer and Trichoderma biofertilizer sprayed on the canopy (P0A1);
e. Trichoderma biofertilizer with husk carrier and Trichoderma biofertilizer spraying on the canopy (P1A1);
f. Trichoderma biofertilizer with biochar as a carrier and Trichoderma biofertilizer sprayed on the canopy (P2A1);

Each treatment was repeated four times, resulting in 24 experimental units. Each experimental unit consisted of one polybag containing one Anjasmoro soybean plant.

## 2.5 Observation Variables

a. Plant height (cm), measured from the ground to the top of the plant every two weeks until the end of the vegetative period;
b. Stem diameter, measured at a point one cm above the soil every two weeks until the end of the vegetative period;

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c. Number of perfect leaves (pieces), counted as the number of perfect leaves on the sample plant. The criteria for perfect leaves on soybean plants are that there are 3-4 leaves on each stem, which are pointed and round or oval in shape. Fallen leaves are not counted;
d. Average time (days) for the appearance of fully developed flowers;

### 2.6 Data Analysis

For weekly population average data, the average of five observed sample copies is calculated to obtain the population average and its error value. For experimental data, analysis is performed using ANOVA at a 5% significance level to determine the presence of differences (answering the hypothesis), followed by a 5% Duncan test to determine the differences between treatments.

## 3 Results And Discussion

### 3.1 Plant height

The results of the analysis of variance showed that the application of Trichoderma biofertilizer to the soil as fertilizer and to the canopy as a spray had no significant effect ($p>0.05$) on the height of Anjasmoro soybean plants 14-56 days after planting (DAP). Meanwhile, the interaction effect was only significant ($p<0.01$) at 56 DAP. The average effect of Trichoderma biofertilizer application in the soil and on the canopy on the height of Anjasmoro soybean plants 14-56 DAP is presented in Table 1.

**Table 1**. Average effect of Trichoderma Biofertilizer Application in Soil and Canopy on Soybean Plant Height of the Anjasmoro Variety 14-56 Days After Planting (DAP) (cm)*

| Treatment | Observation time (HST) | | | |
|---|---|---|---|---|
| | 14 | 28 | 42 | 56 |
| P0S0 | 19.85±1.06 | 32.57±2.78 | 44.04±4.31 | 52.09±2.59 ab |
| P1S0 | 22.21±1.26 | 29.16±1.89 | 36.82±2.95 | 41.70±2.39 a |
| P2S0 | 20.36±1.37 | 31.08±2.08 | 43.41±5.51 | 52.67±1.81 ab |
| P0S1 | 20.09±1.48 | 31.95±1.87 | 43.44±1.46 | 50.29±1.24 ab |
| P1S1 | 23.26±0.52 | 33.52±2.05 | 46.68±5.14 | 53.19±3.51 b |
| P2S1 | 20.18±1.89 | 29.21±2.50 | 39.18±3.24 | 45.99±3.06 ab |
| **BNJ 5%** | **-** | **-** | **-** | **12.30** |

* P0: without biofertilizer, P1: husk biofertilizer, P2: biochar biofertilizer, S0: without biofertilizer but given NPK, and S1: biofertilizer spraying on the canopy without NPK. Numbers followed by different letters in the same column indicate different effects on the 5% BNJ test.

The application of husk formula to Tichoderma biofertilizer combined with spraying a suspension derived from the dilution of biofertilizer formulated in coarse husk flour (P1S1) showed the highest plant response and was able to exceed the response of plants given NPK fertilizer according to general recommendations. This indicates that this combination of treatments provides optimal nutrition and Trichoderma function in meeting plant needs [11, 12]. Trichoderma induces plants to respond to resources in their growing environment, as demonstrated by the optimal elongated growth of soybean plants [13].

### 3.2 Stem Diameter

The application of Trichoderma biofertilizer to the soil as fertilizer had a significant effect ($p<0.05$) on the stem diameter of soybean plants at 42 and 56 days after planting (DAP). The average effect of biofertilizer application at 14-56 DAP is presented in Table 2.

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**Table 2.** Average Effect of Trichoderma Biofertilizer Application in the Soil on the Stem Diameter of Anjasmoro Soybean Plants 14-56 Days After Planting (DAP) (cm)*

| Treatment | Observation time (DAP) | | | |
|---|---|---|---|---|
| | 14 | 28 | 42 | 56 |
| Without biofertilizer, with NPK (P1) | 0.29±0.01 | 0.36±0.01 | 0.42±0.02 ab | 0.52±0.02 b |
| Biofertilizer husk (P1) | 0.30±0.01 | 0.37±0.01 | 0.44±0.01 b | 0.48±0.02 ab |
| Biofertilizer biochar (P2) | 0.29±0.02 | 0.35±0.01 | 0.40±0.01 a | 0.45±0.02 a |
| **BNJ 5%** | **-** | **-** | **0.04** | **0.05** |

* P1 and P2 were not given NPK; numbers followed by different letters in the same column indicate different effects on the 5% BNJ test

Foliar spraying with biofertilizer had no significant effect (*p*>0.05) on stem diameter, but its interaction with the application of biofertilizer in the soil had a significant effect on stem diameter at 42 and 56 days after planting. Table 3 shows the average effect of the interaction between the application of Trichoderma biofertilizer in the soil and spraying at 14-56 days after planting.

**Table 3.** Average effect of Trichoderma biofertilizer application in the soil and on the canopy on the stem diameter of Anjasmoro soybean plants 14-56 days after planting (DAP) (cm)*

| Treatment | Observation time (HST) | | | |
|---|---|---|---|---|
| | 14 | 28 | 42 | 56 |
| P0S0 | 0.29±0.01 | 0.37±0.02 | 0.44±0.02 ab | 0.52±0.02 b |
| P1S0 | 0.29±0.02 | 0.35±0.01 | 0.41±0.02 ab | 0.43±0.02 a |
| P2S0 | 0.29±0.03 | 0.37±0.02 | 0.42±0.02 ab | 0.46±0.02 ab |
| P0S1 | 0.29±0.02 | 0.35±0.01 | 0.41±0.02 ab | 0.52±0.03 b |
| P1S1 | 0.30±0.01 | 0.39±0.02 | 0.47±0.01 b | 0.53±0.02 b |
| P2S1 | 0.29±0.03 | 0.34±0.01 | 0.38±0.01 a | 0.44±0.02 a |
| **BNJ 5%** | **-** | **-** | **0.07** | **0.08** |

P0: without biofertilizer, P1: husk biofertilizer, P2: biochar biofertilizer, S0: without biofertilizer but given NPK, and S1: biofertilizer spraying. Numbers followed by different letters in the same column indicate different effects on the 5% BNJ test

Unlike plant responses in the form of plant height, which showed differences in effect at the end of the observation period, in terms of responses in the form of stem diameter, the effect was evident from 42 days after sowing. This also shows the role of Trichoderma in helping plant growth [14].

## 3.3 Number of leaves

Based on the analysis of variance, it was found that the application of Trichoderma biofertilizer in the soil had no significant effect (*p*>0.05) on the number of soybean leaves, but foliar spraying had a significant effect (*p*<0.05) on the number of leaves at 42 DPA. Meanwhile, the interaction effect of the two factors was significant at 42 and 56 DPA.

The average effect of Trichoderma biofertilizer spraying on the number of leaves at 14-56 days after planting is shown in Table 4, while the interaction effect is presented in Table 5.

**Table 4.** Average Effect of Trichoderma Biofertilizer Spraying on The Canopy on the Number of Soybean Leaves of the Anjasmoro Variety 14-56 Days After Planting (DAP) (cm)*

| Treatment | Observation time (DAP) | | | |
|---|---|---|---|---|
| | 14 | 28 | 42 | 56 |
| Without biofertilizer, given NPK (S0) | 2.67±0.14 | 4.33±0.19 | 6.75±0.57 b | 9.08±0.57 |
| Biofertilizer spraying without NPK (S1) | 2.75±0.13 | 4.17±0.17 | 5.50±0.47 a | 7.92±0.42 |
| **BNJ 5%** | **-** | **-** | **1.06** | **-** |

*Numbers followed by different letters in the same column indicate different effects in the 5% BNJ test

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**Table 5.** Average effect of Trichoderma biofertilizer application on soil and canopy on the number of soybean leaves of the Anjasmoro variety 14-56 days after planting (DAP) (cm)*

| Treatment | Observation time (DAP) | | | |
|---|---|---|---|---|
| | 14 | 28 | 42 | 56 |
| P0S0 | 2.50±0.29 | 4.75±0.25 | 8.75±0.63 ab | 10.25±0.25 b |
| P1S0 | 2.75±0.25 | 3.75±0.25 | 4.75±0.48 a | 7.50±0.29 a |
| P2S0 | 2.75±0.25 | 4.50±0.29 | 6.75±0.48 ab | 9.50±0.29 b |
| P0S1 | 2.75±0.25 | 4.25±0.25 | 4.75±0.25 a | 7.00±0.41 a |
| P1S1 | 3.00±0.00 | 4.50±0.29 | 6.75±0.48 ab | 9.50±0.65 b |
| P2S1 | 2.50±0.29 | 3.75±0.25 | 5.00±0.41 a | 7.25±0.25 a |
| **BNJ 5%** | **-** | **-** | **2.22** | **2.02** |

P0: without biofertilizer, P1: husk biofertilizer, P2: biochar biofertilizer, S0: without biofertilizer but given NPK, and S1: biofertilizer spraying. Numbers followed by different letters in the same column indicate different effects on the 5% BNJ test

As shown in Tables 4 and 5, it appears that the optimal plant response is for soybean growth. Trichoderma provides nutrients from the decomposition of organic matter and extracellular compounds that induce plant growth [15].

### 3.4 Flowering time

The application of biofertilizer as soil fertilization had a significant effect ($p<0.05$) on the average number of days until flowering. Spraying biofertilizer on the canopy surface had no significant effect ($p>0.05$) on the average number of days to flower emergence, nor did its interaction with soil application of biofertilizer have a significant effect ($p>0.05$). The average effect of Trichoderma biofertilizer application in the soil and its interaction with canopy spraying are shown in Tables 6 and 7, respectively.

**Table 6.** Average Effect of Trichoderma Biofertilizer Application in the Soil on the Average Number of Days Until Flowering of Soybean Plants of the Anjasmoro Variety 14-56 Days After Planting (DAP) (Days)*

| Treatment | |
|---|---|
| Without biofertilizer and NPK application (P0) | 36.88±0.30a |
| Biofertilizer husk (P1) | 37.50±0.33 ab |
| Biochar biofertilizer (P2) | 38.25±0.31 b |
| **BNJ 5%** | **1.01** |

* P1 and P2 were not given NPK; numbers followed by different letters in the same column indicate different effects in the 5% BNJ test

**Table 7.** Average Effect of Trichoderma Biofertilizer Application on Soil and Canopy on the Average Number of Days to Flower Emergence in Anjasmoro Soybean Variety 14-56 Days After Planting (DAP) (Days)*

| Treatment | Average |
|---|---|
| Without biofertilizer and given NPK fertilizer (P0S0) | 37.00 ±0.41 |
| Biofertilizer applied to soil and NPK fertilizer (P1S0) | 38.00 ± 0.41 |
| Biochar biofertilizer in soil and given NPK fertilizer (P2S0) | 37.75 ± 0.25 |
| Without biofertilizer in the soil and biofertilizer spraying on the canopy (P0S1) | 36.75±0.48 |
| Biofertilizer husk in the soil and biofertilizer spraying on the canopy (P1S1) | 37.00±0.41 |
| Biochar biofertilizer in the soil and biofertilizer spraying on the canopy (P2S1) | 38.75±0.48 |

P0: without biofertilizer, P1: biofertilizer husk, P2: biofertilizer biochar, S0: without biofertilizer but given NPK, and S1: biofertilizer spraying

The response of plants fertilized with Trichoderma biofertilizer formulated in rice husks and biochar showed good vegetative growth. In line with this, *Trichoderma esperellum* has been proven to be able to live and function normally in saline soil [16]. Trichoderma is capable of degrading organic matter and providing nutrients for plants, as well as protecting plant health from various biotic and abiotic environmental stresses [17].

## 4 Conclusion

Soil fertilization with Trichoderma biofertilizer had no significant effect on plant height, but had a significant effect on stem diameter and stem diameter at 42 and 56 days after planting, and the number of days until the appearance of perfect flowers. Meanwhile, spraying Trichoderma biofertilizer on the canopy had no significant effect on plant height and stem diameter, but had a significant effect on the number of leaves at 42 days after planting in saline soil.

The interaction between Trichoderma biofertilizer application to the soil and biofertilizer spraying on leaves had a significant effect on plant height, stem diameter, and number of leaves at 42 and 56 days after planting, but had no significant effect on the number of days to flower emergence in saline soil. The combination of Trichoderma biofertilizer application with husk carrier material and canopy spraying produced the highest plant response in terms of plant height (53.19±3.51 cm), stem diameter (0.53±0.02 cm), and number of leaves (9.50±0.65 leaves), as well as faster flowering by 37.50±0.33 days.

The application of Trichoderma biofertilizer formulated with rice husk carrier material has the potential to be used in fertilizing soybean plants grown in saline soil media.

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