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Contribution to the allelopathic study of *Melia azedarach* L. on the germination and initial growth of *Fagopyrum esculentum* Moench and *Cyperus iria* L. seeds

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Abstract - Allelopathy can be considered a chemical interference of one or more substances from a plant species in the development of another species, encompassing both inhibition and stimulation effects. In this context, the goal of the current research was to evaluate the allelopathic effect of *Melia azedarach* (chinaberry) leaf extracts on the germination of *Fagopyrum esculentum* (buckwheat) and *Cyperus iria* seeds. The experiment was conducted in a completely randomized design where the seeds were placed on germitest paper containing an aqueous extract of *M. azedarach* leaves at concentrations corresponding to 0, 25, 50, 75, and 100% and kept in a germination chamber in the presence of light. The analyzed variables were: first germination count, germination, length, and dry mass of seedlings. The results showed that, for both species, aqueous extracts at a concentration of 25% affected germination and initial seedling growth differently. The inhibition or stimulation of seed germination and initial growth depended on the extract concentration and varied in different species. *M. azedarach* extracts are not indicated for the control of *C. iria*. Therefore, it recommends removing *M. azedarach* leaves from the fields before sowing to prevent a negative impact on the early development of buckwheat and to stimulate the growth of *C. iria*.

Keywords: Allelopathy. Buckwheat. Weed. Germination process.

Contribuição ao estudo alelopático de *Melia azedarach* L. na germinação e crescimento inicial de sementes de *Fagopyrum esculentum* Moench e *Cyperus iria* L.

Resumo - A alelopatia pode ser considerada uma interferência química de uma ou mais substâncias de uma espécie vegetal no desenvolvimento de outra espécie, englobando tanto efeitos de inibição quanto de estimulação. Neste contexto, o objetivo da presente pesquisa foi avaliar o efeito alelopático de extratos de folhas de *Melia azedarach* (cinamomo) sobre a germinação de sementes de *Fagopyrum esculentum* (trigo mourisco) e *Cyperus iria*. O experimento foi conduzido em delineamento inteiramente casualizado onde as sementes foram dispostas em papel *germitest* contendo extrato aquoso de folhas nas concentrações correspondentes a 0, 25, 50, 75 e 100% e mantidas em câmara de germinação, em presença de luz. As variáveis analisadas foram: primeira contagem de germinação, germinação, comprimento e massa seca de plântulas. Os resultados evidenciaram que, para ambas as espécies, os extratos aquosos de *M. azedarach* na concentração de 25% afetaram de forma distinta a germinação e o crescimento inicial de plântulas. A inibição ou estimulação da germinação e crescimento inicial das sementes dependeu da concentração do extrato e variou nas diferentes espécies. Os extratos de M. azedarach não são indicados para o controle de C. iria. Assim, recomenda-se que as folhas de *M. azedarach* sejam removidas dos campos antes da semeadura a fim de evitar influência negativa no crescimento inicial de trigo mourisco e favorecer o desenvolvimento de *C. iria*.

Palavras-chave: Alelopatia. Trigo mourisco. Planta daninha. Processo de germinação.

Contribución al estudio alelopático de *Melia azedarach* L. sobre la germinación y crecimiento inicial de *Fagopyrum esculentum* Moench y *Cyperus iria* L.

Resumen - La alelopatía puede considerarse una interferencia química de una o más sustancias de una especie vegetal en el desarrollo de otra especie, abarcando tanto efectos de inhibición como de estimulación. En este contexto, el objetivo de la presente investigación fue evaluar el efecto alelopático de extractos de hojas de *Melia azedarach* sobre la germinación de semillas de *Fagopyrum esculentum* (trigo sarraceno) y *Cyperus iria*. El experimento se realizó en un diseño completamente al azar donde las semillas se colocaron sobre papel germitest que contenía extracto acuoso de hojas en concentraciones correspondientes al 0, 25, 50, 75 y 100% y se mantuvieron en una cámara de germinación, en presencia de luz. Las variables analizadas fueron: primer conteo de germinación, germinación, longitud y masa seca de las plántulas. Los resultados mostraron que, para ambas especies, los extractos acuosos de *M. azedarach* a una concentración del 25% afectaron de manera diferente la germinación y el crecimiento inicial de las plántulas. La inhibición o estimulación de la germinación de las semillas y el crecimiento inicial dependió de la concentración del extracto y varió entre las diferentes especies. Los extratos de *M. azedarach* no están indicados para el control de *C. iria*. Por lo tanto, se recomienda que las hojas de *M. azedarach* se retiren de los campos antes de la siembra para evitar una influencia negativa en el crecimiento inicial del trigo sarraceno y favorecer el desarrollo de *C. iria*.

Palabras clave: Alelopatía. Trigo sarraceno. Mala hierba. Proceso de germinación.

Introduction

Allelopathy is the positive or negative effect of plants on other living organisms caused by the release of allelochemicals, which play a significant role in maintaining ecological equilibrium (Muhammad and Majeed 2020; Hickman et al. 2021). In higher plants, they are found in the roots, leaves, stems, flowers, fruits, and seeds, and they can be released as root exudates, volatile compounds, or leaf decay material (Bachheti et al. 2020; Farooq et al. 2020). They impact soil bacteria and other nearby plants, influencing seed germination and root and stem growth (Iqbal et al. 2020).

Allelochemicals exert ecological mechanisms on nearby plants, usually because they cause negative physiological effects such as inhibition of the percentage and speed of germination and reduction of early seedling growth (Li et al. 2019). On the other hand, its effect is not always inhibitory, sometimes showing a positive effect on growth (Thiebaut et al. 2019) when present in lower concentrations (Masum et al. 2018).

Allelopathy is a practice also used in forestry to control weeds. Competition between crops and weeds naturally exists, competing for water, luminosity, nutrients, and space, which can cause a significant reduction in the yield of agricultural crops (Siddhu et al. 2018). As a natural phenomenon, it is a welcome idea that can be used in weed management in cultivated fields (Martina and Onyebuchi 2022) and assist in decision-making about the adoption of new management strategies.

Considering the need for the management of degraded areas and the establishment of successive vegetation, it is essential to know the allelopathic potential of an exotic species as well as the effects of its establishment in these ecosystems. Since *Melia azedarach* L. is a significant tree for timber production, soil rehabilitation, and field protection as shelter belts, it is important to consider the effect of their fallen leaves on the growth characteristics of nearby crops where there may be interference with light capture, soil moisture, and nutrients between your plant debris and other plants.

The species *M. azedarach* (Meliaceae), popularly known as chinaberry, is a deciduous tree originating from China and India, widely cultivated or occurring spontaneously in the south and southeast of Brazil, being widely used as a shade tree (Lorenzi et al. 2003). Its secondary metabolites are flavonoids, terpenoids, and volatile oils (Bibi et al. 2016). Considering that *M. azedarach* is a deciduous species, it can produce a considerable amount of litter which, when covering the soil and decomposing, would release soluble substances with an inhibitory effect to the other species present (Lorenzi 2009).

In the literature, reports are found on the allelopathic effect of species from the Meliaceae family on the germination of *Pennisetum americanum* L. (Bibi et al. 2016); soybean (Gulimar et al 2017); *Ceiba speciosa* A.St.-Hil (Ravenna), *Samanea tubulosa* (Benth.) Barneby & J. W. Grimes, and *Leucaena leucocephala* (Lam.) (Alves et al. 2019); Brassica campestris L. (Gul et al. 2022); *Handroanthus serratifolius* (Vahl) S. Grose (Nascimento et al. 2022), *Abelmoschus esculentus* L. (Moench) and *Raphanus sativus* L. (Pratap et al. 2022), among others. However, no studies were found on *Fagopyrum esculentum* Moench (buckwheat) and *Cyperus iria* L.

Buckwheat is a forage species of great economic importance, belonging to the Polygonaceae family and originally from Central Asia and China. Nutritionally, its seeds do not contain gluten, and it is distinguished from other cereals by the superior quality of its protein and the greater number of vitamins and minerals it contains (Giménez-Bastida et al. 2015; Škrobot et al. 2019). Other research has proved the nutritional potential of its use as an ingredient in animal feed and as an alternative forage (Görgen et al. 2016). Meanwhile, *C. iria* (Cyperaceae) is a weed species responsible for yield loss in rice cultivation, mainly due to competition for environmental resources (Nunes et al. 2018). In addition to the direct negative aspects of competition for environmental resources, it can promote rice lodging or hamper harvesting, affecting crop profitability (Chauhan and Johnson 2010).

Knowledge of the factors that affect the distribution of weed and forage species is an important tool that helps in proposing appropriate management strategies. In this sense, this study raised the hypothesis that the compounds present in the leaves of *M. azedarach* could influence the occurrence of other species (one forage and the other weed). Therefore, the goal of this research was to evaluate the allelopathic effect of *M. azedarach* leaf extracts on seed germination of *Fagopyrum esculentum* and *Cyperus iria*.

Material and Methods

To conduct the experiments were used, leaves of *M. azedarach* collected early in the morning (01/12/2023) in an area of the campus of the Federal University of Santa Maria (29°43'31.1"S 53°43'00.0"W) and non-dormant seeds of *Fagopyrum esculentum*, cultivar IPR 92 Altar (2022 harvest - Agroindustrial e Comercial Pozza Eireli), and *Cyperus iria* (harvest 2022 - 29°43'31.1"S 53°43'00.0"W). The taxonomic identification of species *M. azedarach* was performed by means of an identification manual (Lorenzi 2003). The leaves of *M. azedarach* were crushed in a blender for approximately 2 minutes at a rate of 100 g of fresh leaves to 1 liter of distilled water, which was considered the 100% crude extract (p/v), according to Lima et al. (2020). After 10 minutes, the mixture was filtered through filter paper and allowed to stand for 24 hours (in the dark and refrigerated at ± 5 °C). The design used was completely randomized and composed of different concentrations of extracts: T1 = 0 (control), T2 = 25, T3 = 50, T4 = 75, and T5 = 100%, prepared with distilled water. The pH of the aqueous extracts corresponded to 5.4 (T1), 5.2 (T2), 5.2 (T3), 5.1 (T4), and 5.1 (T5), with no significant difference in pH values between extracts.

To evaluate the allelopathic effect on germination, four repetitions of 50 seeds were distributed on germitest paper moistened with distilled water or extract (2.5 times the weight of the paper), being made into rolls (or in gerbox boxes according to the species) that were stored in a BOD chamber (Biochemical Oxygen Demand) at a temperature of 20 °C (buckwheat) and 35-25 °C (*C. iria*), with a photoperiod of 12 hours. The first count and germination were performed according to Brasil (2009), to the 4-7 (buckwheat) and 7-14 days (*C. iria*).

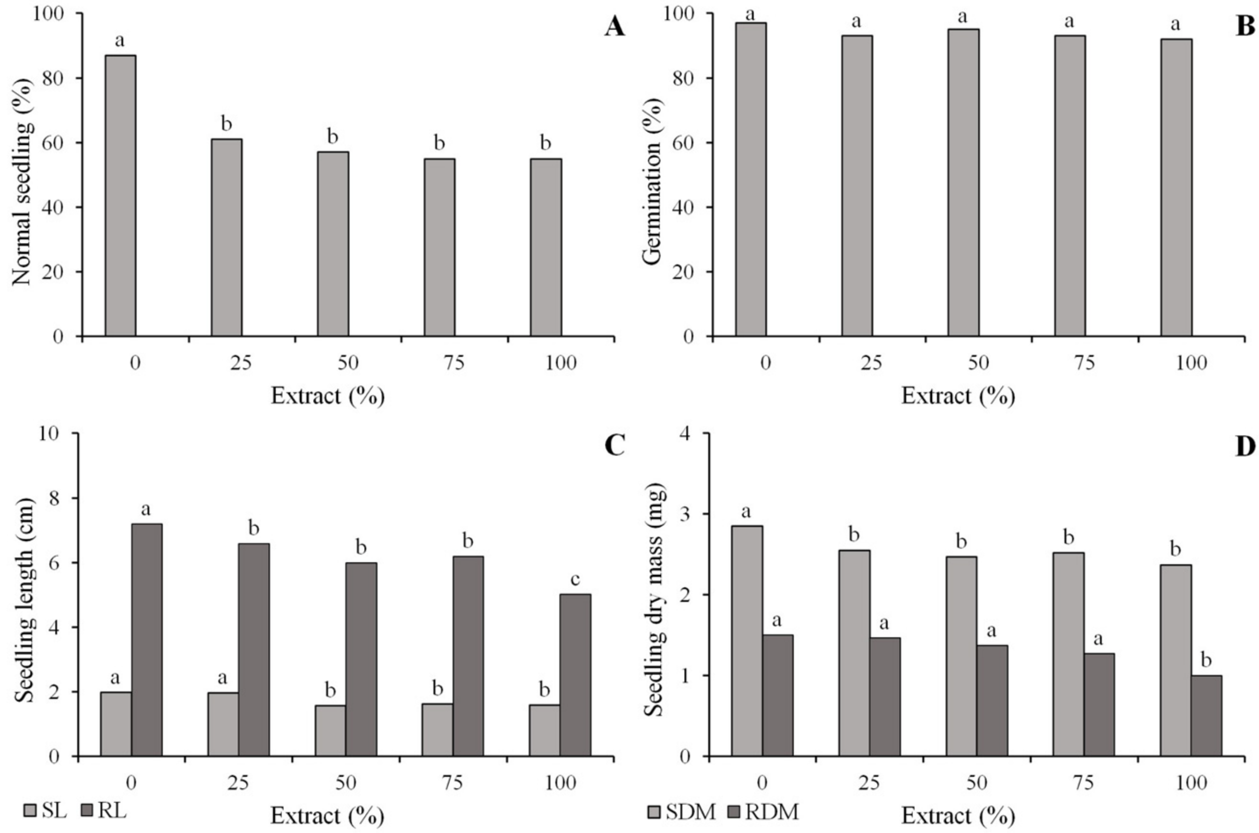
The length (shoot and root) and dry mass of seedlings were measured using the methodology proposed by Krzyzanowski et al. (2020), in which four replications of 20 seeds were sown in two rows in the upper third of the germitest paper and maintained under the same conditions as in the germination test. On the 4th (buckwheat) and 14th (*C. iria*) days after sowing, the average lengths (shoot and root) of 10 normal seedlings of each replication were measured. After drying the material in a forced ventilation oven at 60 ± 5 °C for 48 h, the seedlings were weighed on a precision scale (0.001 g), determining their dry mass (mg). However, in *C. iria*, it was not possible to perform the dry mass test as there were not enough seedlings.

The data were submitted for analysis of variance by the F test ($p < 0.05$) and regression analysis for the different extract concentrations (for each species) using the statistical software Sisvar® version 5.6 (Ferreira 2014).

Results and Discussion

The analysis of variance showed that when the buckwheat seeds were submitted to the different concentrations of *M. azedarach* leaves extracts, there was a reduction in the percentage of normal seedlings in the first count, in the root length, and in the dry mass of the aerial part from the 25% concentration (Figures 1A, 1C, and 1D). In addition, at 50% and 100%, a decrease in shoot length (1.98 for 1.57 cm) and root dry mass (1.5 for 1.0 cm) was observed, respectively. However, no significant difference was observed in the percentage of seed germination (Figure 1B).

Figure 1. First germination count (A), germination (B), shoot (SL) and root (RL) length (C), and dry mass of shoot (SDM) and root (RDM) (D) of buckwheat seedlings under different concentrations of *M. azedarach* extracts. *significant at $p < 0.05$.



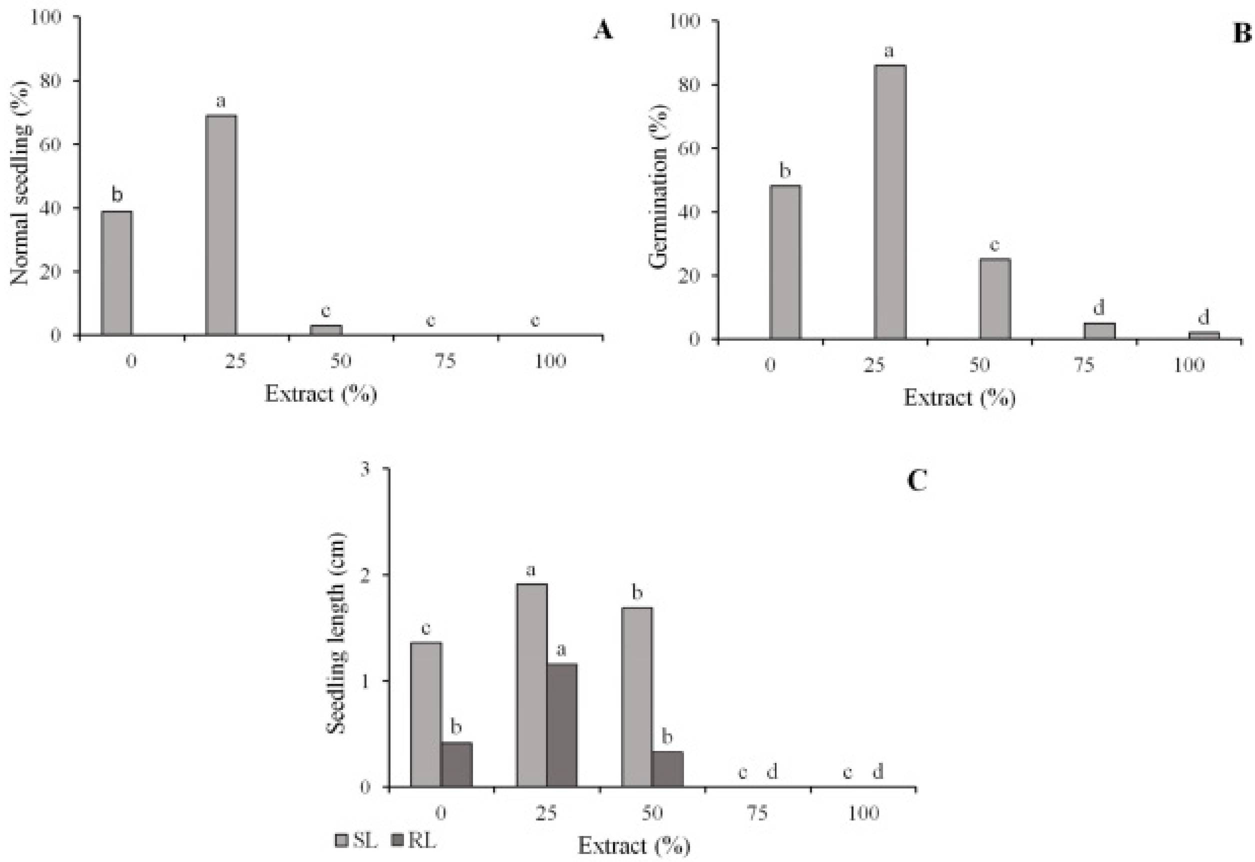
The results obtained in this study confirmed that *M. azedarach* extract has a negative effect on the initial growth of buckwheat seedlings (Figures 1A and 1C). This information is in line with that observed in Brassica campestris L., where different concentrations of aqueous extracts of *M. azedarach* (10, 15, and 20 g 100 mL^{-1}) caused an inhibitory effect on seed germination and seedling growth (Gul et al. 2022). Additionally, in another study (with extract concentrations from 10 to 100%) was verified a harmful effect of *Melia composita* Wild on germination, growth, biomass, and production of *Abelmoschus esculentus* L. (Moench) and *Raphanus sativus* L. (Pratap et al. 2022).

In *Pennisetum americanum* L. and *Handroanthus serratifolius* (Vahl) S. Grose, seed germinability, and seedling length were affected by extract of *M. azedarach* and *Azadirachta indica A*. Juss (family Meliaceae) (Bibi et al. 2016; Nascimento et al. 2022). According to the authors, the observed effect can be attributed to the presence of alkaloids, flavonoids, and phenolic compounds, since such compounds have proven action on seedling development. Similarly, hemp (*Cannabis sativa* L.) leaf extracts at concentrations from 25% to 100% had a negative impact on seed germination and seedling growth of barley (*Hordeum vulgare* L.) cv. Alamo and wheat (*Triticum durum* L.) cv. Mongibello, particularly at sub-optimal temperatures (Patanè et al. 2023).

In other studies, increasing the concentrations (0, 25, 50, 75, and 100%) of the aqueous extracts of *Cyperus difformis* L., *Galinsoga parviflora* Cav., and *Commelina benghalensis* L. caused a reduction in the length of lettuce and tomato roots (Rezendes et al. 2020). When susceptible plants are exposed to allelochemicals, germination, and growth can be affected; consequently, allelopathy can be considered the chemical interference of one or more substances of a plant species in the germination or development of other species, which encompasses both the effects of inhibition and stimulation (Hannachi 2023).

For *C. iria*, the extract at a concentration of 25% resulted in higher values of normal seedlings in the germination, first count, shoot, and root length tests in relation to the control and other treatments (Figures 2A, 2B, and 2C). On the other hand, the leaf extracts, with concentrations starting at 75%, reduced the percentage of germination and completely inhibited the development of the seedlings (48, 86, 25, 5, and 0%, respectively). Thus, it is possible to infer that the extract of *M. azedarach* leaves when used in low concentrations (25%) showed a positive allelopathic effect, while in higher concentrations (75 and 100%), it resulted in the inhibition of *C. iria* seedlings.

Figure 2. Percentage of normal seedlings (G: germination and FC: first count) (A) and shoot (SL) and root (RL) length of *C. iria* seedlings (B) under different concentrations of *M. azedarach* extracts. *significant at p < 0.05.



The allelopathy stimulates or stops the survival of neighboring plants by this ability to produce allelochemicals (Javed 2020). By evaluating the allelopathic potential of *M. azedarach* leaf extract on the germination and growth of seedlings of *Ceiba speciosa* A.St.-Hil (Ravenna), *Samanea tubulosa* (Benth.) Barneby & J. W. Grimes, and *Leucaena leucocephala* (Lam.) in 5 concentrations (0; 2.5; 5; 7.5, and 10%), Alves et al. (2019) verified that the aqueous extract of the leaves had an allelopathic effect on all the studied species, reducing the percentage of germination, speed, length, and dry mass of the seedlings.

By examining seed germination, early growth, and physiological and biochemical aspects of *Amygdalus pedunculata* Pall. subjected to aqueous extracts of four species (*Hedysarum mongolicum* Turez., *Amorpha fruticosa* L., *Sabina vulgaris* Ant., and *Hippophae rhamnoides* L.), Wang et al. (2018) observed a significant positive effect at low concentrations and an inhibitory effect at high concentrations, as observed in this study. Thus, the results of this research corroborate those of Thiebaut et al. (2019) and Masum et al. (2018), who stated that the allelopathic contribution is not always inhibitory, often exhibiting a positive effect on growth and that, when present in lower concentrations, these compounds can act as growth promoters. On the other hand, at higher concentrations, inhibition of seed germination and seedling growth are frequent and associated with allelopathic effects, an essential process in plant interactions in natural environments and agroecosystems (Piršelová et al. 2019).

In the present study, the aqueous extract of *M. azedarach* leaves at different concentrations showed varying levels of inhibition of seed germination of *C. iria* in terms of final percentage and growth. Inhibition was generally minimal at low concentrations (25%) and significant at higher concentrations. This growth inhibition at higher concentrations and stimulation tendency at lower concentrations is widespread in allelopathic research and known as concentration-dependent activity (Islam et al. 2018). In their research, these authors reported that the inhibitory effect of plants depended on the dosage, and the highest concentration showed more ‑inhibitory activity on *Raphanus sativus* L.

Finally, the present study revealed that the inhibition or stimulation of seed germination and initial growth depended on the extract concentration and varied in different species. This information is extremely important for decision-making regarding the presence of C. iria and cultivation of buckwheat in environments where there are plant remains of M. azedarach. Thus, it is recommended that M. azedarach leaves be removed from the fields before sowing in order to avoid a negative influence on the initial growth of buckwheat and favor the development of C. iria. Furthermore, M. azedarach extracts are not indicated for the control of C. iria. A better understanding of the allelopathic mechanisms will help in choosing the appropriate management, helping in agricultural production, and preventing weed infestations.

Conclusions

Melia azedarach extracts at a concentration of 25% negatively influenced the percentage of normal seedlings and the initial growth of buckwheat and positively influenced the development of *C. iria*.

M. azedarach extracts are not indicated for the control of Cyperus iria.

It is recommended that *Melia azedarach* leaves be removed from the fields before sowing in order to avoid a negative influence on the initial growth of buckwheat and favor the development of *C. iria*.

Further studies regarding phytochemical analyses are necessary to comprehend and identify the compounds found in *M. azedarach* extracts.

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