**Enhancing Groundnut's resistance to Cercospora leaf spot by application of bio-agent and essentials oils**

**Abstract**

Groundnut, also known as peanut, is a vital oilseed crop in India. It plays a crucial role in the country's agriculture due to its diverse uses—serving as a source of edible oil, protein-rich food, and high-quality fodder from its foliage or haulm. India holds the distinction of having the largest area under groundnut cultivation globally and ranks second in total production. However, groundnut productivity is frequently challenged by various diseases, with Cercospora leaf spot being one of the most damaging. This disease, caused by the fungal pathogens Cercospora arachidicola and Cercospora personatum, leads to premature leaf fall, reduced photosynthesis, and significant yield losses in both Indian and international groundnut-growing regions. To explore sustainable and eco-friendly disease control options, a field study was conducted during the ***Kharif* season of 2023** to evaluate the effectiveness of biological and natural treatments against Cercospora leaf spot. The experiment tested various treatments and their impact on plant growth and yield under real field conditions. Among all the treatments, **T4 (Seed Treatment with** Pseudomonas fluorescens **+ Foliar Spray with Neem Oil)** showed the most promising results, **Plant Height** showed significant growth, with heights reaching **20.36 cm at 30 days, 33.41 cm at 60 days,** and **42.40 cm at 90 days** after sowing. **Number of Branches** notable increase was observed in branching, with **5.53 branches at 30 days, 6.06 at 60 days**, and **6.13 at 90 days**, indicating improved vegetative vigor and canopy development **Pod Yield** Most importantly, the T4 treatment led to a significant increase in yield, recording **1.88 tonnes per hectare,** far surpassing the untreated control (T0), which exhibited much lower productivity.

**Key words:** *Cercospora arachidicola, Cercospora personatum,* cercospora leaf spot, essential oils, groundnut, neem oil, *pseudomonas fluorescens*.

1. **Introduction**

Groundnut (Arachis hypogaea L.; 2n = 4x = 40) is a globally significant leguminous oilseed crop that plays a unique and multifaceted role in agriculture and human nutrition. Cultivated across approximately 24 million hectares worldwide, groundnut is especially valued for both its high edible oil content (approximately 48–50 %) and substantial protein levels (25–28 %) in the kernels. This exceptional nutritional profile helps position groundnut among the world’s most important oilseed crops—and as the 13th most important food crop globally **(Janila *et al.,* 2013).**

According to the first advance estimates 2023-24, Government of India, *kharif* groundnut crop is estimated at 78.29 lakh tonnes as compared to 85.62 lakh tonnes in 2022-23. Among the states, Gujarat is leading in groundnut production with 36.76 lakh tonnes followed by Rajasthan (18.95 lakh tonnes), Madhya Pradesh (9.61 lakh tonnes), Tamil Nadu (4.47 lakh tonnes), Karnataka (2.57 lakh tonnes) and Telangana (0.10 lakh tonnes) **(groundnut outlook 2023)**.

Groundnut is one of the important oil seed crops in arid and semi-arid area of India. In Uttar Pradesh it is grown as rainfed crop in Eastern Uttar Pradesh and Bundelkhand region. Among all fungal diseases “Cercospoora leaf spot of groundnut” is most common disease, which has early leaf spots (ELS) and late leaf-spot (LLS) i.e., *Cercospora arachidicola*, *Cercospora person*atum is mainly prevalent during the *kharif* than *rabi* season which is imperfect stage of fungi **(Kumar *et al*., 2017)**.

The major diseases of groundnut are due to fungi, which cause seed rots and seedling diseases such as root rot, stem rot, wilts, blight, and leaf spots. Cercospora leaf spots begin to appear in one to two months old plants. Early leaf spot are sub-circular to irregular, 1 to 10 mm diameter. Lesions are commonly dark (reddish) brown to black on the upper surface and light brown on the lower leaf surface. Leaf spot on upper surface is commonly surrounded by a yellow chlorotic halo. Late leaf spot are usually smaller and more nearly circular than early leaf spot lesion, 1 to 6 mm in diameter and are commonly dark gray or black on the lower leaf surface. There is no yellow halo around them. The late leaf spot is usually more severe than early leaf spot. Late leaf spot is almost co-existent with the crop and contributes to significant loss in yield through the world **(Ramesh *et al.*, 2017)**.

Cercospoa leaf spot disease caused by fungus are the major destructive disease of groundnut and could cause a yield loss of up to 50% or more **(Izge *et al*., 2007)**. The disease infects crop directly as well as indirectly and results in huge losses due to leaf defoliation, disruption of photosynthesis process and fewer pods are smaller in size and inferior in quality. Yield losses are even more when crop was not sprayed with the chemicals and bio-agents **(Aslam *et al*., 2018)**.

**2. Materials and Methods**

The present study was conducted at Central Research Field of Sam Higginbottom University of Agriculture Technology And Sciences, Prayagraj, during the *Kharif* season of 2023. The details of the materials used and methods followed for the experiment are described as follows:

**Table 1 Details of Treatments**

|  |  |  |
| --- | --- | --- |
| **S.no.** | **Treatment No.** | **Treatment Name** |
| 1. | **T0** | Control (Untreated) |
| 2. | **T1** | Seed treatment with *Pseudomonas fluorescens* @ 5gm/kg seed + Cloveoil spray @ 1% |
| 3. | **T2** | Seed treatment with *P. fluorescens* @ 5gm/kg seed +Eucalyptus oil spray @ 1% |
| 4. | **T3** | *P. fluorescens* foliar spray @5% |
| 5. | **T4** | Seed treatment with *P. fluorescens* @ 5gm/kg seed + Neemoil spray @ 1% |
| 6 | **T5** | Seed treatment with carbendazim (12%) + mancozeb (63% WP) @ 2gm/kg seed (check) |

**2.1. Sowing of seeds**

On August 11, 2023, TMV (Gn) 13 groundnut seeds were manually sown using the line sowing method. After placing the seeds, they were immediately covered with soil to ensure proper germination. The sowing was done at a depth of 4 to 5 cm, with a spacing of 30 cm between rows and 10 cm between plants (30 × 10 cm) to allow for healthy plant growth and ease of maintenance.

**2.2 Application of bio-agent**

For seed treatment, Pseudomonas fluorescens was applied at a rate of 5 grams per kilogram of seeds to promote healthy germination and protect against soil-borne diseases.

**2.3 Application of fungicide**

For seed treatment, **Sparsh—**afungicide blend containing Carbendazim (12%) *and* Mancozeb (63%)*,* manufactured by **India Chemicals and Fertilizers,** Muzaffarnagar, Uttar Pradesh—was applied at the rate of **2 grams per kilogram of seed.** This helped protect the seeds from fungal infections during early growth stages.

**2.4 Spraying of essential oils**

A natural foliar spray was prepared by mixing **neem oil (4 ml/litre), eucalyptus oil (1 ml/litre),** and **clove oil (1 ml/litre)** in water. To ensure proper blending of the oils with water, a small amount of liquid soap was used as an emulsifier. This spray was applied **three times—**at **45, 60, and 75 days after sowing—t**o help manage pests and support plant health in an eco-friendly way.

**Application of foliar spray:**

|  |  |  |
| --- | --- | --- |
| **S. no.** | **Number of applications** | **Date of application** |
| 1 | Disease appeared on 30 DAS and first application was done after 15 days of disease appearance  | 25th September 2023 |
| 2 | After 15 days of first application second application was done | 10th October 2023 |
| 3 | After 15 days of second application third application was done | 25th October 2023 |

**2.5 Observations recorded in the field:**

* Plant height (cm) at 30, 60 and 90 Days After Sowing (DAS).
* Number of branches per plant at 30, 60 and 90 Days After Sowing (DAS).
* Pod Yield (t/ha)

**2.5.1 Plant height (cm)**

The plant height was recorded from five randomly selected plant in each plot at 30, 60 and 90 DAS. The height was measured from base of the plant to the tip of main shoot and expressed in centimetres.

**2.5.2 Number of branches per plant**

The number of branches was recorded from five randomly selected plant in each plot at 30, 60 and 90 DAS.

**2.5.3 Pod yield (t/ha)**

Plants from net plots area were harvested and developed pods were separated. Soil particles adhered to the pods were removed and sun dried. The pod weight (t/ha net plot) was recorded and used for converting tonnes/ha.

**2.6 Cost of cultivation (₹/ha)**

The cost of cultivation for each treatment was calculated separately taking into consideration of all the cultural practices followed in the cultivation.

**2.7 Gross return (₹/ha)**

The gross return from each treatment was calculated taking into the consideration the market price of the produce.

**2.8 Net profit (₹/ha)**

The net profit from each treatment was calculated separately by using the following formula:

Net profit (Rs/ha) = Gross return – Total cost of cultivation

**2.9 Cost benefit ratio (C:B ratio)**

The benefit cost ratio from each treatment was determined by using the following formula **(Reddy and Reddi, 2004)**.

$$C:B ratio=\frac{Gross return}{Total cost of cultivation}$$

**2.10 Statistical Analysis**

The data obtained were transformed to corresponding are sin or square root values Yates (1984). Statistical analysis was done to test the level of significance and to compare the treatments. The data were subjected to statistical analysis of variance (ANOVA). F-test was used to determine the significant difference.

**3. Results and Discussion**

**3.1 Effect of treatments on Plant height (cm) at 30, 60 and 90 days after sowing**

The average plant height at 30, 60 and 90 DAS and are presented in **Table** **2** and depicted in **Figure** **1**

At 30 days after sowing plant height was significantly increased in T4 – *Pseudomonas fluorescens* @ 5gm+ neem oil @ 1% (20.36cm) followed by T1 – *P. fluorescens* @ 5gm + clove oil @ 1% (18.47cm), T2 – *P. fluorescens* @ 5gm *+* eucalyptus oil @ 1% (17.46cm) and least was observed in T3 – *P. fluorescens* @5%(15.50cm) as compared to T5 – treated check (22.47cm) and T0 - control (10.60cm).

All the treatments are significant over control. Among the treatments T3, T4 and T5 were statistically significant with each other, however treatment (T1 and T2) were non-significant with each other.

At 60 days after sowing plant height was significantly increased in T4 – *Pseudomonas fluorescens* @ 5gm+ neem oil @ 1% (33.41cm) followed by T1 – *P. fluorescens* @ 5gm + clove oil @ 1% (30.36cm), T2 – *P. fluorescens* @ 5gm *+* eucalyptus oil @ 1% (29.57cm), and least was observed in T3 – *P. fluorescens* @ 5%(27.37cm) as compared to T5 – treated check (36.34cm) and T0 -control (20.12cm).

All the treatments are significant over control. Among the treatments T3, T4 and T5 were statistically significant with each other, however treatment (T1 and T2) were non-significant with each other.

At 90 days after sowing plant height was significantly increased in T4 – *Pseudomonas fluorescens* @ 5gm+ neem oil @ 1% (42.40cm) followed by T1 – *P. fluorescens* @ 5gm + clove oil @ 1% (40.77cm), T2 – *P. fluorescens* @ 5gm *+* eucalyptus oil @ 1% (39.78cm), and least was observed in T3 – *P. fluorescens* @ 5%(36.77cm) as compared to T5 – treated check (44.40cm) and T0 - control (28.12cm).

All the treatments are significant over control. Among the treatments T3, T4 and T5 were statistically significant with each other, however treatment (T1 and T2) were non-significant with each other.

In this study, the treatment **T4 –** Pseudomonas fluorescens **(5 g/kg of seed) combined with 1% neem oil** showed the most promising results. Plants treated with this combination recorded a significant increase in height at **30, 60, and 90 days after sowing (DAS).** The improved growth can be attributed to the beneficial traits of P. fluorescens, which is known to promote plant health through **nitrogen fixation, phosphate solubilization, iron chelation**, and **production of plant hormones.** On the other hand, **neem oil** contains active compounds like **azadirachtin, salanin, meliantriol, nimbin, nimbidin, nimbolide, and limonoids**—all of which are known to enhance plant growth and provide natural protection against pathogens.These findings align with previous research by **Meena & Marimuthu (2012), Hol *et al*. (2013), Banjara *et al*. (2018), and Prasanna *et al*. (2022),** who demonstrated that P. fluorescens and neem oil are highly effective in controlling **Cercospora leaf spot,** contributing to better overall plant performance.Although the chemically treated check showed the highest plant height and strong fungicidal action, it may leave behind toxic residues that could harm non-target organisms in the ecosystem. In contrast, the T4 treatment offers a **sustainable and eco-friendly alternative,** not only suppressing the pathogen effectively but also supporting the plant's overall well-being, ultimately leading to increased growth.

**Table 2 Effect of treatments on Plant height (cm) at 30, 60 and 90 days after sowing**

|  |  |  |
| --- | --- | --- |
| **Tr. No.** | **Treatments** | **Plant height (cm)** |
| **30 DAS** | **60 DAS** | **90 DAS** |
| **T0****T1****T2****T3****T4****T5** | Control*Pseudomonas fluorescens* (S.T) @5g/kgof seed +Clove oil (F.S) @1%*P. fluorescens* (S.T) @5g/kgof seed + Eucalyptus oil (F.S) @1%*P. fluorescens* (F.S) @5%*P. fluorescens* (S.T) @5g/kgof seed + Neem oil (F.S) @1%Carbendazim(12%) + Mancozeb(63%) (S.T) @2g/kg of seed (treated check) | 10.6018.47a17.46a15.5020.3622.47 | 20.1230.36a29.57a27.3733.4136.34 | 28.1240.77a39.78a36.7742.4044.40 |
| **S. Ed. (±)** | 0.79 | 0.87 | 0.50 |
| **C.D. (5%)** | 1.66 | 1.84 | 1.06 |

S.T- Seed treatmenrt

F.S- Foliar spray

DAS- Days after sowing

**Figure 1 Effect of treatments on Plant height (cm) at 30, 60 and 90 DAS**

**3.2 Effect of treatments on Number of branches at 30, 60 and 90 days after sowing**

The average number of branches per plant at 30, 60 and 90 days after sowing and are presented in **Table** **3** and depicted in **Figure** **2**

At 30 days after sowing number of branches per plant was significantly increased in T4 – *Pseudomonas fluorescens* @ 5gm+ neem oil @ 1% (5.53) followed by T1 – *P. fluorescens* @ 5gm + clove oil @ 1% (5.13), T2 – *P. fluorescens* @ 5gm *+* eucalyptus oil @ 1% (4.86) and least was observed in T3 – *P. fluorescens* @ 5%(4.33) as compared to T5 – treated check (6.00) and T0 – control (3.26).

All the treatments are significant over control. Among the treatments T3 and T5 was statistically significant with each other, however treatment (T1 and T2) and (T1 and T4) were non-significant with each other.

At 60 days after sowing number of branches per plant was significantly increased in T4 – *Pseudomonas fluorescens* @ 5gm+ neem oil @ 1% (6.06) followed by T1 – *P. fluorescens* @ 5gm + clove oil @ 1% (5.93), T2 – *P. fluorescens* @ 5gm *+* eucalyptus oil @ 1% (5.80) and least was observed in T3 – *P. fluorescens* @ 5%(5.26) as compared to T5 – treated check (6.66) and T0 – control (3.86).

All the treatments are significant over control. Among the treatments T3 and T5 was statistically significant with each other, however treatment (T1, T2 and T4) and (T1 and T4) were non-significant with each other.

At 90 days after sowing number of branches per plant was significantly increased in T4 – *Pseudomonas fluorescens* @ 5gm+ neem oil @ 1% (6.13) followed by T1 – *P. fluorescens* @ 5gm + clove oil @ 1% (6.00), T2 – *P. fluorescens* @ 5gm *+* eucalyptus oil @ 1% (5.87) and least was observed in T3 – *P. fluorescens* @ 5%(5.33) as compared to T5 – treated check (6.73) and T0 – control (3.93).

All the treatments are significant over control. Among the treatments T3 and T5 was statistically significant with each other, however treatment (T1, T2 and T4) and (T1 and T4) were non-significant with each other.

In this study, **Treatment T4—**using Pseudomonas fluorescens (5 g/kg of seed) combined with **1% neem oil**—showed notably positive outcomes. Plants treated with this combination had a significantly greater **number of branches** at **30, 60, and 90 days after sowing (DAS)** compared to other treatments. This boost in plant growth likely stems from the synergy between the two components. P. fluorescens is a well-known plant-growth-promoting rhizobacterium (PGPR) with capabilities such as **nitrogen fixation, phosphate solubilization, iron chelation,** and **phytohormone production,** all contributing to enhanced root and shoot vigor. Neem oil, meanwhile, is rich in bioactive compounds like **azadirachtin, salanin, meliantriol, and nimbin,** along with **nimbidin, nimbolide, and limonoids,** which not only protect against pests and diseases but also stimulate overall physiological health of the plant.

These findings are consistent with previous research. For example, Meena & Marimuthu (2012) demonstrated that P. fluorescens effectively promotes plant growth and suppresses Cercospora-related leaf spot in groundnut—with measurable increases in branches Similarly, Prasanna *et al.* (2022) reported that combining P. fluorescens with neem oil significantly reduced disease incidence of Cercospora arachidicola and C. personatum while increasing plant height, branching, and leaf number **Hol *et al*. (2013) and Banjara *et al*. (2018).**

While the chemical-treated check in your experiment yielded the highest overall growth, it likely relied on synthetic fungicides that could leave toxic residues, with potential negative impacts on beneficial organisms and the wider ecosystem. In contrast, the **T4 treatment offers a sustainable, eco-friendly alternative**—effectively suppressing pathogens, enhancing plant vigor, and promoting a cascade of beneficial effects that result in more branches and leaves, as well as overall plant well-being.

**Table 3 Effect of treatments on number of branches at 30, 60 and 90 DAS**

|  |  |  |
| --- | --- | --- |
| **Tr. No.** | **Treatments** | **Number of branches** |
| **30 DAS** | **60 DAS** | **90 DAS** |
| **T0****T1****T2****T3****T4****T5** | Control*Pseudomonas fluorescens* (S.T) @5g/kgof seed +Clove oil (F.S) @1%*P. fluorescens* (S.T) @5g/kgof seed + Eucalyptus oil (F.S) @1%*P. fluorescens* (F.S) @5%*P. fluorescens* (S.T) @5g/kgof seed + Neem oil (F.S) @1%Carbendazim(12%) + Mancozeb(63%) (S.T) @2g/kg of seed (treated check) | 3.265.13ab4.86a4.335.53b6.00 | 3.865.93ab5.80a5.266.06ab 6.66 | 3.936.00ab5.87a5.336.13ab6.73 |
| **S.Ed. (±)** | 0.21 | 0.17 | 0.23 |
| **C.D. (5%)** | 0.45 | 0.36 | 0.47 |

S.T- Seed treatmenrt

F.S- Foliar spray

DAS- Days after sowing

**Figure 2 Effect of treatments on number of branches at 30, 60 and 90 DAS**

**3.3 Effect of treatments on pod yield (t/ha)**

The data presented in **Table** **4** and depicted in **Figure** **3** revealed that the pod yield of groundnut was significantly increased in T4 – *Pseudomonas fluorescens* @ 5gm+ neem oil @ 1% (1.880 t/ha) followed by T1 – *P. fluorescens* @ 5gm + clove oil @ 1% (1.806 t/ha), T2 – *P. fluorescens* @ 5gm *+* eucalyptus oil @ 1% (1.798 t/ha) and least was observed in T3 – *P. fluorescens* @ 5%(1.343 t/ha) as compared to T5 – treated check (2.180 t/ha) and T0 -control (0.881 t/ha).

All the treatments are significant over control. Among the treatments T3 and T5 were statistically significant with each other, however treatment (T1, T2 and T4) and (T1 and T4) were non-significant with each other.

 In the present study, the treatment **T4 –** Pseudomonas fluorescens **(5 g/kg of seed) combined with 1% neem oil** demonstrated a **significant increase in groundnut yield** compared to the other treatments. This enhanced productivity can be attributed to the **synergistic effects** of the two components, both known for their plant growth-promoting and disease-suppressing properties. P. fluorescens is a beneficial rhizobacterium that supports plant development through mechanisms such as **nitrogen fixation, phosphate solubilization, iron chelation,** and **phytohormone production,** which collectively contribute to improved nutrient uptake and biomass accumulation. Neem oil, on the other hand, contains potent bioactive compounds including **azadirachtin, salanin, meliantriol, nimbin, nimbidin, nimbolide,** and **limonoids,** which not only act as natural protectants against pests and diseases but also enhance overall physiological performance, thereby contributing to higher yield potential.

The current findings are in agreement with earlier research by **Meena and Marimuthu (2012), Kumar *et al.* (2017), Banjara *et al.* (2018),** and **Prasanna *et al.* (2022),** all of whom reported that the combined application of P. fluorescens and neem-based products was highly effective in managing **Cercospora leaf spot** and improving crop performance, including yield.

While the **chemically treated check** recorded the highest absolute yield, it likely achieved this through the use of synthetic fungicides, which—although effective—may leave **toxic residues** that pose risks to non-target organisms and the surrounding ecosystem. In contrast, the **T4 treatment offers a sustainable and eco-friendly alternative,** effectively suppressing pathogens while promoting plant health and productivity without environmental harm.

Thus, the yield increase observed under T4 may be the result of a **cascade of positive physiological effects** driven by biocontrol, nutrient enhancement, and plant vitality—ultimately contributing to the holistic improvement of crop performance.

 **Table 4 Effect of treatments on pod yield (t/ha)**

|  |  |  |
| --- | --- | --- |
| **Tr. No.** | **Treatments** | **Yield (t/ha)** |
| **T0****T1****T2****T3** **T4****T5** | Control*Pseudomonas fluorescens* (ST) @5g/kgof seed +Clove oil (FS) @1%*P. fluorescens* (ST) @5g/kgof seed + Eucalyptus oil (FS) @1%*P. fluorescens* (FS) @5%*P. fluorescens* (ST) @5g/kgof seed + Neem oil (FS) @1%Carbendazim(12%) + Mancozeb(63%) (ST) @2g/kg of seed (treated check) | 0.881 1.806ab 1.798a1.343 1.880ab2.180 |
| **S.Ed. (±)** | 0.09 |
| **C.D. (5%)** | 0.20 |

**Figure 3 Effect of treatments on pod yield (t/ha)**

**Table 5 Cost benefit ratio of groundnut (t/ha)**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Tr.****No.** | **Treatment Details** | **Common Total Cost of Cultivation (₹/ha)** | **Cost of Treatment (₹/ha)** | **Total Cost of Cultivation (₹/ha)** | **Yield****(t/ha)** | **Sale****(t/ha)** | **Gross****return****₹/ha** | **C:B****Ratio** |
| **T0** | Control | 39,100 | - | - | 0.881 | 61,000 | 53,741 | 1:1.37 |
| **T1** | *Pseudomonas fluorescens +* clove oil | 39,100 | 2910 | 42,010 | 1.806 | 61,000 | 110,166 | 1:2.62 |
| **T2** | *P. fluorescens* + eculaptus oil | 39,100 | 4860 | 43,960 | 1.798 | 61,000 | 109,678 | 1:2.49 |
| **T3** | *P. fluorescens* | 39,100 | 2950 | 42,050 | 1.343 | 61,000 | 81,923 | 1:1.94 |
| **T4** | *P. fluorescens* + neem oil | 39,100 | 1310 | 40,410 | 1.880 | 61,000 | 114,680 | 1:2.83 |
| **T5** | Carbendazim (12%)+Mancozeb (63%) (treated check) | 39,100 | 414 | 39,514 | 2.180 | 61,000 | 132,980 | 1:3.36 |

**Figure 4 Cost benefit ratio of groundnut**

**3.4. Cost benefit ratio of groundnut (t/ha)**

Observations regarding the economics of treatments are shown in the **Table 5 and Figure 4.** Higher gross return value (**₹**114,680) and C:B Ratio (2.83) was observed in T4 - *Pseudomonas fluorescens* (ST) @5g/kg of seed + Neem oil (FS) @1% as compared to T0 (control) recorded lowest gross return value (**₹**53,741) and C:B Ratio (1.37).

**CONCLUSIONS**

Based on field observations, the present study concluded that the combination of Pseudomonas fluorescens **and neem oil** was highly effective in managing **Cercospora leaf spot** in groundnut. Given the growing global demand for **organically grown and sustainably managed agricultural products**, these findings hold strong promise for promoting **eco-friendly and residue-free crop protection strategies.** The results were particularly effective under the **agro-climatic conditions of Prayagraj,** indicating that this approach could serve as a practical alternative to chemical fungicides in similar environments. However, to further validate these outcomes and ensure broader applicability, **additional field trials across diverse regions** are recommended. This would strengthen the evidence base for integrating such biological treatments into sustainable groundnut production systems for the future.

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