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Molecular Identification of the Iraqi Aquatic Fern *Azolla pinnata* and Evaluation of its Efficiency in Reducing CO_2 under Various Environmental Conditions

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ABSTRACT

Background: Azolla is one of the fern plants that has great importance in many fields, such as its value as animal feed and its possession of a number of active substances with antimicrobial and antioxidant properties, in addition to its role in consuming carbon dioxide, which has an important role in climate modification.

Methods: This study was conducted to determine the optimal conditions for *A. pinnata* growth and its reduction of carbon dioxide, which has a positive effect in reducing global warming on planet Earth. *Azolla pinnata* was isolated from the Tigris River-IRAQ and molecularly identified using nucleotide sequence analysis of ITS within 5.8S rRNA gene.

Result: *Azolla* isolated for the first time from the Iraqi environment was molecularly identified to the species level *Azolla pinnata* strain IRAQ-Tikrit.Has,A.A and registered in NCBI under the accession number PQ836460.1. The results showed a significant increase in CO_2 concentration in *A. pinnata* culture over time when incubated in the dark at 10-40°C. On the other hand, the results revealed a continuous decrease in CO_2 concentration with increased light intensity over time. The interaction between *A. pinnata* growth duration and light intensity, the lowest CO_2 concentration reached 375.14 and 362.38 ppm when incubated at light intensity of 10000 lux for 10 days at 20 and 3°C, respectively. The results also showed that there was an increase in the growth of *A. pinnata* with an increase in the depth of the liquid nutrient medium up to 50 cm, the growth indicators such as absolute growth rate (AGR) and Standing *A. pinnata* crop (yt) reached 10.53 g/m^2/day and 106.42 g/m^2 with a decrease in CO_2 to 325.64 ppm, with no significant effects on the growth indicators and CO_2 concentrations when *A. pinnata* growing at depths of 60-80 cm. Azolla is one of the biological factors that play a role in reducing carbon dioxide, as it plays an important role in modifying the climate of the Earth's surface if the standard environmental conditions for its growth are established.

Key words: *Azolla pinnata,* Climate change, CO_2 Reduction, Light intensity.

INTRODUCTION

Azolla is an aquatic fern under the family Azollaceae which can easily be grown in a variety of water types including fresh water of lakes, ponds, swampy lands, rice paddies and slow moving waterways. It is described by the fact that it develops on water with the formation of small flat structure akin to leaves floating on the water surface.Azolla is a heterosporous fern and is mutualistic with bacteria related to cyanobacteria, nitrogen-fixing bacteria that dwell within the dorsal cavities of Azolla's fronds (Rames, 2019). It is characterized by its ability to float on the water's surface, forming small, flat, leaf-like structures that rest atop the water. *Azolla* is classified as a heterosporous fern and establishes a symbiotic relationship with nitrogen-fixing bacteria (cyanobacteria), which reside within the dorsal cavities of its fronds (Rames, 2019). Tropical and subtropical environments are suitable for the growth and reproduction of Azolla, however, it is distributed almost all over the region of North and South, America, Indian continent, African countries and Australia as mentioned by Korsa *et al*. (2024). From among such varieties of Azolla, the most common are *Azolla pinnata*, *Azolla nilotica* and *Azolla mexicana*. Among them, *Azolla pinnata* has been considered the most important biofertilizer due to its varieties, short Generation time and requirement of nutrients (Nasir *et al*., 2022).

Azolla is considered a promising application of biotechnology that can be scientifically utilized to mitigate environmental pollution. Its rapid growth rate and low

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production cost make it highly efficient, with the ability to double its biomass within a period of 3 to 6 days (Rehman *et al*., 2022). Due to its symbiotic relationship with the nitrogen-fixing cyanobacterium *Anabaena azollae*, Azolla efficiently captures CO_2 and converts it into organic matter through rapid growth. Studies indicate that Azolla can sequester up to 1.1 tons of CO_2 per hectare annually, making it a promising tool for climate change mitigation. Additionally, its high photosynthetic rate and ability to thrive in aquatic environments enhance its potential for bioremediation and sustainable agriculture (Wagner, 1997; Brouwer *et al*., 2018).

The photosynthetic rates obtained in different experiments vary widely, probably due to differences in species or strain, biomass per unit area and other conditions of experimentation. Reported photosynthetic rates for *A. caroliniana* ranged from 40 to 123, µmoles CO_2 mg^{-1} chlorophyll hr^{-1}. Net photosynthesis of *A. pinnata* varied from 2.0 to 12 ,µmol CO_2 m^{-2} S^{-l} (Daniel and Bartholomew, 1993). By reducing carbon dioxide emissions, Azolla plays a crucial role in mitigating air pollution and enhancing overall environmental quality (Hamdan and Hour, 2022). Additionally, Azolla serves as a valuable dietary supplement for various livestock species due to its rich content of essential vitamins and minerals, providing vital nutrition for animals (Ahmed *et al*., 2016; Bhatt *et al*., 2020).

In many developed and developing countries, Azolla is used as a substitute for chemical and nitrogen-based fertilizers, owing to its symbiotic relationship with nitrogen-fixing bacteria. This unique trait has positioned Azolla as a crucial component in agricultural practices (Chandrababu and Parvathy, 2025). Moreover, Azolla biomass can be used as a biofertilizer to increase the organic content of certain soil types, contributing to improved soil quality and enhanced agricultural productivity. This application also reduces agricultural costs and alle*via*tes the need for the production and use of organic fertilizers, which may have negative environmental impacts due to their high nitrogen content. Additionally, Azolla's ability to absorb nitrogen from the atmosphere further strengthens its role in promoting environmental sustainability and minimizing the negative impacts of agriculture (Baviskar *et al*., 2023). Carbon dioxide levels in the atmosphere can be reduced by absorbing large amounts through major ecosystems such as forests, which play a crucial role in lowering emissions. Additionally, several strategies can be employed to mitigate elevated carbon dioxide levels, including controlling and reducing emissions from fossil fuels, or removing carbon dioxide from the atmosphere through various methods known as carbon sequestration. This process encompasses both biological and non-biological techniques (Ussiri and Lair, 2017). Among the biological methods, Azolla, in its various species, plays a significant role in regulating the Earth's climate by absorbing large amounts of atmospheric carbon. Azolla is characterized by its rapid growth and ability to double its biomass within a short period, ranging from five to six days, offering a substantial opportunity to sequester some of the carbon dioxide emitted into the atmosphere. It is noted that the flourishing of *Azolla* in ancient times significantly contributed to the reduction of carbon dioxide levels, making it an effective tool for capturing large quantities of carbon and lowering its concentration in the environment (Kosesakal and Yildiz, 2019). The biomass produced by Azolla can subsequently be stored to sequester carbon and remove it from the active carbon cycle. Studies have indicated that 1 hectare of Azolla can capture approximately 21,266 kg of carbon dioxide. Research also shows that carbon dioxide emissions have slowed in their growth since the early 21st century (Hamdan and Hour, 2022).

Climate change is an environmental global event that has a great impact on the ecosystem and human health. Arguably one of the main drivers of climate change through carbon dioxide (CO_2) emissions with the removal or reduction in these CO_2 emissions using absorption of excess CO_2 being a vital solution to climate change. So it is highly required, you find other alternatives to remove CO_2 and Azolla offers one, various environmental conditions are key factors influencing Azolla's growth. Typically, rapid growth is associated with Azolla's production of carbon dioxide. Therefore, the current study aims to assess the impact of different light intensities, temperature levels and exposure durations on the effectiveness of Azolla in reducing CO_2 emissions.

MATERIALS AND METHODS

This study was conducted at the College of Agriculture - Tikrit University - Iraq, during 2022-2024.

Isolation of *Azolla pinnata*

A. pinnata was collected from the Tigris River-IRAQ , It was diagnosed phenotypically to the genus level by the herbarium of the College of Agriculture, Tikrit University

Molecular identification of *A. pinnata*

For DNA extraction 0.5 g of fresh, young leaves of for A. pinnata were extracted following the protocol of the Genomic DNA mini kit (plant) extraction kit provided from Geneaid. The entire ITS region (ITS1, 5.8S gene and ITS2) was amplified *via* polymerase chain reaction (PCR) using primers ITS1 and ITS4 (White *et al*., 1990). The mixture of reaction (25 µL) consisted of the following components: 1.5 µl DNA, 5 µl Taq PCR PreMix, forward and reverse primers each at a concentration of 10 pc/ml (1 µl) then make up to 25 µl with distilled water. PCR system consisted of a total of 37 cycles, with each round including the following:

1. Initial denaturation at 95°C for 5 minutes-one cycle.
2. Denaturing at 95°C for 45 seconds + annealing at 58°C for 45 seconds + first extension at 72°C for 45 seconds-35 cycles.

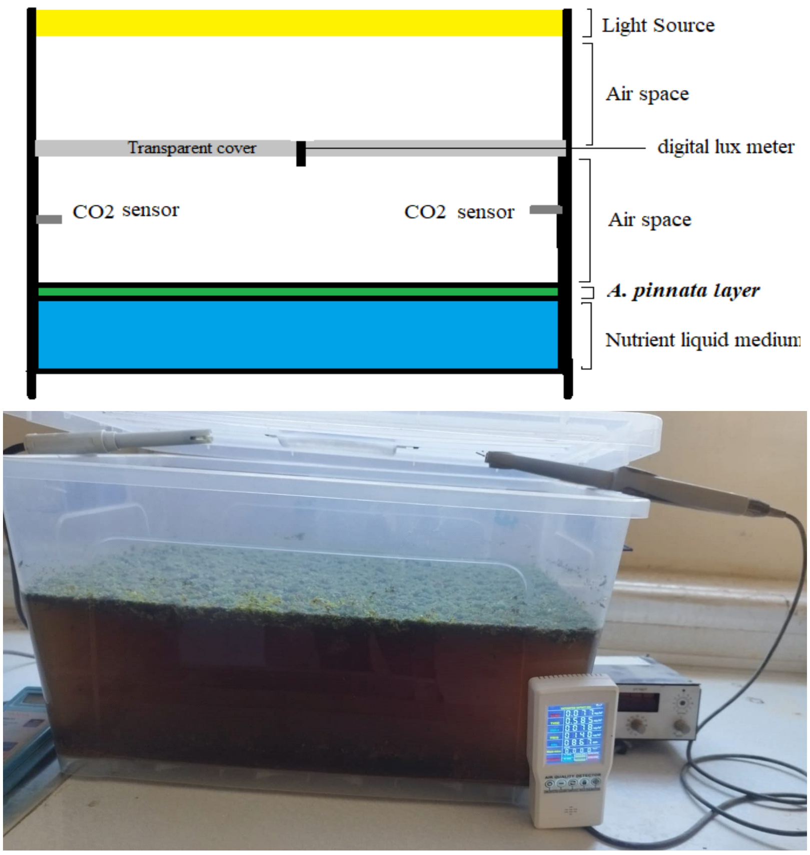
3. Final extension at 72°C for 7 minutes- one cycle (Hassan and Al-Qiassi, 2022).

Gene amplification was performed using a thermocycler (Applied Biosystem Gene-amp PCR System 9700). After amplification, PCR products were analyzed by electrophoresis using a 1.5% agarose gel, then the bands were visualized under ultraviolet (UV) light at a wavelength of 302 nm after treating with dye (Intron Korea red stain). Nucleotide sequences of genes were determined in the Korean company Biotechnology Lab using (Applied Biosystem 3730XL, DNA Sequencer). The results were compared to the database at the National Center for Biotechnology Information (NCBI) using a specialized local alignment search tool (BLAST) to align with nucleotide sequences of the targeted gene from plant isolates in this study and identify their species based on matches in the aforementioned database. After completing the diagnosis of *Azolla* spp., the identified species was recorded in NCBI and a phylogenetic tree was constructed using the Maximum Likelihood method *via* MEGA 11 software (Kumar *et al*., 2018).

Nutrient culture medium and growth conditions

60 × 40 × 40 cm) length × width × depth (of plastic container was used for growth of *A. pinnata*, the container was supplied by Espinas and Watanabe's medium (EWM), consisted of macronutrients included; $CaCl_2$ (40 ppm), KH_2PO_4 (20 ppm), $MgSO_4$ (40 ppm) and KCl (40 ppm) and micronutrients $MnSO_4$ (0.5 ppm), Na_2MoO_4 (0.15 ppm), H_3BO_3 (0.2 ppm), $ZnSO_4$ (0.01 ppm), $CuSO_4$ (0.01 ppm) and Ferric citrate (2 ppm) the pH was adjusted at 6.5 (Dawar and Singh, 2002). 10 g fresh wt. of washed *A. pinnata* plants were taken as initial inoculum.

The containers were provide with white UV-Free Sun Lamp (verilux light bulbs) United States Digital lux metrer



Light source : White UV-Free Sun Lamp (verilux light bulbs) United States
Air space : About 50 cm hight
Digital lux metrer ; Digital Illuminance/Light Meter, 0-200,000 Lux Luxmeter (LX1330B).
Bestone Industrial Limited/ China
Azolla pinnata layer: growth about 1-3 cm
Nutrient liquid medium , 20 cm depth
CO_2 sensor of Air quality detector

Fig 1: Above; diagram of the components of the cultivation of *A. pinnata*, below; *A. pinnata* grown in the Nutrient liquid medium.

and Air quality detector (RoSH model JSM-131-China) for measurement of CO_2 (Fig 1).

Effect of temperature on the CO_2 level

The containers contained nutrient liquid medium separately incubated at 10, 20,30 and 40C for 10 days with 0 (dark) - 10000 lux. CO_2 was measured at interval 2 days for 10 days.

Determination of growth parameters

According to (Brouwer *et al*., 2018), the absolute growth rate (AGR) was determined as (dry matter weight (g)/m^2/day).

$$y_t = \text{Standing } A.\ pinnata \text{ crop} = \text{AGR.t+b}$$

b = Standing crop at the start of the linear growth (g/m^2).
t = Time (days).

Statistical analysis

Factorial experiments were carried out in this study and analysis of variance was conducted using the program (SPSS). The means were compared according to Duncan's multiple range test at a level of significance of 0.05 (Al-Rawi and Khalaf Allah, 1980).

RESULTS AND DISCUSSION

Molecular identification of *Azolla pinnata*

Azolla pinnata was molecularly identified using ITS within 5.8S rRNA gene, *Azolla pinnata* coded *Azolla pinnata* strain IRAQ-Tikrit.Has,A.A. registered in NCBI under the accession number PQ836460.1, the phylogentic tree on Fig 2 showed the *Azolla pinnata* strain IRAQ-Tikrit.Has,A.A. was the nucleotide sequence of the ITS region of Azolla was most identical to the American Azolla isolate (*Azolla pinnata* subsp. *pinnata* voucher Ap7, accession number JN604551.1) with a match percentage of 99.27. E-value and per cent identity were zero and more than 90% with all *Azolla pinnata* registered in NCBI, while they were more than zero and

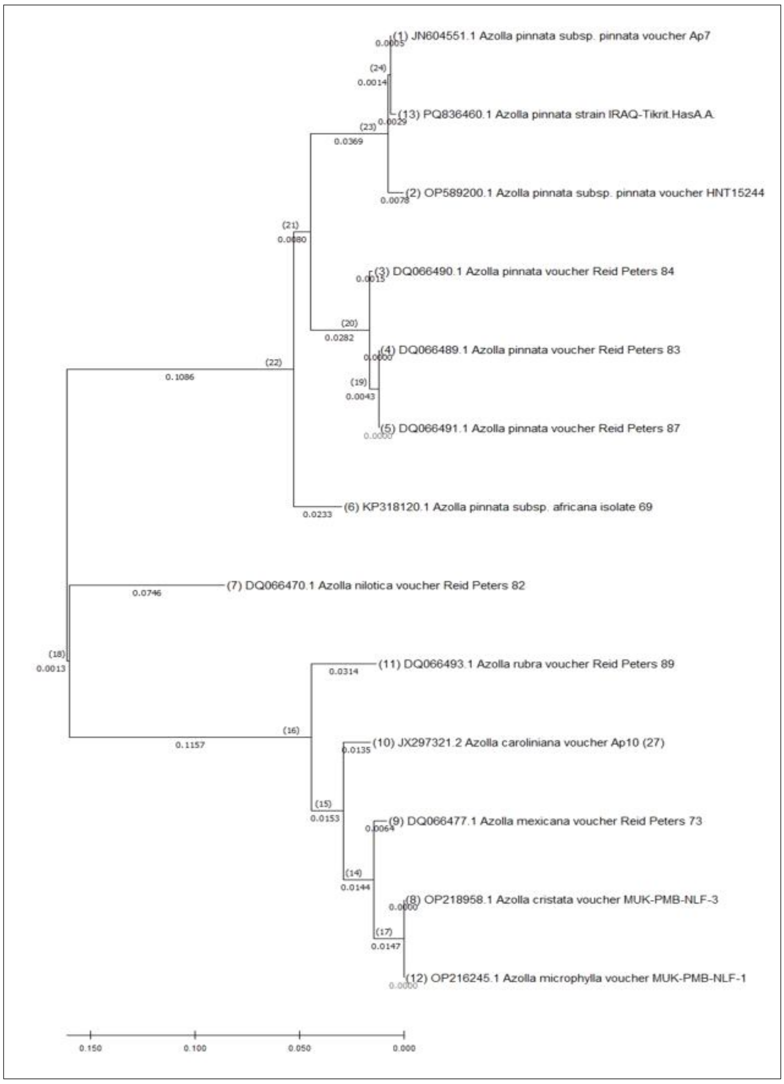


Fig 2: Evolutionary analysis of *Azolla* spp. by maximum likelihood method.

less than 80% with other *Azolla* spp. like *Azolla nilotica*, *Azolla mexicana*, *Azolla caroliniana*, *Azolla microphylla, Azolla cristata* and *Azolla rubra*. These results confirm that the precise classification of *Azolla* isolated for the first time from the Iraqi environment belongs to the *Azolla pinnata.*

Effect of temperature and Light Intensity on CO_2 Concentration for 10 days growth of *A. pinnata*

Fig 3 shows the effect of temperature and light intensity on CO_2 concentration produced from *A. pinnata* in the culture medium at a depth of 20 cm for 10 days. The results showed no significant differences in CO_2 concentrations during incubation in darkness for 10 days, with the highest concentrations recorded ranging from 827.43 to 894.74 ppm across temperatures from 10°C to 40°C. In contrast, incubation under light conditions revealed a consistent decrease in CO_2 concentrations with increasing light intensity, reaching the lowest concentration at 10,000 lux., at this intensity, temperatures of 20°C and 30°C had a significant effect in reducing CO_2 levels, with the lowest values recorded at 375.14 and 362.38 ppm, respectively, compared to 416.81 and 410.25 ppm at 10°C and 40°C.

When comparing the percentages of CO_2 reduction, it is noted from Fig 4 that the highest percentage of CO_2 reduction was when *A. pinnata* grew at temperatures of 20 and 30°C, while the lowest percentage of CO_2 reduction was when growing at temperatures of 10 and 40°C. On the other hand, the same figure shows that the highest percentage of reduction was when *A. pinnata* grew at a light intensity of 10,000 lux compared to other light intensities.

Effect of nutrient medium depth on CO_2 concentration and growth parameters of *A. pinnata*

The effect of the depth of the nutrient medium on the concentration of CO_2 gas and growth indicators of *A. pinnata*. Table 1 shows the effect of the depth of the liquid nutrient medium on the growth of *A. pinnata* and its effect on the concentrations of CO_2 gas. The results showed that there was an increase in the growth of *A. pinnata* with an increase in the depth of the liquid nutrient medium up to 50 cm, as the value of the growth indicators AGR and yt reached 10.53 g/m^2/day and 106.42 g/m^2 with a decrease in CO^2 gas to 325.64 ppm, with no significant effects on the growth indicators and gas concentrations when growing at depths of 60-80 cm.

When *Azolla* was incubated under dark conditions, an increase in carbon dioxide (CO_2) levels was observed throughout the experiment period (ten days) across all studied temperatures. This can be explained firstly by the non-photosynthetic character of *A. pinnata* in conditions of

Table 1: Impact of nutrient liquid medium depth of *Azolla pinnata* growth (at 30°C) and Co_2 concentration at 10000 lux.

Nutrient liquid medium depth (cm)	AGR* of *A. pinnata* (g/m^2/day)	y_t ** (g/m^2) for 10 days	CO_2 concentration (ppm) for 10 days
10	7.32	77.32	388.57
20	8.11	85.22	362.38
30	8.43	88.42	354.21
40	9.34	97.52	342.91
50	10.23	106.42	325.64
60	10.26	106.72	323.97
70	10.27	106.82	322.35
80	10.27	106.82	322.33
LSD 0.05	0.31	4.24	9.07

*AGR = Absolute growth rate (dry matter weight (g)/m^2/day).
**y_t = Standing *A. pinnata* crop = AGR.t+b (Brouwer *et al.*, 2018).
b = Standing crop at the start of the linear growth (g/m^2).
t = Time (days).

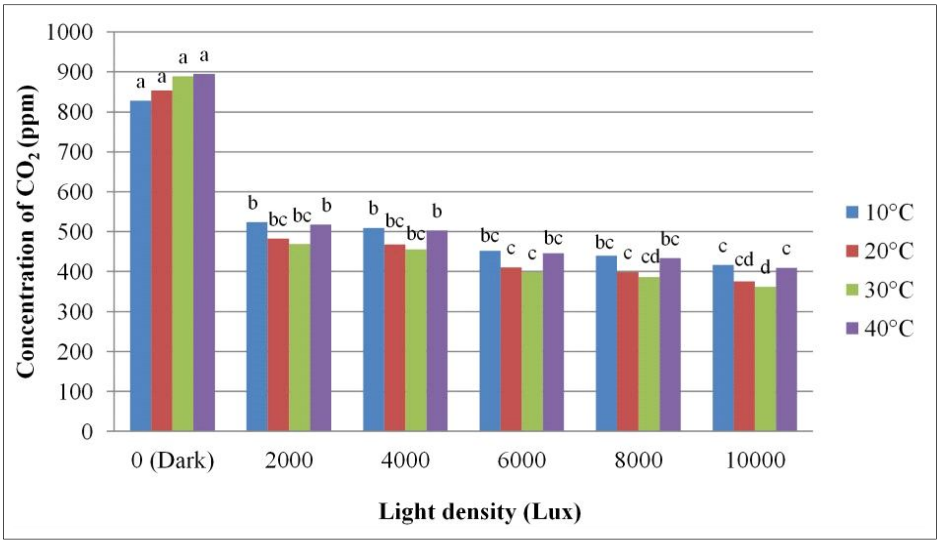


Fig 3: Impact of temperature and Light Intensity on CO_2 Concentration for 10 days growth of *A. pinnata*.

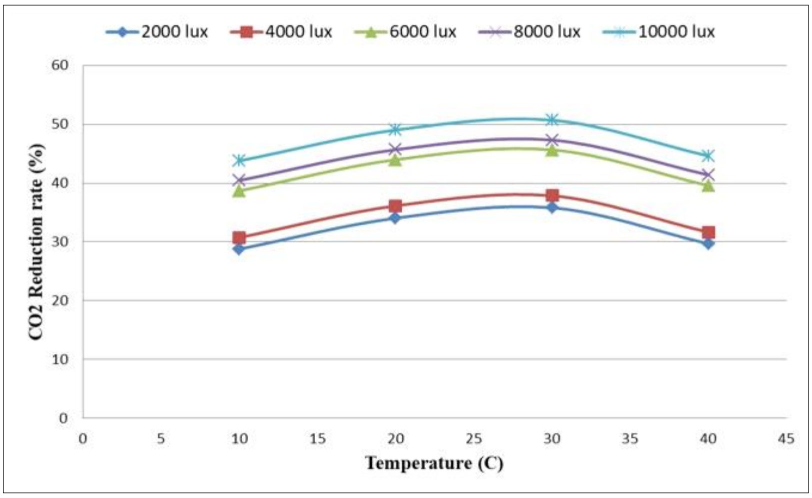


Fig 4: Percentage of CO_2 reduction by *A. pinnata* grown at different light intensity, temperatures and time periods.

the darkness and secondly, by other physiological processes in Azolla's metabolism, predominantly respiratory, involved in the increases of CO_2 amount. Conversely, under light conditions, the results showed a significant and continuous decrease in CO_2 levels with increasing temperature and light intensity up to 10,000 lux throughout the incubation period (ten days).

This continuous decrease in carbon dioxide may be attributed to the photosynthesis process, as previous studies have proven an increase in carbon dioxide retention from the atmosphere with the increase in the biomass of Azolla, which results in an increase in the photosynthesis process, which works to convert carbon dioxide into glucose and water with the release of oxygen in the presence of light. Therefore, there is a direct relationship between the rates of photosynthesis of Azolla and the increase in CO_2 retention in the atmosphere, which made Azolla An important vital factor in combating global warming and controlling climate change, in addition to its effective role in improving the quality of the water in it and the sustainability of biodiversity (Hamdan and Hour, 2022). The results showed that the best vegetative growth of Azolla was recorded at temperatures of 20-30°C, as slow growth of Azolla was observed at temperatures below 20°C, while the effect of temperatures above 30°C is negative on the vegetative growth of Azolla in the presence of light. The results of our study agreed with Cheng *et al*. (2010), De *et al*. (2015) and Hossain *et al*. (2021), who stated that the optimum temperatures for Azolla growth are in the range of 20-30°C and that temperatures above 30°C negatively affect the growth of Azolla, as previous studies recorded the death of Azolla at a temperature of 45°C (Pereira and Carrapico, 2009). Photosynthesis occurring in the optimal conditions (20-30°C) for the growth of Azolla is the main activity that works to sequester carbon dioxide by increasing the biomass of the plant (Pereira and Carrapico, 2009; Hamdan and Hour, 2022; Sarkar *et al*., 2023). A significant increase in carbon dioxide consumption was recorded with increasing light intensity during the incubation period (ten days). A significant decrease in CO_2 levels was observed with increasing light intensity up to 10,000 lux. This is in agreement with Hossain *et al*. (2021) and Sara *et al*. (2023), who recorded a decrease in CO_2 levels with increasing light intensity levels between 8,000-10,000 lux. The importance of light intensity lies in its direct effect on the photosynthesis process of Azolla and the process of building carbohydrates that are important in plant metabolism, in addition to Its direct effect on the symbiotic relationship between Azolla and nitrogen-fixing bacteria, which leads to an increase in the vegetative growth of Azolla plants, which is noticeable at radiation intensity ranging between 5000-10000 lux. This is what our study proved, which recorded the highest reduction in CO_2 levels at light intensity of 8000-10000 lux, while recording the best vegetative growth of Azolla plants at these ranges. The depth of the medium represents the nutritional content available for the growth of the Azolla plant. The higher the nutritional content, the more we expect the growth to increase and thus the biomass of the Azolla plant to increase, which is reflected in an increase in the photosynthesis process and an increase in the consumption of carbon dioxide. The results showed that the best depth of the medium was 50 cm, which recorded the best vegetative growth of Azolla, in addition to recording the highest percentage of decrease in CO_2 levels, while depths greater than 50 cm did not affect the vegetative growth of Azolla negatively or positively or on the consumption of carbon dioxide, which is consistent with the results of Sadeghi *et al*. (2012).

CONCLUSION

The results from this study refer to the promising potential of the fern plant *Azolla pinnata* in reducing CO_2. In the case of indoor cultivation of Azolla, the growth of *A. pinnata* can increase, producing high biomass while consuming high levels of CO_2 if it is incubated at a light intensity of 8000-10000 lux, at temperatures of 20-30°C and at a depth of 50 cm.

In the future studies, it is planned to implement this study in large basins on a large scale in geographical areas suffering from high levels of carbon dioxide, especially in industrial areas and we will monitor the levels of this gas throughout the year.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

The manuscript does not contain animal experiments.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

REFERENCES

Ahmed, H.A., Ganai A.M., Beigh Y.A., Sheikh G.G., Reshi P.A. (2016). Performance of growing sheep on Azolla based diets. *Indian Journal of Animal Research*. **50(5):** 721-724. doi: 10.18805/ijar.9642.

Al-Rawi, Mahmoud, K. and Allah, K., Muhammad, A. (1980). Design and analysis of agricultural experiments. Dar Al-Kutub for Printing and Publishing, University of Mosul.

Baviskar, P., Kharat, S. and Gawande, S. (2023). Biochemical characterization and fertilization potential of composts produced from invasive plants in the Senegal River Valley. *Environmental Science and Pollution Research.* https://doi.org/10.1007/s11356-023-26748-7.

Bhatt, N., Tyagi, N., Chandra, R., Meena, C.D., Prasad, K.C. (2020). Growth performance and nutrient digestibility of azolla pinnata feeding in sahiwal calves (*Bos indicus*) by replacing protein content of concentrate with azolla pinnata during winter Season. *Indian Journal of Animal Research*. **55(6):** 663-668. doi: 10.18805/ijar.B-4004.

Brouwer, P., Schluepmann, H., Nierop, K.G., Elderson, J., Bijl, P.K., van, der Meer I., de Visser, W., Reichar,t G.J., Smeekens, S., van der Werf, A. (2018). Growing Azolla to produce sustainable protein feed: The effect of differing species and CO_2 concentrations on biomass productivity and chemical composition. *J. Sci Food Agric*. **98:** 4759-4768. https:// doi. org/ 10. 1002/ jsfa. 9016.

Chandrababu, A.K., Parvathy, U. (2025). Water Fern- Azolla: A Review. *Agricultural Reviews*. **46(1):** 138-142. doi: 10. 18805/ag.R-2529.

Cheng, W., Saka, H., Matsushima, M., Yagi, K. and Hasegawa, T. (2010). Response of the floating aquatic fern phosphorus levels. *Hydrobiologia*. **656(1):** 1-14 .

Daniel, J.N. and Bartholomew, D.P. (1993). Growth and Photosynthesis of three Azolla species in response to irradiance. *Biotronics.* **22:** 1-14.

Dawar, S. and Singh, P.K. (2002). Comparison of soil- and nutrient-based medium for maintenance of Azolla cultures. *Journal of Plant Nutrition*. **25(12):** 2719-2729.

De, A.K., Bera, S. and Adak, M.A. (2015).physiological changes of duck weed fern (*Azolla pinnata* R.Br.) under nitrogen and phosphorus depletion. *Genomies and Applied Biology*. **5(9):** 1-9.

Hamdan, Z.H. and Hour, A.F. (2022). CO_2 sequestration by propagation of the fast-growing Azolla spp. *Environmental Science and Pollution Research*. **29:** 16912-16924.

Hassan, A.A. and Al-Qiassi, A.R.M. (2022). Isolation of the medicinal mushroom ganoderma resinaceum in Iraq, morphological, molecular identification and production of basidiocarps on novel media. *Trends in Sciences*. **19(14):** 4949. https://doi.org/10.48048/tis.2022.4949.

Hossain, M.A., Shimu, S.A., Sarker, M.S.A., Ahsan, M.E. and Banu, M.R. (2021). Biomass growth and composition of *Azolla* (*Azolla pinnata* R.B.R.) supplemented with inorganic phosphorus in outdoor culture. *SAAR Journal of Agriculture*. **19(1):** 177-182.

Korsa, G., Alemu, D. and Ayele, A. (2024). *Azolla* plant production and their potential applications. *International Journal of Agronomy*. 12. http://doi.org/10.1155/2024/1716440.

Kosesakal, T. and Yildiz, M. (2019). Growth performance and biochemical profile of *Azolla pinnata* and *Azolla caroliniana* grown under greenhouse conditions. *Ecological Modelling*. **243:** 7-8. https://doi.org/10.1016/j.ecolmodel.2012.06.011.

Kumar, S., Stecher, G., Li, M., Knyaz, C. and Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution.* **35:** 1547-1549.

Nasir, N.A. Zakarya, N.M., Kamaruddin, I.A., S.A. and Islam, A.K.M.A. (2022). Advances and future prospects on biotechnological approaches towards *Azolla* for environmental sustainability. *Pertanika Journal of Tropical Agricultural Science*. **45(3):** 595-609.

Pereira, A. and Carrapico, F. (2009). Culture of *Azolla filiculoides* in artificial conditions. *Plant Biosystems*. 431-434. https://doi.org/10.1080/11263500903172110.

Rames, K.V. (2019). Environmental microbiology. Chennai: MJP Publishers. 406 pages.

Rehman, A., Ma, H., Ozturk, I. and Ulucak, R. (2022). Sustainable development and pollution: The effect of CO_2 emission on population growth, food production, economic development and energy consumption in Pakistan. *Environmental Science and Pollution Research*. **29(12):** 17319.

Sadeghi, R., Zarkami, Sabetraftar, K. and Van Damme, P. (2012). Application of classification trees to model the distribution pattern of a new exotic species *Azolla filiculoides* (Lam.) at Silken Wildlife Refuge, Anzali Wetland, Iran. *Ecological Modelling*. **243:** 7-8. https://doi.org/10.1016/j.ecolmodel.2012.06.011.

Sara, C., Agnese B., Maria C.V., Francesco D., Lara R., Federico B. and Francesco P. (2023). Impact of high light intensity and low temperature on the growth and phenylpropanoid profile of Azolla filiculoides. *International Journal of Molecular Sciences*. **24(8554).** https://doi.org/10.3390/ijms2408554.

Sarkar, A., Bhakta, J.N. and Bhakta, B.O.K. (2023). Evaluating growth-dependent enhanced carbon dioxide sequestration potential of *Azolla pinnata* using cattle wastes (cow dung and cow urine). *Heliyon*. **9(3):** e14610. https://doi.org/ 10.1016/j.heliyon.2023.e14610.

Ussiri, D.A.N. and Lair, G.J. (2017). Carbon sequestration for climate change mitigation and adaptation. Cham: Springer International Publishing. https://doi.org/10.10071978-30319-53844-7.

Wagner, G.M. (1997). Azolla :A review of its biology and Utilization. *Bot Rev.* **63:** 1-26. http //doi.org /10.1007 /BF02857915.

White, T.J., Bruns, T., Lee, S. and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protocols: A Guide to Methods and Applications. California: Academic Press Inc.