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First assessment of the anti-cyanobacterial potentialities of the invasive weed *Verbesina encelioides* against *Microcystis aeruginosa* growth

M. Lahlali[a], N. Manaut[b], M. Loudiki[c], M. Douma[a,*]

[a]*Natural Resources Engineering and Environmental Impacts Team, Multidisciplinary Research and Innovation Laboratory, Polydisciplinary Faculty of Khouribga (FPK), Sultan Moulay Slimane University, Khouribga, Morocco, emails: douma_mountasser@yahoo.fr (M. Douma), meryemlahlali00@gmail.com (M. Lahlali)*
[b]*Faculty of Sciences Semlalia, Laboratory of Microbial Biotechnology, Agrosciences and Environment, Cadi Ayyad University, Labeled Research Unit-CNRST N°4, Marrakesh, Morocco, email: manaut_najat@yahoo.fr*
[c]*Laboratory of Water, Biodiversity and Climate Change, Phycology, Biotechnology and Environmental Toxicology Research Unit, Department of Biology, Faculty of Sciences Semlalia, Cadi Ayyad University, Marrakesh, Morocco, email: loudiki@uca.ac.ma*

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ABSTRACT

This work aims to assess the anti-cyanobacterial potentialities of the invasive weed *Verbesina encelioides* against *Microcystis aeruginosa* growth. In an experimental bioassay, the aqueous extract of the aerial parts of *V. encelioides* (AEVE) was tested to investigate its activity on *M. aeruginosa* growth. Several growth parameters and physiological indicators were assessed. To reveal the potentially allelochemicals, phenolic and flavonoid contents were quantified in AEVE. Results demonstrated that AEVE inhibit the growth of *M. aeruginosa* in a concentration dependent way. Furthermore, under both highest concentration of AEVE (0.75 and 1 mg/mL), the inhibitory rate (IR) reaches 71% and 79% only after 4 d (d) of experimentation, respectively. The highest IR (93%) was achieved at the highest concentration (1 mg/mL) on 12-d. Thus, the inhibition rates were confirmed by powerful IC50 and IC90 values (0.37 and 0.8 mg/mL), respectively. Additionally, during the experimental period, all four-treatment groups (0.25–1 mg/mL) showed a significant decrease in the content of Chlorophyll-a and carotenoids compared to the control. Overall, the results demonstrate the anti-cyanobacterial effect of AEVE on *Microcystis* growth. Moreover, the invasive weed *V. encelioides* might be proposed as a potential environmentally friendly anti-cyanobacterial agent to control *Microcystis* blooms in the eutrophic water bodies.

Keywords: *Microcystis aeruginosa*; Blooms; Inhibition; Algaecide; *Verbesina encelioides*; Terrestrial invasive plant; Green approach

1. Introduction

Harmful cyanobacterial blooms (CyanoHABs) have become a serious problem in drinking water sources and recreational purposes worldwide. *Microcystis* spp. are the most involved cyanobacterial species in CyanoHABs [1]. *Microcystis* blooms cause severe water quality deterioration due to scum formation, hypoxia, taste, and odors [2,3]. Furthermore, they are often toxic and produce hepatotoxins (Microcystins), which consequently contaminate drinking water and cause adverse effects on the health of various living organisms [4].

To reduce noxious effects of toxic cyanobacteria in situ and/or in water treatment plants, diverse physical methods have been used such as artificial mixing and thermal destratification [5–7], filtration, ultraviolet, and ultrasound treatments. These last are usually high cost and take a long time [8–10]. Chemical methods using chemical oxidants are

* Corresponding author.

highly efficient and low-cost methods. However, chemicals generate secondary pollution, which directly or indirectly affects the health of both ecosystems and humans [11,12]. Therefore, plant-based alternatives as green approaches to control *Microcystis aeruginosa* growth have widely used macrophytes [13,14] and medicinal plants [15–18]. In various works devoted to the research of the algicidal and allelopathic potentialities of plants, plant-derived polyphenolics were the most common allelochemicals in HABs control [18,19]. The phytochemical investigation of extracts allowed the identification of several compounds. They are mainly flavonoids, glycosides, terpenoids, saponins and several phenolic acids [18,20].

Recent bioassay researches have become interested in the use of invasive plants, with anti-cyanobacterial potential to control harmful algal blooms as a 2-fold innovative solutions: first, to reduce the produced invasive plant biomasses; and second, to eliminate the proliferation of toxic cyanobacteria by a green agent [14,19–21]. However, the use of invasive alien plants remains little limited; despite the benefits that can offer in controlling *Microcystis* blooms [14,21].

Otherwise, invasive plants is one of the main emerging problems in terrestrial ecosystems, namely in agroecosystems where they cause crop damage. The excessive biomass produced by invasive plants presents a challenge for the managers of agroecosystems [22]. A sustainable alternative can consist of turning a presumably "useless" biomass toward an economic and/or ecological valorization [19].

V. encelioides (Cav.) Benth. & Hook. Filex Gray (Asteraceae) is a perennial herb, which belongs to the Asteraceae family. It is an indigenous species from North and South America [23], and now found in numerous countries worldwide [24]. It is often invaded in vast expanses of pastures, orchards, and forest areas in tropical and subtropical regions [25]. In Mediterranean region, as one of the most invasive weeds, it commonly spread and colonizes wastelands, roadside borders and field crops [26,27]. Several reports have demonstrated their antimicrobial [28], antiviral [29], antioxidant [30], and anticancer [31,32] activities.

V. encelioides can occur in different soil types due to its high competition potential using the release of allelochemicals as the main invasion factor [33]. Its allelopathic effects have been demonstrated on various plants in particular *Raphanus sativus* L. (radish), *Zea mays* L. (maize), *Pennisetum glaucum* (L.) R. Br. (pearl millet), *Triticum* sp. (wheat), *Oryza* sp. (rice), *Lens culinaris* M. (gram), *Raphanus sativus* L [34,35]. However, no indication in the literature has reported its allelopathic potential in the biocontrol of harmful algae.

Phytochemical analysis of *V. encelioides* revealed the presence of various potential allelochemicals including phenolic acids, flavonoids, sesquiterpenes, galegine, and triterpenoids [31,36]. Most of these plant allelochemicals are considered as the source of green algaecides because both of their biodegradability and efficiency in the inhibition of neighbor plant growth [37].

This work aims to investigate, for the first time, the anti-cyanobacterial potentialities of the weed *V. encelioides* on *M. aeruginosa* growth in an experimental bioassay.

2. Materials and methods

2.1. Biological materials

M. aeruginosa was sampled from the eutrophic Lalla Takerkoust reservoir, Morocco, (31°21′36″ N; 8°7′48″ W) in August 2020. It was isolated and maintained as a monoalgal non-axenic strain under aseptic laboratory conditions at 25°C ± 1°C under light intensity of 70 μE/m²·S, with a light/dark cycle of 15/9 h.

V. encelioides was collected in May 2021 from a wild land, in the locality of El Jouala (Province Kalaâ des Sraghna, Morocco) (31°88′79″ N; 7°44′15″ W). Aerial parts were rinsed several times with distilled water to remove debris, dried away from sunlight at ambient temperature (25°C), and then crashed into powder prior to extraction.

2.2. Preparation of plant extracts

The aqueous extraction of the aerial plants was carried out according to the method described by Chen et al. [38], with small modifications. Briefly, 10 g of dried biomass powder leaves was placed in 100 mL distilled water under agitation (45°C; 48 h). After that, the macerate was autoclaved and maintained as an aqueous extract at 4°C.

2.3. Quantification of total phenolic and total flavonoids in extracts

Total phenolic (TPs) concentration was determined with the Folin–Ciocalteu method [39]. Total flavonoids (TFs) content was determined by the method described by Kim et al. [40].

2.4. Anti-cyanobacterial bioassay

Five groups of Erlenmeyer flasks (500 mL) containing Z8 medium [41] to a final volume (300 L) were used to contain 5 concentrations (0 (control), 0.25, 0.5, 0.75, 1; V/V%) of the AEVE whish are equivalents to 0, 0.25, 0.5, 0.75, 1 mg·DW/mL, respectively. A volume of *M. aeruginosa*, in exponential growth phase, was added to each flask to make an initial density (0.99×10^6 cell/mL). The cultures were incubated at 25°C ± 1°C, illuminated in 15/9 h light-dark cycle with fluorescent tubes (70 μE/m²·S) within 12 d. All groups were conducted in triplicate. *Microcystis* growth under different treatments was quantified, each 2 d, using Malassez hemocytometer.

2.5. Inhibition parameters

The effects of the AEVE on *Microcystis* growth were expressed using three parameters: inhibitory rate (IR), the half-maximal inhibitory concentration (IC50) and the IC90. IR of *Microcystis* growth was determined according to Eq. (1):

$$\mathrm{IR}(\%) = \left[\frac{N_0 - N}{N_0}\right] \times 100 \quad (1)$$

where N_0 and N (cells/mL) are the cell density in the control and treatment cultures, respectively. IC50 and IC90

are calculated based on the concentration range (x) used according to the inhibition rates (Y) recorded at the end of the experiment.

2.6. Morphological modification

M. aeruginosa growth under different treatments was examined during the experiment using an optical microscope with a camera (Motic BA210) under 400× magnification. Several morphological criteria (cell diameter, form and condensation of colonies, pigmentation, and vacuoles density) were elucidated. Images were taken and any discrepancies within the culture were documented.

2.7. Pigments determination

The concentrations of Chlorophyll-a and carotenoids were quantified by spectrophotometry according to Lichtenthaler and Wellburn [42]. They extracted with ethanol 95% at 4°C for 48 h, and then determined using a spectrophotometer (TOMOS V-1100) at 470, 649, and 665 nm. The following formulas were used to calculate the concentrations (μg/mL): [Chlorophyll-a] = 13.95 × DO665 – 6.88 × DO649; [Carotenoïds] = [(1,000 × DO470) – (2.05 Chl-a)]/229.

2.8. Statistical analysis

Data with three replicates were statistically analyzed by two-way analysis of variance (ANOVA 2) with Tukey's test to assess differences between exposure concentrations and control at $p = 0.05$. Correlation coefficients were calculated between cellular density and TPs and TFs, concentrations in the end of experimentation.

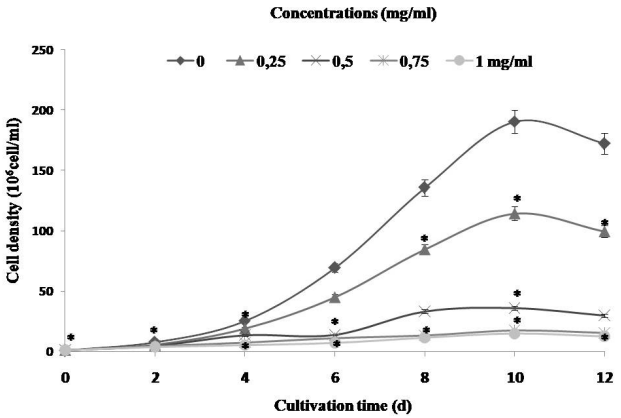

Fig. 1. Effect of different concentrations of AEVE on *Microcystis aeruginosa* growth. Error bars represent the standard deviation ($n = 3$). *<0.05 indicates significant differences compared to the untreated culture (ANOVA two-way).

3. Results

3.1. Assessment of the inhibitory effect on growth of *M. aeruginosa*

Fig. 1 shows the growth of *M. aeruginosa* under the AEVE. In control group, the cell densities remained between 0.99 × 10^6 and 190.3 × 10^6 cell/mL as un optimum value at 10-d. In contrast, *Microcystis* cell densities were significantly reduced ($p < 0.05$) during the bioassay period at the different tested concentrations (0.25, 0.5, 0.75, and 1 mg/L).

The IR appeared to be dose dependent. Overall, IRs exceed 40% only after 2-d at the three tested concentrations (0.5, 0.75 and 1 mg/mL) (Table 1). Under both highest concentrations of AEVE (0.75 and 1 mg/mL), the IRs reach 71% and 79% after 4 d of experimentation, respectively. The highest IRs (¨ > 90%) were achieved after the 8 d at the highest concentrations (0.75 and 1 mg/mL). The optimum IR was obtained at the 12 d under 1 mg/mL concentration.

Thus, the bioassay results were expressed in terms of the inhibitory concentrations. Both the IC50 and IC90 were calculated. In the end of the experimentation, the IC50 and IC90 mentioned two values 0.37 and 0.8 mg/mL, respectively (Fig. 2).

3.2. Effects on morphological changes in *M. aeruginosa*

The morphological changes in *M. aeruginosa* cultures under treatments are elucidated in Fig. 3. In control groups (0%), *M. aeruginosa* cells were appeared clearly structured

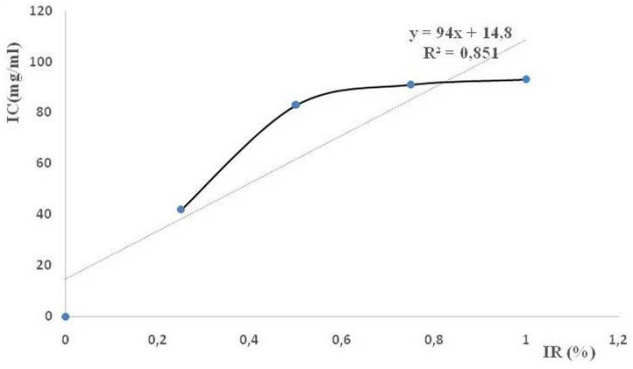

Fig. 2. Inhibitory concentrations recorded according to the IRs during the bioassay.

Table 1
Inhibitory effects expressed as inhibitory rate (%) of AEVE on *Microcystis aeruginosa* growth

Treatments (mg/mL)	0	2	4	6	8	10	12
0.25	0	28 ± 0.25	25 ± 0.02	35 ± 0.05	38 ± 0.02	40 ± 0.01	42 ± 0.03
0.5	0	40 ± 0.08	47 ± 0.1	80 ± 0.02	76 ± 063	81 ± 0.03	83 ± 0.07
0.75	0	44 ± 0.03	71 ± 0.15	84 ± 0.04	90 ± 0.04	91 ± 0.02	91 ± 0.00
1	0	52 ± 0.16	79 ± 0.03	90 ± 0.02	92 ± 0.01	92 ± 0.18	93 ± 0.01

with regular surfaces. For these last, cell forms were rounded and pigmented, with cell diameter between (2–2.3 µm) in the end of the treatment period (Fig. 3. C, C.I). However, under high concentrations (0.75 and 1 mg/mL), *M. aeruginosa* cells lose their standard and regular form to a cell clusters (1.4–1.7 µm cell diameter), forming sediment aggregates, with anaform, destroyed and shrinking, especially, in the end of the test period (Fig. 3. T, T.I). These morphological changes were accompanied by coagulation and sedimentation of cyanobacterial cells, especially after 4 d of exposure, with yellowing cell colors.

3.3. Effects on photosynthetic pigments

In order to assess the physiological modification, two photosynthetic pigments were measured (Chl-a and carotenoids) as physiological indicators of *Microcystis* growth in the bioassay. During the 12-d experimental period, all four-treatment groups (0.25–1 mg/mL) demonstrated a significant decrease ($p < 0.05$) in the content of Chl-a and carotenoids, compared to the control. With the increase in extract concentrations, the pigment contents appear to be strongly inhibited.

After 6 d of bioassay, Chl-a and carotenoid contents, at both the highest concentrations (0.75–1 mg/mL) showed significant and important inhibition, with values ranging from (47%–66%) and (45%–65%), respectively (Fig. 4).

3.4. Phytochemical characterization

The results of the phytochemical characterization are shown in Table 2. AEVE exhibited important values on TPs, TFs. As well, a high-significant correlations have been well obtained between the IRs of the three high concentration (0.5%–1%) and TPs, and TFs concentrations (0.95, 0.94), respectively.

4. Discussion

This study is the first assessment of the anti-cyanobacterial potentialities of *Verbesina encelioides*. As is obtained, AEVE act negatively on the *M. aeruginosa* growth where the inhibitory effect appeared dose dependent (Fig. 1). Under the highest concentrations (0.75 and 1 mg/mL), the IRs reach 71% and 79% on day 4 of experimentation, respectively. After, it was excessed (90%) on 8-d (Table 1). The maximal IR (90%) was achieved under 1 mg/mL concentration on day 12. Thus, the inhibition rates are confirmed by powerful IC50 and IC90 values (0.37 and 0.8 mg/mL), respectively (Fig. 2 and Table 1).

This strong inhibition demonstrated the high anti-cyanobacterial potential of AEVE against *M. aeruginosa*. These results are in agreement with those observed in other previous works under similar concentration (0.1–2 mg/mL) of aqueous extracts: *Ailanthus altissima* (66.3%–91.8%) on 5 d [43], *Thalia dealbata* (92.7%) on 7-d [44], *Nymphaea tetragona*, *Typha orientalis*, *Nelumbo nucifera* and *Iris wilsonii*, (75%–82%) during 19 d [38], *Thymus satureioides*, *Achillea ageratum*, *Artemisia herba-alba*, and *Origanum compactum* (88%–95%) after 8 d [16,17]. Lahlali et al. [21] demonstrated that the aqueous extract of the invasive weed *Oxalis pes-caprae* L. effectively inhibited the growth *M. aeruginosa* on 10 d (86%).

In our experimental study, growth inhibition is accompanied by a decrease in the two photosynthetic pigments

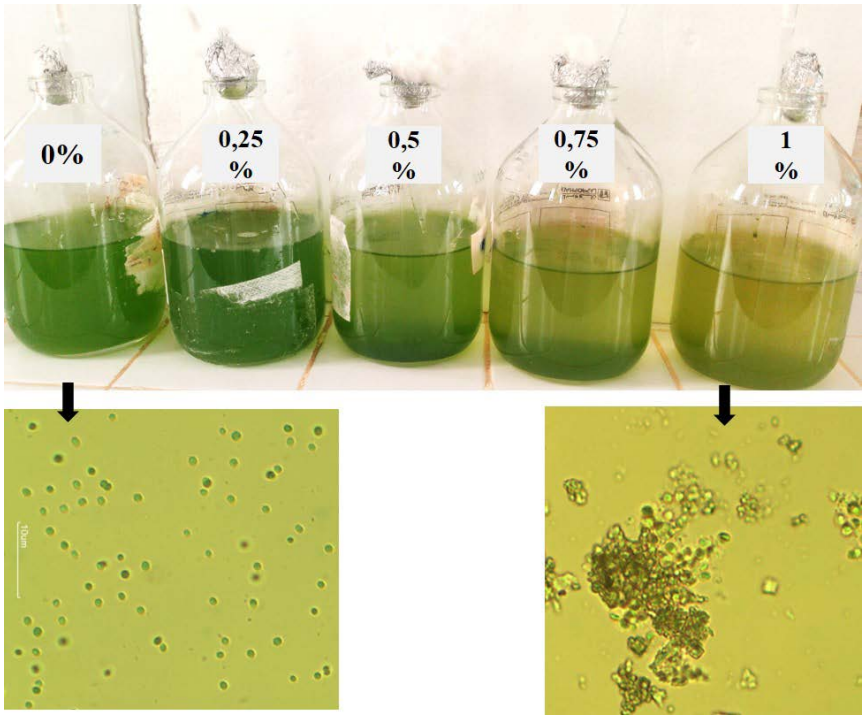

Fig. 3. Visual and microscopic observations of *Microcystis aeruginosa* cells in the control (0%; mg/mL) and treatment groups (0.25%–1%) of *Verbesina encelioides* L. aqueous extracts (Gr. x 40), with aggregated and sedimented cells, and yellow and pale colors in decomposed process.

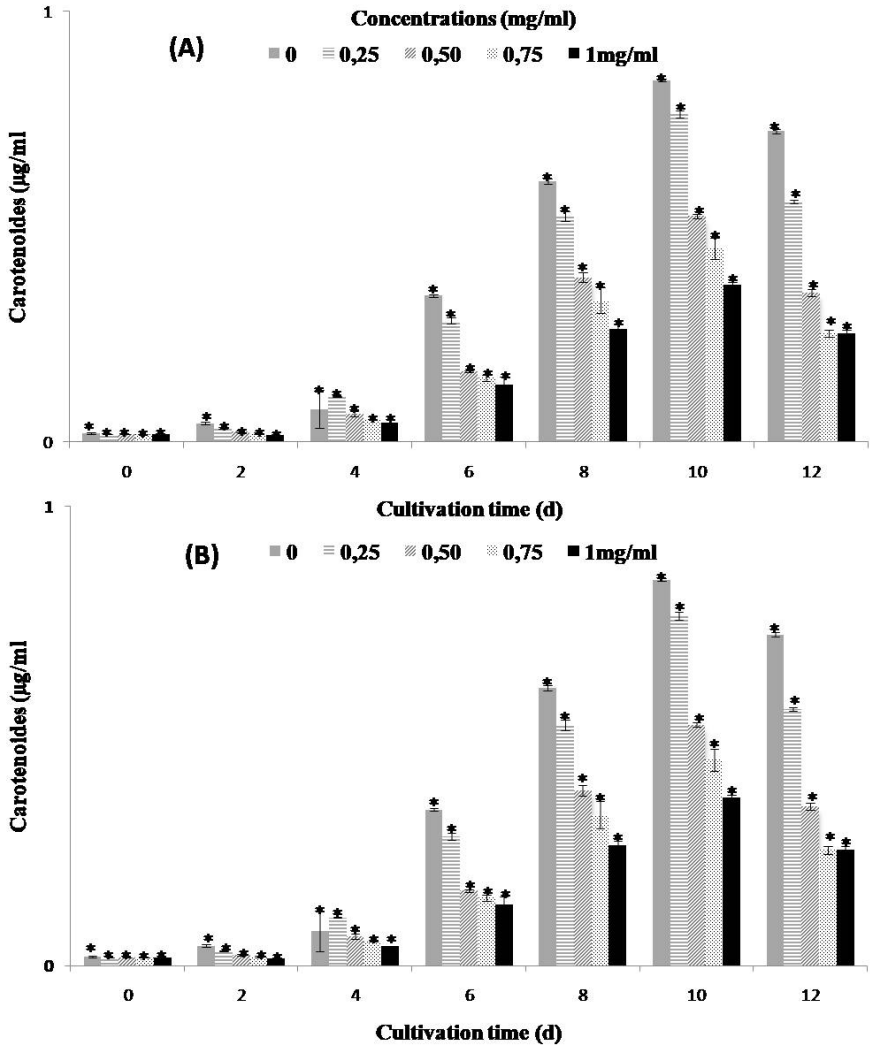

Fig. 4. Effect of AEVE on Chl-a (A) and carotenoids (B) in *Microcystis aeruginosa* cultures, respectively. Each value is the mean ± SD of three replicates. *indicates significant differences at $p < 0.05$ compared to the untreated culture (ANOVA two-way).

Table 2
Total phenolic, total flavonoids amounts in AEVE; and correlations between all amounts and IRs of the three high concentration (0.5%, 0.75%, 1%) after 12 d of exposure

	TP[a]	TF[b]
Concentrations	470 ± 0.5	71 ± 1
Coefficient of correlation	0.95	0.94

[a]µg gallic acid equivalent mL/aqueous extract;
[b]µg catechin equivalent/mL aqueous extract.

(Chl-a and carotenoids), as well as morphological changes in treatment groups (Figs. 3 and 4). These criteria are mainly indicators of physiological alterations occurring in a stressful environment. In this sense, previous studies have demonstrated the negative effect of plant extracts on Chl-a and carotenoids contents [16,17,45]. Its decrease demonstrates the disruption of photosynthesis, which affects *M. aeruginosa* growth and reproduction [46].

This inhibitory effect could be related to the potential allelochemicals released by *V. encelioides*. Previous works have indicated that plant-derived polyphenolics were the most common allelochemicals in CyanoHABs control [18,44,47]. They are mainly flavonoids, glycosides, terpenoids, saponins and several phenolic acids [38,45,48].

The phytochemical characterization of *V. encelioides* aqueous and organic extracts indicated that the main chemical compounds of this plant are phenolic acids, flavonoids, sesquiterpenes, galegine, and triterpenoids [31,36]. The most of these bioactive compounds were known by their antioxidant and antimicrobial activities, and belong to the common allelochemicals [19].

From the obtained results, the important values of TPs and TFs may play potentially role in the inhibitory activity (Table 2). These results are in agreement with previous works indicated the effect of TPs and TFs in the *M. aeruginosa* inhibition [44,49,50]. There is well known that the allelochemical compounds inhibited the growth of the cell by altering both the physiological state and cellular

structure [49,51]. Phenolic acids exhibit cell-permeability features because of their amphiphilic and lipophilic nature [52]. Wang et al. [53] demonstrated that p-coumaric acid and ferulic acid disrupted the cell membrane integrity of *M. aeruginosa*. Furthermore, during stressful situations, reactive oxygen species (ROS) act on cell membranes by degrading unsaturated phospholipids that increase the permeability of the membranes [42]. Thus, disruptions in the antioxidant defense system inhibit photosynthesis and oxygen evolution due to interactions with PS II components [54], which ultimately induce the cell death [15].

5. Conclusion

The ability of the AEVE plant to suppress the growth of *M. aeruginosa* is demonstrated in this study. This effect is dose dependent. The highest IR (93%) was achieved on 12-d at the highest concentration (1 mg/mL). Thus, the inhibition rates were confirmed by powerful IC50 and IC90 values (0.37 and 0.8 mg/mL), respectively. Additionally, during the 12-d experimental period, all four-treatment groups (0.25–1 mg/mL) demonstrated a significant decrease in the content of Chlorophyll-a and carotenoids compared to the control. TPs, TFs, characterized may be the main responsible allelochemicals.

Consequently, *Verbesina* plant can be recommended as an environmentally friendly agent to treat waters contaminated by *M. aeruginosa* blooms. Other approaches will be required in the future to identify the dominant and specific allelochemicals, as well as to study its potential effects on aquatic ecosystems.

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Conflict of interests

The authors declare no conflict of interests.

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