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Original Research

Biocidal Properties of Silver-Nanoparticles in Water Environments

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Abstract

Nanotechnology offers manufacturing and use of structures in which at least one dimension is expressed in nanometers. An increasing number of everyday products contain some ingredients in molecules size. One of them – Ag-nanoparticles (nanosilver) – is commonly used due to its biocidal properties. There is a serious risk of nanoparticles being released into the environment, especially to the soil or surface water. Because of low selectivity of silver-nanoparticles in water it is not an environmentally-friendly product (destroying aquatic microflora and fauna).

The aim of this study was to determine the effect of Ag-nanoparticles on the growth and survivability of three water plants: *Oedogonium* sp., *Versicularia dubyana*, and *Lagarosyphon madagascariensis*.

Plants were treated with silver nanoparticle solutions of concentrations ranging from 0.0 to 10.0 $mg \cdot dm^{-3}$ Ag (NANO SILVER product by NANOCO Corp.). Light conditions were controlled through 16-hour light cycle using a 15W Tungsram lamp. The whole experiment was carried out over 10 days. Changes in plant morphology and growth in various Ag-nanoparticle concentrations were observed.

Ag-nanoparticles showed biocidal activity for *Oedogonium* sp. after 24 hours of incubation in solutions with a concentration of 1.0-10.0 $mg \cdot dm^{-3}$ Ag. Plants were dark, destroyed, and sank to the bottom. In samples with higher plants, nano silver caused blackening and some plant deaths at concentrations of 5.0 and 10.0 $mg \cdot dm^{-3}$ Ag, or inhibited their growth in lower concentrations.

Selective activity of nano silver may be useful for eliminating unwanted plants. However, its uncontrolled release into the environment may be harmful to aquatic organisms and devastating for entire ecosystems.

Keywords: silver nanoparticles, water, plants, *Oedogonium* sp., *Versicularia dubyana*, *Lagarosyphon madagascariensis*

Introduction

Nanotechnology enables manufacturing of materials in atom scale (in the range from 1 to 100 nm). Their primary use is for electronics and electrical engineering, but more often they are finding applications in pharmacy, cosmetology, and medical techniques. They are characterized by a number of unusual properties such as: mechanical (strengthening other materials), electrical (emission and conductivity), magnetic, and biological. Nanoparticle applications are currently so common that consumers are often not aware that they are using them. The variety of

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techniques allows nanoparticle manufacturing in an increasingly easier and more efficient way [1-3].

Apart from the polymorphic or complex nanostructures, products containing single nanoelements also can be used. Moreover, elements in nanoscale are often distinguished by different or modified (enhanced) chemical and physical properties compared to the "normal" metal forms [4]. Nanoparticle metal colloids are characterized by bigger active surface areas even at low concentrations, so they exhibit high biochemical activity and bioavailability. For example, nanosilver is known for its antibacterial, antifungal, and even antiviral properties [5].

Biocidal properties of silver have been known since ancient times, when silver cups were used to drink water in order to protect from various diseases. At that time no one knew about the existence of pathogenic microorganisms, and silver was used more intuitively, guessing that it protected against bacteria, molds, fungi, and their spores. These features of silver were recognized at the beginning of the 19th century, without full knowledge about the mechanism of its action. Today it is known that silver ions that operate directly on single cells are toxic to microorganisms. Catalytic properties of metal destroy genetic material in cell nucleus [6]. The antibacterial, antifungal, and antiviral characteristics have already been confirmed widely [7-11].

The basic forms of silver-nanoparticle utility are powders and water liquids. Powders are used for spraying plastics, textiles, and lining materials, whereas liquids are used for spraying work surfaces and equipment as well as (in concentrated form) in disinfection [12]. It is considered that the high reactivity and microscopic dimensions of nanoparticles may be dangerous to the environment, especially in the case of their uncontrolled release. Silver applied to the soil or water reservoir as well as applied to large areas or woven into fibers migrates to all of a biosphere's components. It reacts with natural microflora and fauna, which destroys them. Because of the low selectivity of silver-nanoparticles it is not environmentally friendly [13].

The need to describe ecotoxicology of silver-nanoparticles and their influence on the whole environment results also from legal aspects, e.g. soil, air, and water protection laws, including waste and management policy [14].

Water (especially its surface resources) is considered to be the most vulnerable to contamination from all of the biosphere components. It is involved in many technical processes or constitutes an environment in which they are conducted while sludge is removed from the environment in varying degrees of purification.

As a component of each closed ecological system, including living organisms, water becomes a carrier in which contaminants are going to circulate [15]. Particularly harmful and dangerous are those components that do not dissolve in water like, for example, heavy metals. Chemical sorption stores them into various deposits, avoiding biodegradation or following distribution [16], leading to accumulation in bottom sediments as well as to passive/active transfer through many biological food chains [17]. Due to the increasing prevalence of nano-silver application and its possibility of surface water penetration, safe concentration limits of nanoparticles in aquatic environments should be established [3].

The aim of this study was to determine the effects of molecular silver on the survivability and development of aquatic plants exposed to its activity. The possibility of hydrophyte use as a water pollution indicator also was examined.

Experimental Procedures

Three species of aquatic plants: *Oedogonium* sp. (Fuzz algae), *Versicularia dubyana* (Java moss), and *Lagarosyphon madagascariensis* (Madagascar lagarosiphon) were used in our research. Select plants represented three common hydrophytes in surface waters: filamentous algae, bryophytes, and higher plants [18-20]. Algae were collected from the Oder River in Poland. Other specimens were derived from the university aquarium collection.

Oedogonium sp. is a stringy, free-floating plant. Generally, it exists in stagnant or slow-flowing waters, well exposed and oxygenated. It commonly forms colonies characterized by a complex structure. Its body is elongated, translucent, and fixed to the base with small hooks produced by specially constructed pear-shaped cells [21].

Versicularia dubyana is small, richly leaved with a well-selected main body axis and small branches. This typical amphibian habitat area includes moss forest streams. It grows well both in submergence and on water surface, in humid air conditions. It easily clings to rocks and roots, rises quickly, and forms bushy turf. It accepts any kind of water (even slightly salty), any type of lighting, with a wide range of temperature tolerance [22].

Lagarosyphon madagascariensis is a medium-sized plant, green and leafy. It is similar to waterweed, but does not produce verticals, and its leaves densely standing bent downwards are placed in a distributed manner as a spiral around the stem. Lagarosiphon comes from around Madagascar, as well as from other tropical areas of Africa and South America. It is a popular aquarium plant. It prefers clean, warm, well-oxygenated, and aerated water. Except for strong lighting, it has no other special requirements [23].

The plants were cleaned up, cut into 1 cm pieces, and placed in plastic containers with Hoagland solution containing silver-nanoparticles. Concentrations of nano silver were as follows: 0.0, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, and 10.0 $mg \cdot dm^{-3}$ Ag. The source of nanoparticles was NANO SILVER preparation (Nanoco, Poland). This product is an aqueous suspension of Ag-Si nanocomposite containing 2.0 $g \cdot dm^{-3}$ silver.

Light conditions were controlled through 16-hour light cycle (15W Tungsram lamp for regular lighting of 1200 lux in accordance with the plants' light requirements). The experiment was conducted for 10 days at 18ºC (±2ºC), in triplicate [1].

An effect of different silver-nanoparticle concentrations on plant morphology and growth were observed. Changes in plant length were measured by electronic caliper (0.05 mm accuracy).

Table 1. Average growth [mm] and survivability of *Oedogonium* sp. incubated for 10 days at a concentration of nano silver solutions 0.0-10.0 mg·dm^{-3} Ag.

Silver concentration [mg·dm^{-3} Ag]	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
0.0	1.00 (nc)	1.04 (nc)	1.10 (nc)	1.16 (nc)	1.23 (nc)	1.28 (nc)	1.37 (nc)	1.39 (nc)	1.43 (nc)	1.46 (nc)
0.1	1.00 (nc)	1.03 (nc)	1.07 (nc)	1.13 (A_1)	1.20 (A_1)	1.24 (A_1)	1.30 (A_1)	1.33 (A_1)	1.35 (A_1)	1.37 (A_1)
0.2	1.00 (nc)	1.01 (nc)	1.05 (A_1)	1.09 (A_1)	1.14 (A_1)	1.20 (A_1)	1.24 (A_1)	1.27 (A_1)	1.35 (A_1)	1.34 (A_1)
0.5	1.00 (nc)	1.01 (nc)	1.05 (A_1)	1.09 (A_1)	1.14 (A_1)	1.20 (A_1)	1.21 (A_1)	1.26 (A_1)	1.30 (A_1)	1.30 (A_1)
1.0	1.00 (A_2)	1.00 (A_2)	1.01 (A_2/B)	1.02 (A_2/B)	1.04 (A_2/B)	1.04 (A_2/B)	1.04 (A_2/B)	1.04 (A_2/B)	1.04 (A_2/B)	1.05 (A_2/B)
2.0	1.00 (A_2/B)	1.00 (A_2/B)	1.01 (A_2/B)	1.01 (A_2/B)	1.01 (A_2/B)	1.01 (A_2/B)	1.01 (A_2/B)	1.01 (A_2/B)	1.01 (A_2/B)	1.01 (A_2/B)
5.0	1.00 (A_3/B)	1.00 (A_3/B)	1.01 (A_3/B)	1.01 (A_3/B)	1.01 (A_3/B)	1.01 (A_3/B)	1.01 (A_3/B)	1.01 (A_3/B)	1.01 (A_3/B)	1.01 (A_3/B)
10.0	1.00 (A_4/B)	1.00 (A_4/B)	1.00 (A_4/B)	1.00 (A_4/B)	1.00 (A_4/B)	1.00 (A_4/B)	1.00 (A_4/B)	1.00 (A_4/B)	1.00 (A_4/B)	1.00 (A_4/B)

A – plant color change (A_1 – dark green, A_2 – gray, A_3 – brown, A_4 – black), B – plant death, nc – no morphological changes

Table 2. Average growth [mm] and survivability of *Versicularia dubyana* incubated for 10 days at a concentration of nano silver solutions 0.0-10.0 mg·dm^{-3} Ag.

Silver concentration [mg·dm^{-3} Ag]	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
0.0	1.00 (nc)	1.04 (nc)	1.12 (nc)	1.17 (nc)	1.25 (nc)	1.29 (nc)	1.36 (nc)	1.40 (nc)	1.43 (nc)	1.43 (nc)
0.1	1.00 (nc)	1.04 (nc)	1.11 (nc)	1.17 (nc)	1.24 (nc)	1.28 (nc)	1.36 (nc)	1.38 (nc)	1.43 (nc)	1.43 (nc)
0.2	1.00 (nc)	1.02 (nc)	1.09 (nc)	1.14 (nc)	1.20 (nc)	1.24 (nc)	1.30 (nc)	1.35 (nc)	1.40 (nc)	1.40 (nc)
0.5	1.00 (nc)	1.01 (nc)	1.05 (nc)	1.08 (nc)	1.14 (A_1)	1.18 (A_1)	1.23 (A_1)	1.26 (A_1)	1.37 (A_1)	1.37 (A_1)
1.0	1.00 (nc)	1.01 (A_1)	1.05 (A_1)	1.09 (A_1)	1.15 (A_2)	1.18 (A_2)	1.23 (A_2)	1.25 (A_2)	1.27 (A_2)	1.27 (A_2)
2.0	1.00 (A_1)	1.01 (A_1)	1.05 (A_1)	1.07 (A_2)	1.13 (A_2)	1.19 (A_2)	1.20 (A_2)	1.20 (A_2)	1.20 (A_2)	1.20 (A_2)
5.0	1.00 (A_2)	1.00 (A_2)	1.03 (A_2/B)	1.03 (A_2/B)	1.03 (A_2/B)	1.03 (A_2/B)	1.03 (A_2/B)	1.03 (A_2/B)	1.03 (A_2/B)	1.03 (A_2/B)
10.0	1.00 (A_2)	1.00 (A_2/B)	1.00 (A_2/B)	1.00 (A_2/B)	1.00 (A_2/B)	1.00 (A_2/B)	1.00 (A_2/B)	1.00 (A_2/B)	1.00 (A_2/B)	1.00 (A_2/B)

A – plant color change (A_1 – dark green, A_2 – gray), B – plant death, nc – no morphological changes

Statistica 10.0 was used for the statistical analysis, especially for an analysis of variance and Duncan's *post-hoc* test. Statistical differences were determined at $p<0.05$.

The box-plot graphs made in Statistica 10.0 show solutions with average±std deviations (boxes), and average±1.96×std deviations (plots).

Results and Discussion

The results obtained in the study are presented in Tables 1-4 and Figs. 1 and 2.

The change in Ag-nanoparticle solution color to gray was observed one day after establishing the experiment, which was caused by nano silver oxidation. This effect was confirmed in various synthesis of silver nanoparticles from different donors [24, 25]. The intensity of solution staining increased with the concentration of Ag and continued until the fifth day of the experiment. After this period, test solutions gradually lost their color. The observed water color changes into brown-black were considered a normal effect, but strong and dark color gives reason to believe that the high concentration of nanoparticles limits the permeability of light in water, which may be a lethal factor for aquatic organisms (limited photosynthesis ability, low oxygen production, plant death).

Oedogonium sp. samples immersed in 2.0-10.0 mg·dm^{-3} Ag solutions became obscured and plants were completely invisible. The first signs of plant death were noticed within 24 hours in solutions with concentrations of 1.0 mg·dm^{-3} Ag or higher. The plants fell to the bottom, losing live green color and became gray (in 1.0 and 2.0 mg·dm^{-3} Ag), brown (5.0 mg·dm^{-3} Ag) or black (10.0 mg·dm^{-3} Ag). In other solutions, in addition to darkening fronds, there were no dying

Table 3. Average growth [mm] and survivability of *Lagarosyphon madagascariensis* incubated for 10 days at nano silver solution having a concentration of 0.0-10.0 mg·dm^{-3} Ag.

Silver concentration [mg·dm^{-3} Ag]	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
0.0	1.00 (nc)	1.14 (nc)	1.21 (nc)	1.37 (nc)	1.44 (nc)	1.58 (nc)	1.66 (nc)	1.78 (nc)	1.85 (nc)	1.97 (nc)
0.1	1.00 (nc)	1.09 (nc)	1.20 (nc)	1.30 (nc)	1.41 (nc)	1.50 (nc)	1.66 (nc)	1.74 (nc)	1.83 (nc)	1.95 (nc)
0.2	1.00 (nc)	1.08 (nc)	1.12 (nc)	1.24 (nc)	1.30 (nc)	1.44 (nc)	1.50 (nc)	1.65 (nc)	1.70 (nc)	1.80 (nc)
0.5	1.00 (nc)	1.05 (nc)	1.09 (nc)	1.15 (nc)	1.23 (nc)	1.38 (nc)	1.43 (A_1)	1.56 (A_1)	1.60 (A_1)	1.71 (A_1)
1.0	1.00 (nc)	1.04 (nc)	1.05 (A_1)	1.09 (A_1)	1.15 (A_1)	1.20	1.23	1.27	1.30	1.50
2.0	1.00 (A_1)	1.03 (A_1)	1.06 (A_1)	1.09 (A_2)	1.14 (A_2)	1.19 (A_2)	1.20 (A_2)	1.22 (A_2)	1.26 (A_2)	1.35 (A_2)
5.0	1.00 (A_2)	1.02 (A_2)	1.04 (A_2/B)	1.05 (A_2/B)	1.09 (A_2/B)	1.06 (A_2/B)	1.07 (A_2/B)	1.09 (A_2/B)	1.09 (A_2/B)	1.11 (A_2/B)
10.0	1.00 (A_2)	1.02 (A_2/B)	1.03 (A_2/B)	1.05 (A_2/B)	1.05 (A_2/B)	1.06 (A_2/B)	1.06 (A_2/B)	1.07 (A_2/B)	1.07 (A_2/B)	1.07 (A_2/B)

A – plant color change (A_1 – dark green, A_2 – gray), B – plant death, nc – no morphological changes

Table 4. Differences resulting from Duncan's test in day 10 of the experiment with various concentrations of Ag-nanoparticles.

Silver concentration [mg·dm^{-3} Ag]	0.1	0.2	0.5	1.0	2.0	5.0	10.0
	M=1.5833	M=1.5133	M=1.4600	M=1.2733	M=1.1867	M=1.0500	M=1.0233
0.0	0.841229	0.582866	0.425681	0.099889	0.046265*	0.012129*	0.009664*
0.1		0.702539	0.525990	0.131465	0.062532	0.016749*	0.013472*
0.2			0.770879	0.224032	0.113132	0.032236*	0.026260*
0.5				0.315142	0.168593	0.050853	0.042214*
1.0					0.636733	0.256593	0.218935
2.0						0.458776	0.402889
5.0							0.884167

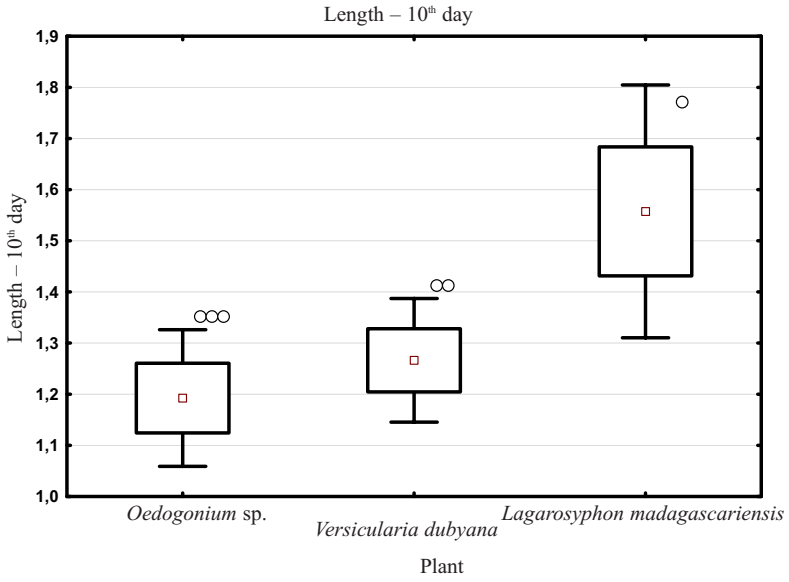
*statistically significant ($p < 0.05$)

algae. From day three the differences in algae color in 0.2 mg·dm^{-3} were found, whereas from days four to 10 of incubation this effect also was observed in ≥0.1 mg·dm^{-3} Ag. The influence on plant death was noticed on the first day in ≥2.0 mg·dm^{-3} Ag, and from day three to 10 also in 1.0 mg·dm^{-3} Ag samples.

Almost no degradation and color changes were noted in the case of *Versicularia dubyana* and *Lagarosyphon madagascariensis*. Only in the case of *Lagarosyphon madagascariensis* were no color change differences found in 5.0 mg·dm^{-3} Ag on days five and six. The plants were destroyed and firmly ashen after two days of incubation at the two highest concentrations, and from day two also in 1.0 mg·dm^{-3} Ag. In 0.5 mg·dm^{-3} Ag a different color was perceived only on day five. The plants in 0.0-2.0 mg·dm^{-3} Ag retained a weak green color (compared to controls). What is important is that these plants did not die until the end of the experiment. The first deaths of *Versicularia dubyana* and *Lagarosyphon madagascariensis* were observed on day two in 10.0 mg·dm^{-3} Ag, and in 5.0 mg·dm^{-3} Ag on day three.

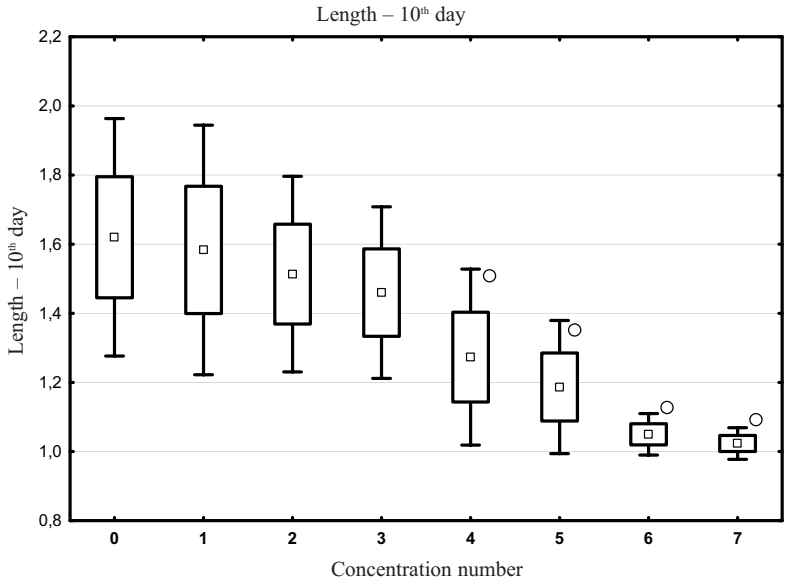
The survivability of plants exposed to Ag-nanoparticles was inversely proportional to the applied metal concentration. This fact was previously confirmed also for potato (*Solanum tuberosum* L.), tomato (*Lycopersicon esculentum* Mill.), Arabidopsis (*Arabidopsis thaliana*), cucurbits, and others. It is suggested that proper use of low-dose Ag-nanoparticles may even stimulate growth and survival of seedlings or plant juvenile phases, exhibiting concurrently a sterilizing effect against pathogenic microorganisms from their environment to protect plants against diseases in their early stages of life [26, 27].

The growth differences were observed between examined groups of plants compared to the control group. Variance analysis of plant length in subsequent days of the study allowed for post-hoc tests in relation to used Ag nanoparticle concentrations and plant species. Plants in control solutions (without the addition of nano silver) were growing normally in contrast to the experimental groups. Just from day two, the plant growth disorders were noticed in proportion to the metal concentration; silver was the only



○ – statistically significant ($p < 0.05$)

Fig. 1. The differences in the average length of the plants on day 10 of the experiment [cm].



○ – statistically significant ($p < 0.05$)

Fig. 2. Differences in the average length of plants in the following concentrations of Ag 0-0.0, 1-0.1, 2-0.2, 3-0.5, 4-1.0, 5-2.0, 6-5.0, and 7-10.0 mg·dm^{-3} Ag.

limiting factor of plant growth and survivability. The highest length was noted for *Lagarosyphon madagascariensis* and the smallest for *Oedogonium* sp.

No significant correlations were found for *Oedogonium* sp. length in the subsequent days of the experiment, and this is because of the biggest limitation of this species even in the lowest concentration of Ag-nanoparticles. There was a negative correlation ($p<0.05$) for *Versicularia dubyana* starting from day two, and for *Lagarosyphon madagascariensis* only from the day five. This demonstrates the sensitivity on the investigated species to various Ag-nanoparticles concentrations. The biggest differences between the concentrations were noted in the case of growth on day four. In day 10 the correlations between the species, concentration, and length were significant ($p<0.05$).

Starting day two there were significant ($p<0.05$) differences between *Oedogonium* sp. and *Lagarosyphon madagascariensis*. However, significant differences between *Versicularia dubyana* and *Lagarosyphon madagascariensis* ($p<0.05$) were observed only in days two and 10 (Fig. 1).

There was also significant difference between control and examined groups (in 2.0-10.0 mg·dm^{-3} Ag samples). This effect remained for the whole experimental period. There were no significant differences between the control group and the group with the two lowest concentrations of Ag (Fig. 2).

High nano silver additives may significantly reduce growth of plants (leaves, roots, stems) without destroying them completely. Moreover, physiological changes were also reflected in limited metabolism, chlorophyll activity, or changes in the quantity and composition of the essential plant oils [28-30].

Growth inhibition effect in higher plants or biocidal activity of Ag-nanoparticles against green algae can be an advantage. Because of the selective action of silver it may limit excessive gain of green plants in water reservoirs and thus reduce the possibility of unwanted expansion [1, 2, 13, 16].

Taking into consideration common Ag-nanoparticles used in various branches of technology, industry, and public consumption, there is serious need to assess the scale of water (but not only) contamination in order to describe effective methods for its elimination.

Conclusions

- Ag-nanoparticles limit the growth and survivability of some aquatic plants
- *Oedogonium* sp. strongly reacts to the content of nano silver in contrast to *Versicularia dubyana* and *Lagarosyphon madagascariensis*

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