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# Managing *Sitobion avenae* Fabricius (1775) by *Rosmarinus officinalis* and *Artemisia herba-alba* aqueous extracts in soft wheat field

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**Abstract**
Plants with insecticidal effects are becoming more and more important and used in biological management programs instead of chemicals. In fact, aqueous extracts of the two aromatic plants leaves which are the rosemary *Rosmarinus officinalis* and the white wormwood *Artemisia herba-alba*, were tested against the cereal aphid *Sitobion avenae* with 2.5%, 5% and 10% doses compared with a reference product and a control. Consequently, the two aqueous extracts of the two plants tested were efficient against aphids. The aphid population density was significantly reduced with mortality rates reaching 67.43% for rosemary and 51.79% for white wormwood. The highest doses were exhibited as the more lethal for aphids. It is noticed that the aqueous extracts could be more efficient if the aphids' population densities were lower.

**Keywords:** Aqueous extract, *Rosmarinus officinalis*, *Artemisia herba-alba*, *Sitobion avenae*

## 1. Introduction

Aphids are phloem suckers. They consume the essential nutrients for plant growth and reproduction [1]. These pests cause great damages for plants as yields decrease and spoiled baking quality. Also, they transmit many viruses [2, 3]. Dedryver [3] mentioned that aphids are vectors of 50% of plant viruses. For cereals, the barley yellow dwarf virus BYDV is commonly encountered. It can be spread by a restricted aphids' numbers. Its infection induces fewer ears, lower number and weight of grains [1, 4]. *S. avenae* (F.) is commonly called the English grain aphid. It is considered as one of the most injurious cereal aphid species on small grains in most countries growing cereals. It is known especially in temperate climates of the northern and southern hemispheres. Its feeding damages make it a dreadful species for two reasons. It has a strong preference for feeding on the heads particularly to the forming grains [8–12]. Also, it has a high efficiency in transmitting the BYDV [13, 14]. *S. avenae* shows the most active dispersal behaviour between plants in comparison with other aphids' species [15]. It usually arrives later in the season and reaches maximum numbers at the earing stage [16, 17]. In Tunisia, according to the study of Boukhris-Bouhachem *et al.* [6], *S. avenae* considered as the third abundant aphid attacking cereals with an occurrence rate reaching 22%. Several natural enemies can control aphids [18]. The Hymenopterous group (Aphidiinae) and Coccinellids are the most efficient enemies. Chrysopids, Syrphids and Cecidomyids larvae have secondary effectiveness [19]. Insecticide sprays are commonly used; in the crop production areas over the world. They protect crop from pests to reduce yield and quality losses. These sprays are usually impractical, ineffective, highly influenced by weather conditions. Samely, they have important negative impacts on the human health and the environment [20–23]. Furthermore, many pesticides are toxic to natural enemies [20, 21, 23]. The use of plants extracts (essential oils or aqueous extracts) as biopesticides empirical methods to control pests. These plants hold in their tissues bioactive components which have an insecticidal or acaricidal effects [24–26]. This work aims to study the efficiency of two bioinsecticides to control *S. avenae*. Two plants *Artemisia herba alba* and *Rosmarinus officinalis* were chosen to evaluate their aqueous extracts.

## 2. Material and Methods

### 2.1. Plants' collection and aqueous extracts preparation

The rosemary *Rosmarinus officinalis* L. (1753) and the wormwood *Artemisia herba-alba* Asso (1779) leaves were collected from Djebal Zaghouan (36°21′07″N, 10°06′43″E). Leaves were dried in the open area for several days and then grounded to powder. The aqueous extracts (AE) are prepared by maceration for 24 hours from powder leaves. The choice of doses was based on the study of Nia *et al.* [27]. The following doses of 25 (2.5%), 50 (5%) and 100 (10%) g/litre of water were tested. Then, the mixture was filtrated to have an aqueous solution without impurities and waste.

### 2.2. Treatments and experiment design

The experiment was carried out on an attacked field of soft wheat *var. Hidra* by aphids *S. avenae* (Figure 1). Two areas; of one hectare each one, were chosen. Each area was divided into 5 plots to be used for different treatments: one for the control (C), three for doses plants and one for the reference product. The aqueous extracts were symbolized by R1, R2 and R3 for *Rosmarinus officinalis* and A1, A2, A3 for *Artemisia herba-alba*. The reference product used during this trial was D-cis (Deltamethrin) with a concentration of 15 ml/l of water. Each plot was about 100m in length and 20m in width. The first application of the treatment (T1) was performed on April 15$^{th}$ 2019 followed by a second one (T2) after 7 days. Aphids' population monitoring and product efficacy evaluation were conducted after 24 hours, 4 days and 7 days. Sampling was carried out by sampling 10 wheat plants from each plot with one sweeping net.


**Fig 1:** Aphids' colonies of *S. avenae* on heads (a), damages caused by aphids' colonies (b)

### 2.3. Efficacy and mortality rates

The efficacy of aqueous extracts and reference insecticide on *S. avenae* was evaluated using the Abbott formula [28]:
Efficacy (%) = (T0 - Tt / T0) * 100; with T0 (control) = number of alive aphids on untreated plants; Tt = number of live aphids on treated plants.
Mortality was calculated and corrected according to the Abbott formula [29]:
Mc (%) = ((Me-Mt)/(100-Mt)) *100; Mc = adjusted percentage of mortality; Me = mortality of the tested product; Mt = mortality in untreated control.

### 2.4. Statistics analysis

The analysis data was made by SPSS statistical software version 24. to compare means by Duncan's test and independent t-test.

## 3. Results

### 3.1. *Artemisia herba-alba* aqueous extract test

Generally, the control showed that the aphid population is continuously changing during the study compared to those of the aqueous extracts and the reference product (Figure 2). After the first application of AE, the different doses A1, A2 and A3 of *A. herba-alba* showed a severe decrease in aphids' populations. This decline is followed by a huge increase in the 4th day after the treatment. This pattern of the aphids' colonies was the same again after the second application. For the reference product, aphid populations are in gradual decline interrupted by small increases as well as being the lowest compared to those counted for all three doses. In contrast to sampling, the required results from sweeping net revealed that the control showed a decrease in counted aphids followed by an increase from the date separating the two treatments towards the end of the period. The A2, A3 and reference product doses have approximately the same behaviour as the control with low individuals' numbers in (T1+7 days). The A1 dose appears to be less effective than other doses during the 4$^{th}$ and 7$^{th}$ days of the first treatment. An increase in the number of individuals in (T1+7 days) was rapidly reduced by the effect of the second treatment. As for the reference product, it could almost eradicate aphids after 24 hours to reach 25 individuals. Beyond this date, the aphids' numbers were slightly instable. In addition, the second treatment added a certain stability of the values from the first treatment for all the doses tested of the aqueous extract. However, there was an increase in numbers for all doses towards the end of the period which is explained by the normal evolution of aphids presented by the control curve.

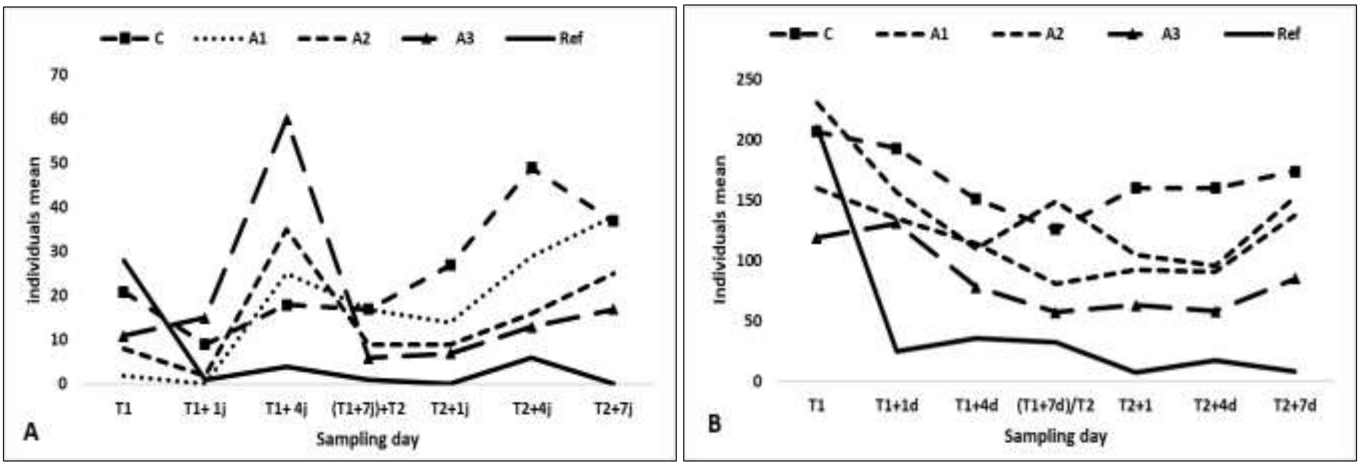


**Fig 2:** Aphids' population monitoring for plants' sampling (A) and sweeping net (B) in treated plots by different AE doses of *A. herba alba* (A1: dose1, A2: dose 2 and A3: dose 3) compared to reference product (Ref) and control (C).

The results of plant sampling (Table 1) revealed that there is no significant differences regarding efficacy rates between the three tested doses of *A. herba-alba* and that of reference product. Whereas the results obtained in sweeping net showed that there is no significant difference between A1 and A2 but there is a difference between the two previous doses compared to A3 and reference product.

**Table 1:** Efficacy rate (%) of different doses of *A. herba-alba* extract used against *S. avenae* (Duncan test with $p<0.05$).

| Treatment | Efficacy rates% | |
|---|---|---|
| | Plants Sampling | Sweeping net |
| A1 | 24,56 ± 48,75 [a] | 19,17 ± 20,66 [a] |
| A2 | 32,81 ± 64,43 [a] | 32,83 ± 9,02[a] |
| A3 | - 4,69 ± 121,81 [a] | 51,83 ± 11.41[b] |
| Reference product | 82,6 ± 21,96 [a] | 86,33 ± 9,07[c] |
| | DF=3; F=1.45; p=0.26 | DF=3; F=28,24; p=0.00 |

Duncan test at α<0.05 is applied to compare means, means followed by same letter were not significantly different.
AE doses of *A. herba-alba* (A1: dose1, A2: dose 2 and A3: dose 3) compared to reference product (Ref).

### 3.2. Rosemary aqueous extract test

The results of the sampling (Figure 3) revealed that the R1, R2 of rosemary AE and Ref, as well as the control, resulted in peaks after 4 days of the 1[st] treatment. After the second treatment, there was some stability in values up to the 4th day for these same doses mentioned above followed by an increase in numbers when D-cis led to the eradication of aphids. However, the R3 dose showed contradictory effects to those of the other doses and Ref. It caused the decline of the aphids' population after 24 hours of the 1[st] treatment and it continues to slightly decrease their numbers till the end. On the other hand, the data obtained from sweeping net is completely different from what is externalized from the plants' sampling by approving the efficacy of rosemary extract for approximately all doses. Indeed, rosemary has ensured a decrease in aphid numbers approximately stable over time from the first 24 hours. The R3 dose showed that it is the most effective compared to the other rosemary doses since it recorded values comparatively close to those of the Reference product.

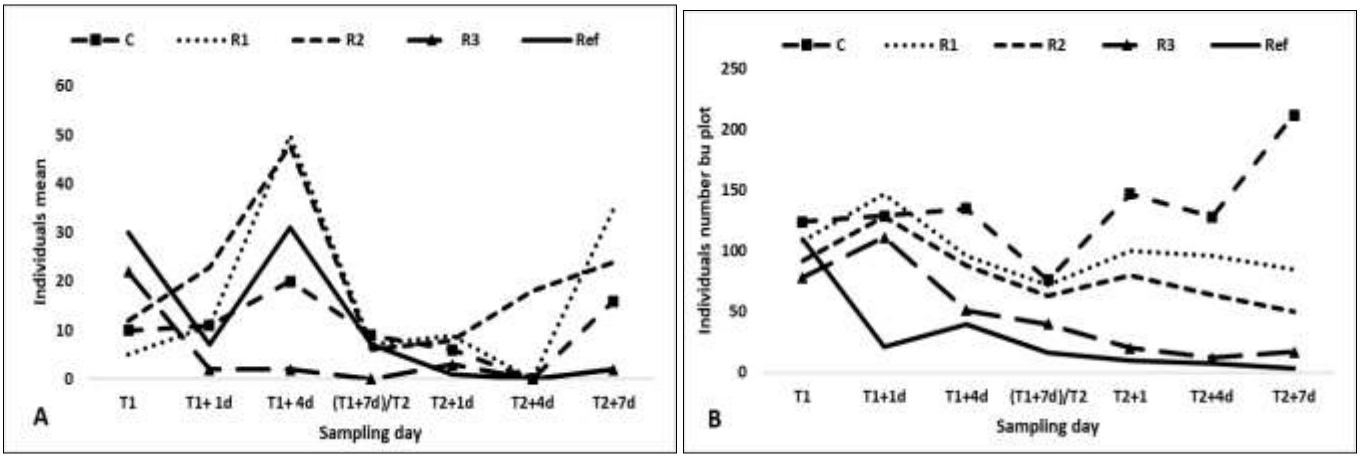


**Fig 3:** Aphid population monitoring for plants' sampling (A) and sweeping net (B) in treated plots by different AE doses of *R. officinalis* (R1: dose1, R2: dose 2 and R3: dose 3) compared to reference product (Ref) and control (C).

Obtained results (Table 2) showed that there is no significant difference between R1 and R2 doses also between the R3 dose and reference product, which means that they have the same efficacy. However, the set of two doses R1 and R2 are significantly different from the set of R3 and Ref. The efficacy rates confirms that the R3 of the rosemary AE have an efficiency close to that of Ref.

**Table 2:** Efficacy rates of different doses of rosemary extract used against *S. avenae* (Duncan test with $p<0.05$).

| Traitement | Efficacy rates% | |
|---|---|---|
| | Plants sampling | Sweeping net |
| R1 | 4,75 ± 80,73 ª | 25,5 ± 20,97 ª |
| R2 | -63,18 ± 61,11 ª | 39,76 ± 23,02 ª |
| R3 | 59,19 ± 64,37 ᵇ | 67,43 ± 27,01 ᵇ |
| Reference product | 45,74 ± 58,12 ᵇ | 86,94 ± 10,69 ᵇ |
| | df= 3; F= 4,08; p= 0,21 | df= 3; F= 10,04; p=0.00 |

Duncan test at $p<0.05$ is applied to compare means, means followed by same letter were not significantly different.
AE doses of *R. officinalis* (R1: dose1, R2: dose 2 and R3: dose 3) compared to reference product (Ref).

Obtained results showed that *A. herba-alba* and *R. officinalis* AE exhibited an insecticidal effect against *S. avenae* with high doses. Indeed, sweeping net makes it possible to obtain better results than destructive sampling, which is influenced by the heterogeneity of aphids' colonies on plants within the same plot as well as the climatic conditions. In addition, rosemary AE showed lower aphids' numbers after treatment than those obtained with white wormwood AE.

**3.3. Mortality rates of *A. herba alba* and *R. officinalis* aqueous extracts:** The natural rates of mortality in the control were low during the experiment (Figure 4). The mortality rates of aqueous extract showed a progressive increase on the basis the increase of doses. For each aromatic plant, the mortality rates showed a significant difference between the different tested doses, the control and the reference. Comparing between plants *A. herba-alba* and *R. officinalis*, it is found that there is no significant difference between the two extracts for each dose. This means that both plants have the same dose level from which mortality rates were increased by increasing the dose. The D3 dose of both plants was the most lethal against aphids with 64.81% and 46.62% mortality rates for rosemary and white wormwood, respectively. These results seem to be acceptable because of the high densities of aphids' populations.

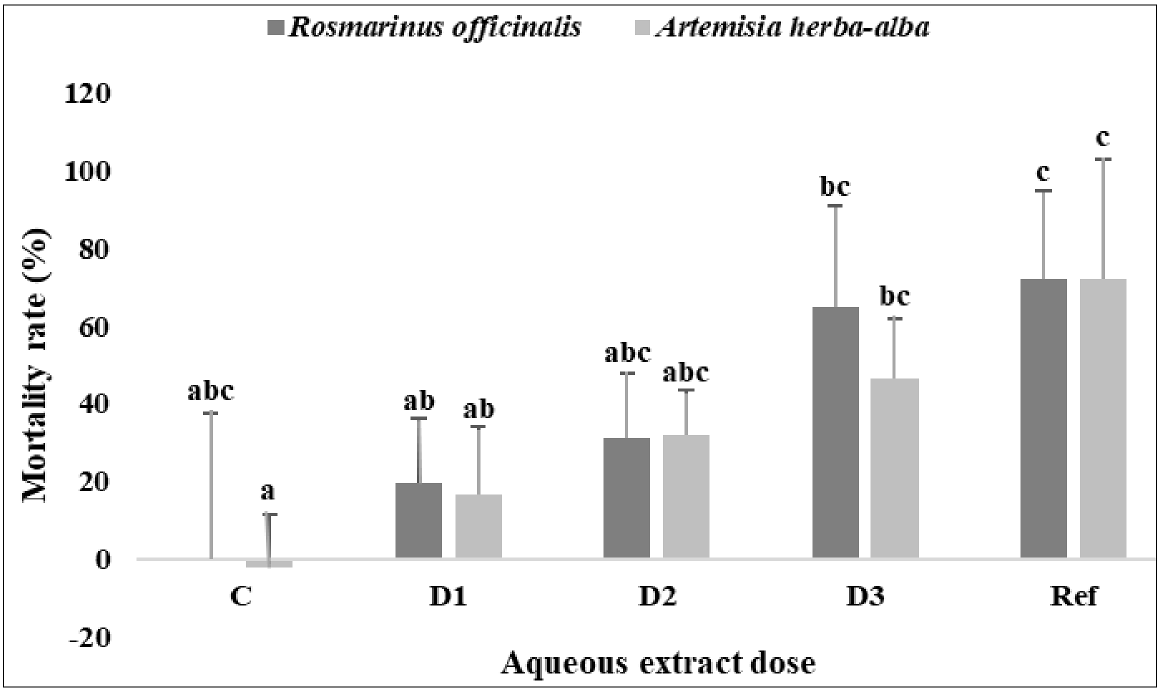


**Fig 4:** Mortality rates of different aqueous extracts' doses of both *R. officinalis* and *A. herba-alba* used against *S. avenae* (Duncan test with $p<= 0.05$) with C: control, D1: dose 1, D2: dose 2, D3: dose 3 and Ref: reference product. (Values followed by the same letters are not significantly different at $p\leq0.05$).

## 4. Discussion

*S. avenae* is known as a dreadful aphid species for its injuries on cereals [30, 31]. Fereres *et al.* [32] accorded that the severity of damage of this pest on winter wheat can be enhanced by the water stress even at low aphid densities. In Tunisia, the cereal agriculture is carried under rainy conditions which means that cereals are always under water stress due to the irregularity and the scarcity of rainfall. The damage threshold is defined differently from a study to another to start spraying [32]. In Britain, five or more aphids on flowering heads were considered as an alarming sign for rapid proliferation [33] while the critical infestation was fixed to 15 to 20 individuals per tiller in the same stage in Belgium and France. However, the critical infestation in the Netherlands is presented the percentage of attacked tillers which reach 70% [34]. There is no study indicating the threshold level for *S. avenae* in Tunisia. This study was conducted as a curative management because the aphids' populations were already well installed in the culture. Many botanical plants were experimented in phytomedicine for their repellent and confusant characteristics against pests, also for their capacity to limit their development or their feeding [35–38]. Among the aromatic plants which known for their insecticidal or acaricidal properties are *A. herba-alba* and *R. officinalis* [39–43]. Nia *et al.* [27] noted that the effect of these two plant extracts on aphids was not well studied. Nevertheless, other studies conducted on *S. avenae* showed that the mortality rates reached 77.56% and 90.58% at 3% concentration for neem oil and of turmeric powder respectively after 24 hrs [44]. The majority of researches consist in studying the effect of essential oils of *R. officinalis* and *A. herba alba*. In the present work, we aim to evaluate the efficacy of aqueous extracts of these two plants. The tested AE of *A. herba-alba* and *R. officinalis*; for curative management against high densities population of *S. avenae*, revealed efficient at the dose D3 (10%) with efficacy rates 51.79% and 67.43% respectively and mortality rates 46.62% and 64.81% respectively. It is demonstrated that the rosemary was more efficient and lethal than the white wormwood. It supposed that it could be more efficient if these AE were applied on low aphid densities to eradicate the population before its proliferation. Only Aziz *et al*. [45] studied the toxic effect of *A. herba-alba* AE towards Indian and Saudi Arabian strains of *Anopheles*, *Aedes* and *Culex* mosquitos. The AE showed a toxicity against larvae and it is attributed to flavonoids and saponins which are characterising the AE [45]. This result is the opposite of essential oil where the dominant component was terpenoids. It is still that the AE composition is efficient to control pests. In fact, flavonoids were approved to influence the arthropod growth and reproduction [46, 47]. Also, saponins have an effect on insect mainly feeding behaviour, growth regulation with a high toxicity on various pests such as *Tetranychus urticae*, *Spodoptera littoralis* and *Culex pipiens* [48–52]. These components can be present in the AE compositions of *A. herba alba* and *R. officinalis* used in this work and they can be the factors of population mortality of *S. avenae*. An experiment on *M. persicae* was conducted to evaluate the essential oils of *R. officinalis* and *A. herba-alba*. These essential oils were extracted with different solvents (petroleum ether, ethanol and distilled water) and applied in different concentrations (1, 2.5, 5, and 10%). Results showed that the etheric extract of all plants was effective and caused mortalities over 60% at 10% after 24hrs, while ethanolic and aqueous extracts did not show any significant insecticidal effect [27]. The phytochemical screening showed the richness of etheric extract in terpenes. The results obtained suggest that we can make bioinsecticides based on leaves etheric extracts from these plants for use in integrated pest management [27]. The use of *A. herba-alba* oil against *Aphis gossypii* showed a high toxicity ($LC_{50}$=0.023%) with 90.44% population mortality [53]. Applying the oil extract with different solvents of *A. herba-alba* against *Chrysomyia albiceps* showed that all of them had toxic effects on larvae [54]. Other wormwood similar to white wormwood used to control aphids. *A. absinthium* and *A. seiberi* were used against *M. persicae* and *Eriosoma lanigerum* respectively and showed highly toxic [24, 55]. Further, Sharifian *et al*. [40] have reported that the *A. herba-alba* EO had good insecticidal activity against various stored-product beetles, including *Callosobruchus maculatus* (F.), *Rhyzopertha dominica* (F.), and *Tribolium castaneum* (Herbst). Recently, Amel *et al*. [42] noted that *A. herba-alba* essential oil showed ovicidal activity against *Ephestia kuehniella* Zeller moths. As well as white wormwood, rosemary essential oil demonstrated efficient against insects and mites. From seven essential oil used on *Brevicoryne brassicae*, that of rosemary was considered the most lethal [56]. In other application against *Tetranychus urticae*, it has caused the complete mortality of the mite population [57]. The rosemary essential oils can be efficient against Coleopterans' pests. Its ethanolic essential oil caused the mortality of 91.2% of the *Acanthoscelides obtectus* after 7 days at concentrations of 50% and 100%, and 81.7% adult mortality at 1% concentration on *Corythucha ciliate* [58]. Furthermore, they induced the decline of the longevity, fecundity, and fertility *Callosobruchus maculatus* [59]. On Hemipterans' pests, the essential oil were lethal at 81.7% adult mortality at 1% concentration on *Corythucha ciliate* [60]. Indeed, it was effective in reducing the severity of attacks of *Bemisia tabaci* in cabbage after 24 and 48 h with killing nymphal and adult stages [61, 62]. Further, the oil concentration and the time of exposure are key factors in mortality. Thus, an experiment conducted on *Musca domestica* demonstrated that the higher oil concentration of rosemary rated the highest mortality with the longest time of exposure [63]. During the experiment, there were fluctuations of the aphid numbers recorded in the different doses of rosemary and wormwood. This can be explained by the distribution heterogeneity of aphid colonies on randomly sampled plants. As well, the aphids' proliferation stands can be the climatic conditions just after the application of the treatment. They were characterized by a windy weather and irregular rains which can prevent the fixation of the AE droplets on pest populations. Nevertheless, many works showed the richness in terpenoids of *A. herba-alba* and *R. officinalis* essential oils [43, 64–70]. The components of these aromatic plants can have effects in different aspects. They can lead to the mortality of adult, larvae and eggs as well as their biological activities. Also, they can play the role of repellent pests [71, 72].

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