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Influence of Pollution by Penconazole Pesticide on Some Microalgae Isolated from Qarun Lake, El-Fayoum, Egypt

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ABSTRACT

The microalgae sensitivity to exogenous contaminants such as pesticides increases their use as model organisms to appreciate the ecotoxicity of pollutants in aquatic environments and its effect on human health. This study focused on the influence of penconazole pesticide on the chlorophyll *a* content, cell count, and the biochemical content of *Synechocystis aquatilis*, *Chlorella vulgaris*, and *Scenedesmus dimorphs*. The data indicated that at low pesticide level (0.1, 0.2, 0.4, 0.8mg/ l), the effects declined compared to control, while at higher concentrations (1.6, 2 and 4 mg /l), the effects were visible. The pesticide had a significant impact on the biochemical contents of the tested algae, particularly at higher concentrations, compared to the control. The three tested species exhibited distinct behaviors in comparison with their controls. *Synechocystis aquatilis* showed a significant increase in growth between 24 to 28 days, followed by *Chlorella vulgaris*, and *Scenedesmus dimorphus*, which exhibited this effect between 32 to 36 days, with samples being taken at four-day intervals. This pattern indicates that *Synechocystis aquatilis* was the most sensitive microalgae species in response to pesticide pollution in the Qarun Lake habitats.

INTRODUCTION

Water pollution has grown to be of a great interest in the world, however direct wastewater discharge poses problems to the aquatic ecosystems through causing eutrophication and effects on their physico-chemical characteristics. Moreover, wastewater is fundamentally enriched with recalcitrant toxic substances that decompose harmful effects on the receiving environments **(Dig *et al.*, 2021)**. Phosphorus and nitrogen are found with high quantity in wastewater **(Bhat *et al.*, 2017; Solovchenko,**

2019), as well as wastewater contains different quantities of heavy metals, pesticides, endocrine disruptors, and pathogens **(Ahmad *et al.*, 2019; Sarkar *et al.*, 2019).**

The continued rise in the global population has led to a quick development in agriculture worldwide. For the yield protection and crops quality, the use of agrochemicals has annually increased **(Qu *et al.*, 2020; Qiu *et al.*, 2022)**. Pesticides include herbicides, fungicides, insecticides, rodenticide, acaricides, nematicides, plant growth regulators, defoliants, antirodent drugs, among others **(Köck *et al.*, 2010; Rasmussen *et al.*, 2015; Tsaboula *et al.*, 2018)**. The sales of triazole fungicide all over the world reached 3.47 billion in 2014, and its use in the USA reached 2.9 million kg in 2016 due their high efficiency in fungi treatment **(Wang & Zhang, 2017; Toda *et al.*, 2021; Wang *et al.*, 2021)**. Pesticides usage in Egypt has increased according to FAO report (Food and Agriculture Organization) from 4931 tons in 2000 to 13.178 tons in 2019 (**FAO, 2021)**. Fungicides inhibiting the biosynthesis of ergosterol have been approved to improve the effect of green algae (Chlorophyta) and photobacteria in aquatic environment (**Liu *et al.*, 2013)**. Penconazole (1-(2,4-dichloro-b-propylphenethyl)-1H-1,2,4-triazole) is among the widely used triazole fungicide in the world, which may be used to control fungal and improve agricultural production, which is usually sprayed directly on the plants surface **(Husak *et al.*, 2017)**. It's scattered to the soil and water environment, causing certain environmental safety risks **(Ming *et al.*, 2021)**. The field effectiveness test results indicate that the treatment has a superior control influence on grape white rot, as reported by **Pose-Juan *et al*. (2010)**.

The domestic and agricultural wastes discharged into Qarun Lake from its two main drains (EL-Bats and El-Wadi drains) represent the fundamental factor influencing its water quality **(Ibrahim *et al.*, 2021)**. Its components are polluted with a wide range of pesticides **(Mansour & Sidky, 2003; Ali *et al.*, 2008)**. The lake suffers from critical water pollution problems due to absolute solid, liquid domestic, and industrial waste throwing practices, as well as agrochemical contamination and lack of prospective wastewater management **(Shaaban *et al.*, 2016)**. Therefore, preventing drainage of pesticides from aquatic ecosystems is mandatory nowadays to maintain the balance and protect biodiversity **(Harit, 2019)**.

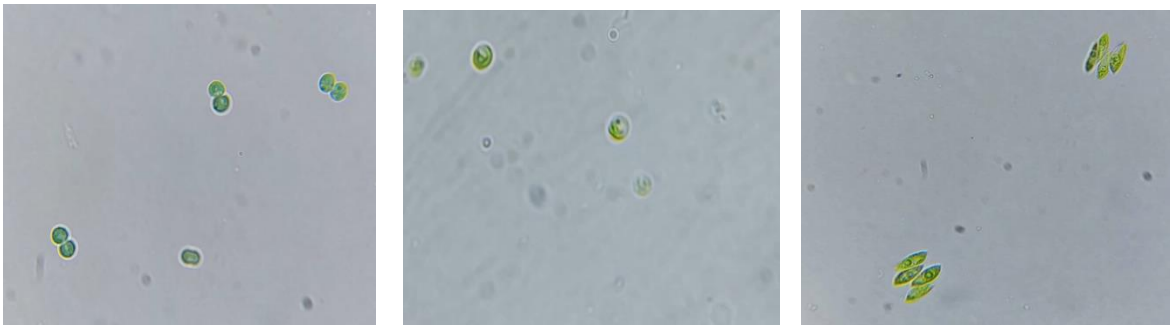
Aquatic microorganisms are considered to be the main producers, consumers, and decomposers of natural water environments; moreover, they preserve the stability of aquatic ecosystems by sharing in the nutrient cycle **(Lu *et al.*, 2019; Zhang *et al.*, 2021)**. The aquatic microorganism's sensitivity to exogenous contaminants, such as antibiotics, nanoparticles, herbicides, and fungicides, raises their use as a model organism to appreciate the ecotoxicity of pollutants in aquatic environments and health **(Lu *et al.*, 2020; Lu *et al.*, 2021; Deng *et al.*, 2022)**. Microalgae have the ability to grow in various climatic and environmental conditions, thus providing an alternative option for wastewater remediation **(Kadir *et al.*, 2018; Vernes *et al.*, 2019)**. Application of microalgae can play a critical role for the treatment of different classes of pesticides from

contaminated environments. However, some microalgae species are known to degrade complex pesticides into less toxic compounds **(Massoud *et al.*, 2008)**. In modern days, microalgae technology has reached considerable success in the effective remediation of pesticides pollution **(Jing *et al.*, 2020)**.

The occurrence of pesticides in water bodies is a significant concern due to their toxicity and environmental persistence. Microorganisms, being a vital part of aquatic ecosystems, are among the species most affected by these pollutants. In our study, we aimed to investigate how penconazole, a commonly used pesticide, impacts the microbial community in aquatic environments.

MATERIALS AND METHODS

The tested species in the present study were isolated from Qarun Lake with a series of work in the isolation and purification of the species using a solid agar. The isolated species were *Synechocystis aquatilis* (blue green algae) which was grown in Zarrouk medium **(Zarrouk, 1966)**, as well as *Chlorella vulgaris* and *Scenedesmus dimorphs* (green algae), which were grown in MBL medium **(Nichols, 1973)**.



A B C

Fig. 1. Photos of the selected algal strains **A:** *Synechocystis aquatilis*. **B:** *Chlorella vulgaris* and **C:** *Scenedesmus dimorphs*

Experimental design

The pure culture cells inoculated were around 5 x10^4 cells ml^{-1} of the stock culture at the end of the log (logarithmic) phase. The three microorganisms were subjected to concentrations of 0.1, 0.2, 0.4, 0.8, 1.6, 2 and 4mg of penconazole pesticide (a triazole fungicide). All culture flasks were kept under a light intensity of 3700 lux at 28± 1ºC for the blue green algae and 23± 1ºC for the green species. Samples were taken after every four days intervals up to forty-eight days for the evaluation of the growth curve of the tested algae in terms of cell count and chlorophyll *a* content.

The triazole fungicide used in this study is commercially available as penconazole, chemical name [1-(2,4-dichloro-β-propylphenethyl)-1H- 1,2,4-tri-azole]

which was obtained from an agrochemical company, Egypt (10 % active ingredient). The chemicals utilized in the preparation of the standards and other reagents are manufactured by BDH chemicals (1td.Pools England), Sigma (St.Louis, M.O. 63178 U.S.A), A.R. chemicals (Mallianckrodt Chemical Works, St. Louis, M.O. 63147 U.S.A.) and MERCK (61 Drmstadt Germany).

The algal growth was estimated by cell count (using a standard haemocytometer under an Olympus BH-2 light microscope) and chlorophyll *a* content (**Metzner *et al*., 1965)**. The biochemical contents of tested algae were determined by the detection of carbohydrates (total, soluble and insoluble) and proteins. For the carbohydrate determination in algal pellets, the anthrone sulphuric acid method, which was performed by **Fales (1951)** and **Schlegel (1956)** and confirmed by **Irigoyen *et al.* (1992)**, was used. All carbohydrates were colorimetrically determined. Finally for protein, the method of **Bradford (1976)** was carried out.

Statistical analysis

Data were statistically analyzed via the one-way analysis of variance (ANOVA test) using SPSS Statistical Package Program **(SPSS, 2015)** version 23. Means of treatments were compared by using Duncan multiple range test when the differences were significant **(Duncan, 1955)**. Level of significance in all tests was $P \leq 0.05$. The results are expressed as means ± standard error (SE).

RESULTS

Influence of penconazole pesticide on growth of the tested species

Analysis of Qarun Lake water indicated the presence of twenty types of pesticides detected at different sites of the lake during the present study, as represented in Table (1). Penconazole pesticide was selected to test its effect on the isolated algal species under controlled laboratory conditions since it was found with the highest concentrations in the lake.

The influence of the penconazole pesticide was detected on the growth of three microalgae species (*Synechocystis aquatilis*, *Chlorella vulgaris* and *Scenedesmus dimorphs*) by cell count and chlorophyll *a* content. The data indicated that at low pesticide concentrations (0.1, 0.2, 0.4, 0.8mg/ l) the effects were more or less decline, while at higher concentrations (1.6, 2 and 4mg/ l) the effects appeared clearly. Figs. (2, 3) illustrate that lower concentrations of penconazole (0.1, 0.2, 0.4, 0.8mg/ L) resulted initially in a significant increase in growth across the tested microalgae species. *Synechocystis aquatilis* showed the highest growth increase within 24 to 28 days, while *Chlorella vulgaris* and *Scenedesmus dimorphus* recorded their highest growth between 32 to 36 days. However, after these time intervals, there was no further increase in growth,

followed by a noticeable decline as the exposure period extended beyond 28 days for *Synechocystis aquatilis* and 36 days for the other two species. Higher concentrations of penconazole (1.6, 2, and 4mg/ L) generally led to a significant decrease in growth across the tested microalgae species compared to the control. However, there was a slight increase in growth observed at later time intervals: 40 to 44 days for *Chlorella vulgaris* and *Scenedesmus dimorphus*, and 36 to 40 days for *Synechocystis aquatilis*. Additionally, the Duncan multiple range test, as shown in Fig. (7), indicated that by day 24 (the midpoint of the experiment), *Synechocystis aquatilis* exhibited a significant increase in growth across all concentrations, except at 4 mg/L. At this highest concentration, *Chlorella vulgaris* and *Scenedesmus dimorphus* showed a more significant increase in growth compared to *Synechocystis aquatilis*.

On the other hand, Figs. (4, 5) reveal that there is no effect of penconazole pesticide on the cell shape of *Synechocystis aquatilis* and *Chlorella vulgaris* at higher concentrations compared to the control. However, Fig. (6) shows an obvious change in the shape of the cells of *Scenedesmus dimorphs* at higher concentrations compared to the control, as the cell shape converted from filiform to circular.

The biochemical content of the tested species was also investigated in this study to discuss the influence of the pesticide on the microalgal biochemical contents, including total, soluble and insoluble carbohydrates, as well as the total proteins contents. The data in Fig. (8) clearly displays the great influence of pesticide on the carbohydrate contents of the tested algae compared to the control, especially at high levels. For *Synechocystis aquatilis*, the data assessed that the soluble and total carbohydrate content increased significantly in the control, and the highest values were 5.48 and 10.18mg/ g fresh weight, while the lowest content (0.29 and 0. 76mg/ g fresh weight) was found at 4mg/ l, and their values were at their highest at all the concentrations compared to the other species, except at 4mg/ l, where *Chlorella vulgaris* was the highest, followed by *Scenedesmus dimorphs* and *Synechocystis aquatilis*. For *Chlorella vulgaris*, the data indicated that the soluble and total carbohydrate content was the highest at 0.2mg/ L and in the control, with values of 4.62 and 8.58mg/ g fresh weight, respectively. In contrast, *Scenedesmus dimorphus* had its highest values of soluble and total carbohydrate content at the control, with 2.23 and 7.91mg/ g fresh weight, respectively. The lowest carbohydrate content was observed at 4mg/ L, with values of 0.97 and 1.45mg/ g fresh weight for *C. vulgaris*, and 0.50 and 1.07mg/ g fresh weight for *S. dimorphus*.

On the other hand, *Scenedesmus dimorphus* exhibited higher insoluble carbohydrate content compared to the other species. Its lowest values were recorded at 4mg/ L for *Synechocystis aquatilis* (0.02mg/ g), *C. vulgaris* (0.48mg/ g), and *S. dimorphus* (0.57mg/ g fresh weight). The highest insoluble carbohydrate content was

recorded at 0.1mg/ L for *Synechocystis aquatilis* (5.01mg/ g fresh weight), while for *C. vulgaris* and *S. dimorphus*, the highest values (4.85 and 5.68mg/ g fresh weight, respectively) were observed in the control samples. On the other hand, Fig. (9) shows that the high increase in the cells protein content was recorded in *Synechocystis aquatilis*, followed by *Chlorella vulgaris* then *Scenedesmus dimorphs*, which increased significantly at all the concentrations, except at the higher concentration of 4mg/ l, where the protein content of *Chlorella vulgaris* and *Scenedesmus dimorphs* cells increased significantly compared to *Synechocystis aquatilis.*

Table 1. Different types of pesticides recorded at the selected sites of Qarun Lake with their concentration mg/l during the period of autumn 2020 - summer 2021

	Pesticide	**St.1**	**St.2**	**St.3**	**St.4**	**St.5**	**St.6**	**St.7**	**St.8**
1	Ethoprophos	0.0166	N.D	0.0116	N.D	0.0127	0.0151	0.0124	0.0114
2	Terbufos	0.0082	0.0077	0.0080	0.0078	0.0083	0.0084	N.D	N.D
3	Diazonon	N.D	0.0091	0.0133	N.D	0.0210	0.0135	0.0604	0.0286
4	Pirimicarb	0.0159	0.0134	0.0109	0.0094	0.0041	0.0166	0.0209	0.0122
5	Parathion-methyl	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
6	Pirimiphos-methyl	0.0056	N.D	N.D	N.D	N.D	N.D	N.D	N.D
7	Malathion	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.0053
8	Chlorpyrifos	0.0238	0.0161	N.D	0.0167	N.D	0.0181	N.D	N.D
9	Cyprodinil	0.0018	0.0013	N.D	N.D	0.0064	0.0026	0.0052	0.0037
10	Penconazole	0.2070	0.1858	0.2410	0.2107	0.1027	0.2104	0.0111	0.1000
11	Chlorefnvinphos (Z+E)	0.0944	0.0109	0.0117	0.0093	0.0159	0.0093	0.0640	0.0378
12	Profenofos	N.D	N.D	N.D	N.D	N.D	0.0864	N.D	N.D
13	Carboxin	N.D	N.D	N.D	N.D	N.D	0.0187	0.0195	0.0217
14	Kresoxim methyl	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
15	Chlorfenapyr	N.D	0.0054	0.0062	N.D	0.0055	N.D	0.0121	N.D
16	Diniconazole	0.0162	0.0073	0.0084	N.D	N.D	0.0108	0.0130	N.D
17	Ethion	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
18	Epoxyconazole	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
19	Bifenthrin	N.D	N.D	4.1400	N.D	0.0046	N.D	0.0095	N.D
20	Fenpropathrin	0.0392	0.0490	0.1001	0.0474	0.1003	0.2014	0.0304	0.0916

*St: Site - N.D: not detected.

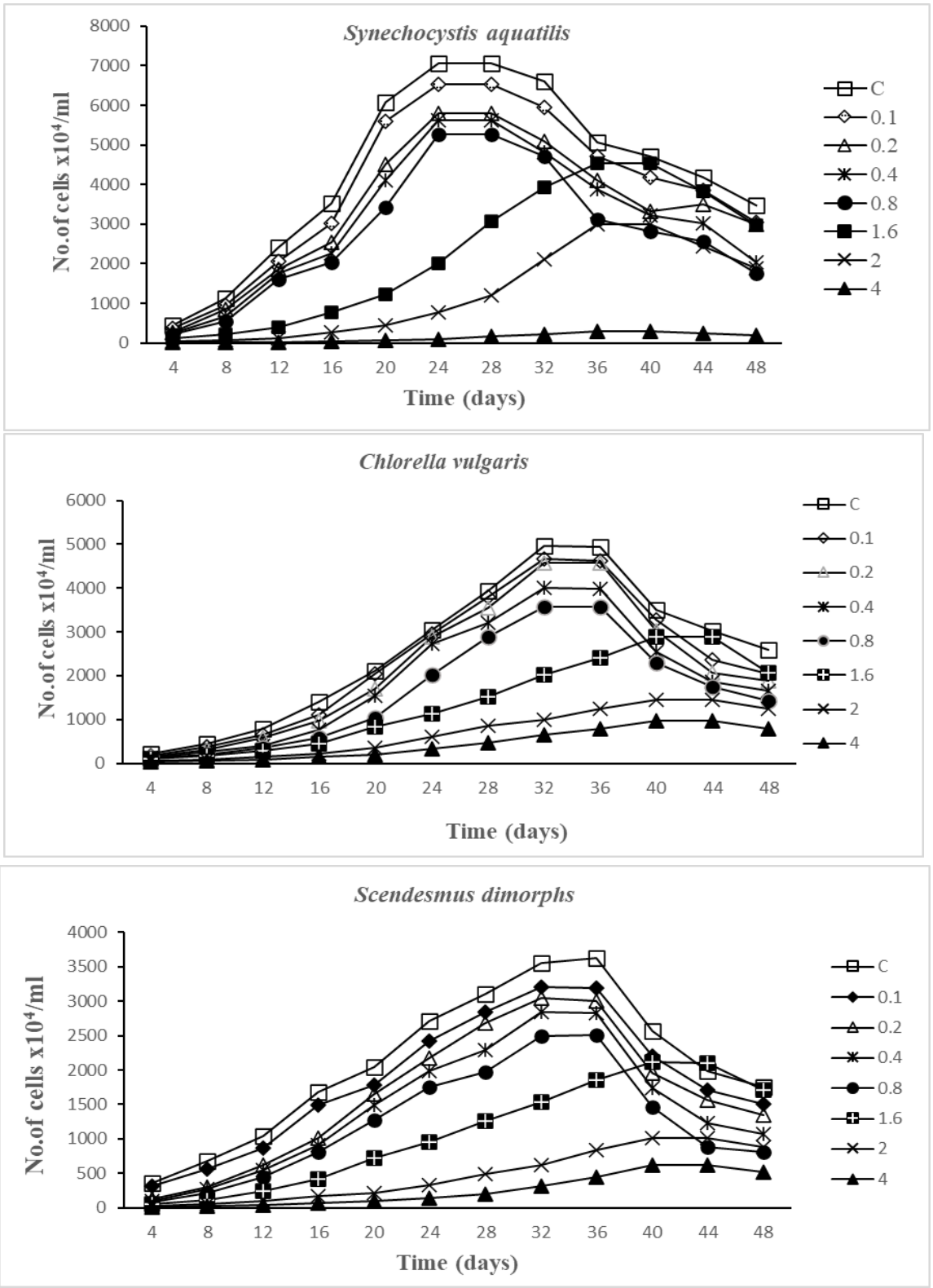


Fig. 2. Effect of different concentrations of penconazole pesticide on growth of the tested algae in terms of cell count (No. of cells x10^4 /ml)

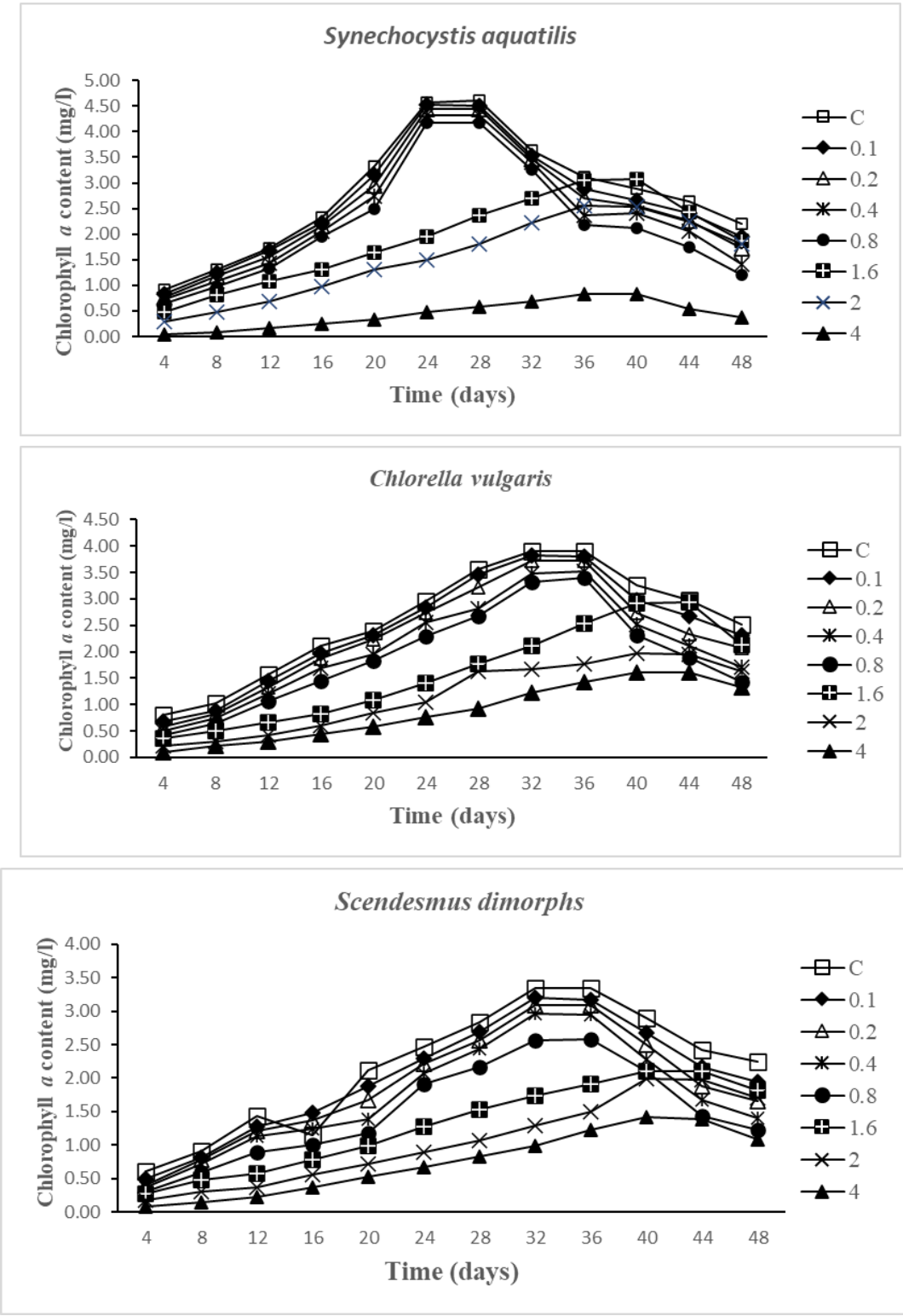
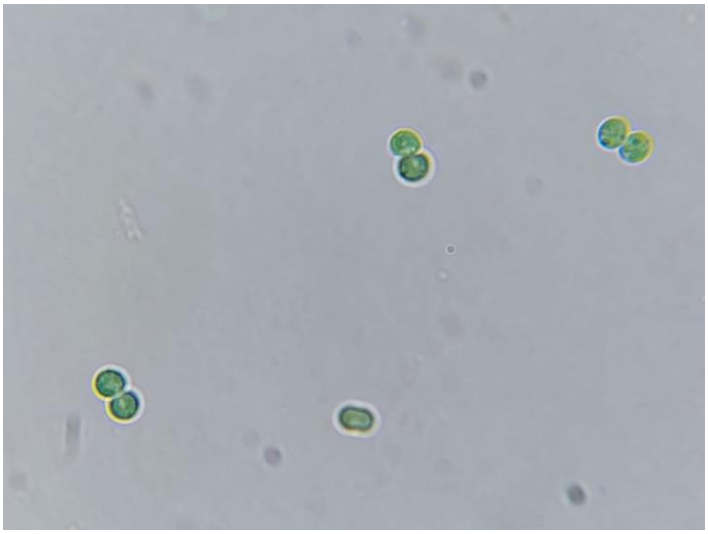
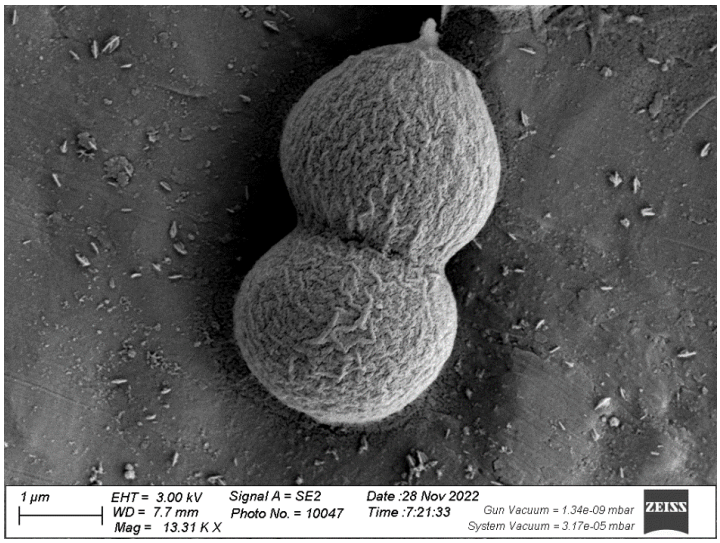


Fig. 3. Effect of different concentrations of penconazole pesticide on growth of the tested algae in terms of chlorophyll *a* content (mg/l)



A



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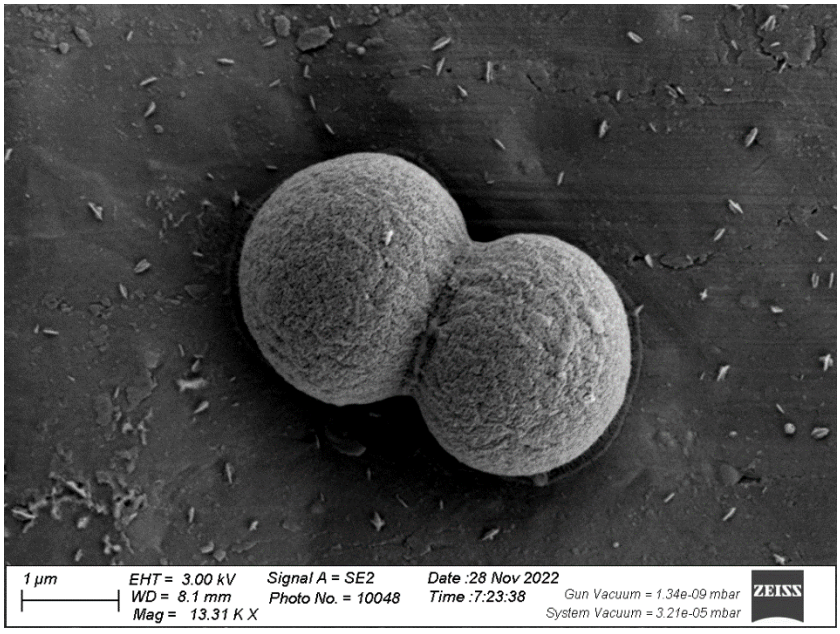
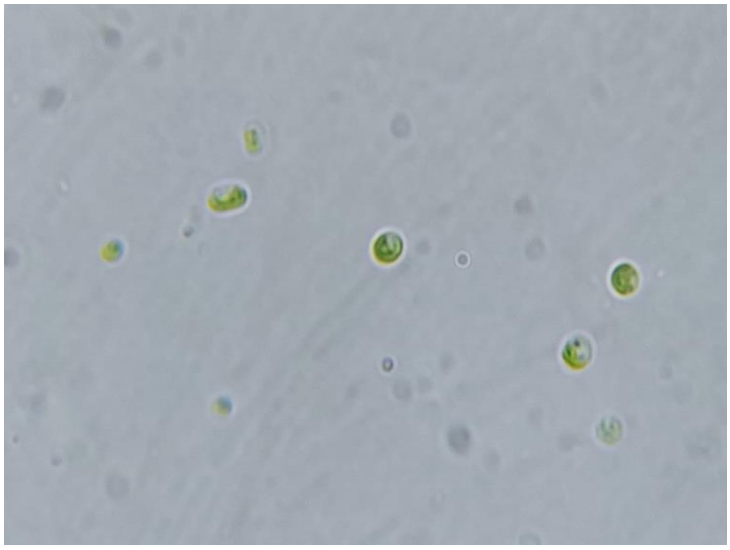


C

Fig. 4. (A) *Synechocystis aquatilis* under the light microscope at the control phase, **(B)** *Synechocystis aquatilis* under the scanning electron microscope at the control phase, and **(C)** *Synechocystis aquatilis* under the scanning electron microscope at 1.6mg /l concentration of penconazole pesticide



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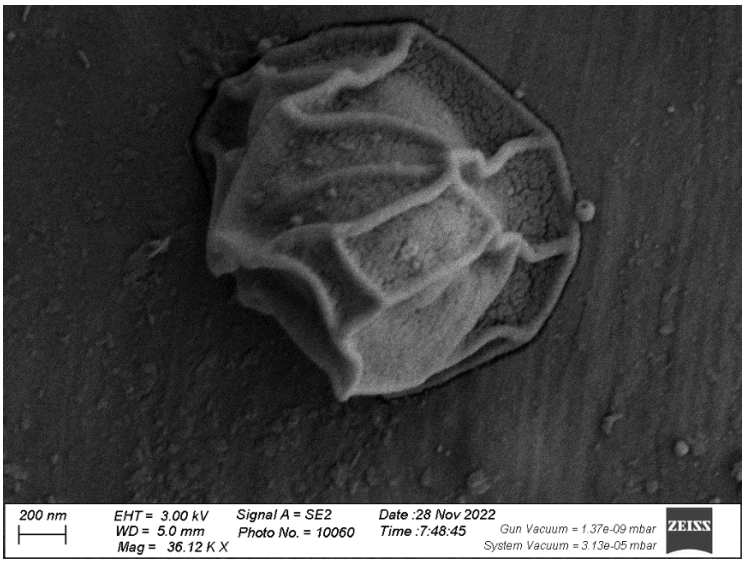
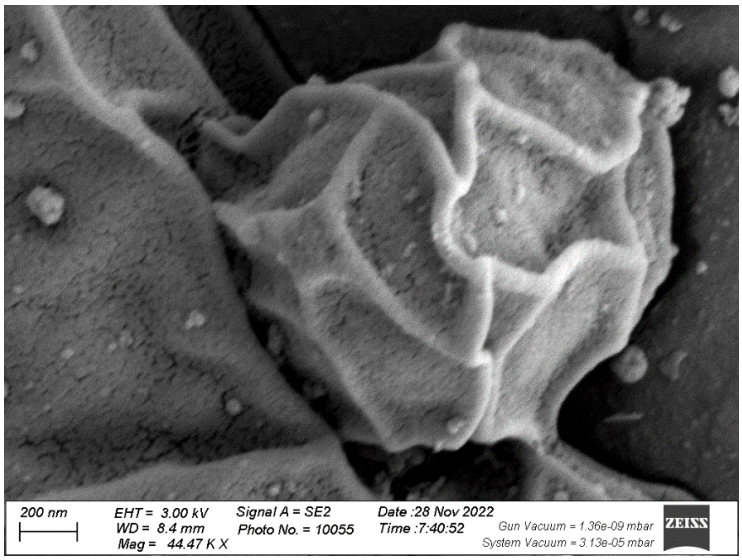
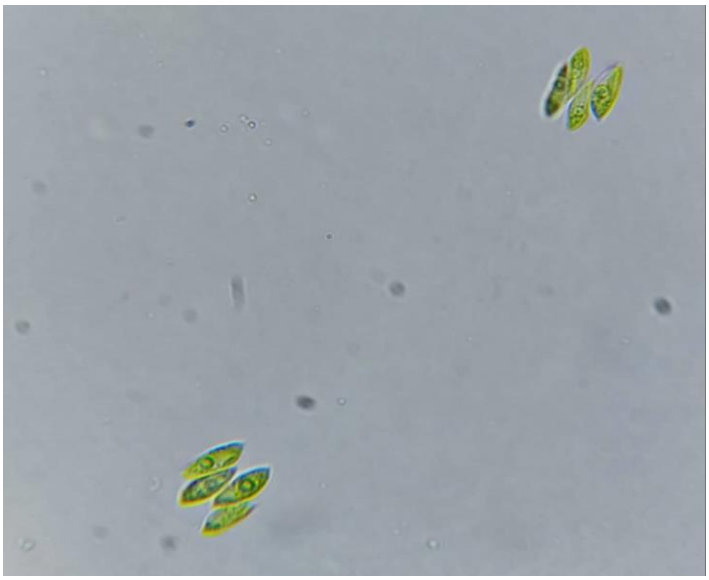


B



C

Fig. 5. (A) *Chlorella vulgaris* under the light microscope at the control phase, **(B)** *Chlorella vulgaris* under the scanning electron microscope at the control phase, and **(C)** *Chlorella vulgaris* under the scanning electron microscope at 1.6 mg/l concentration of penconazole pesticide



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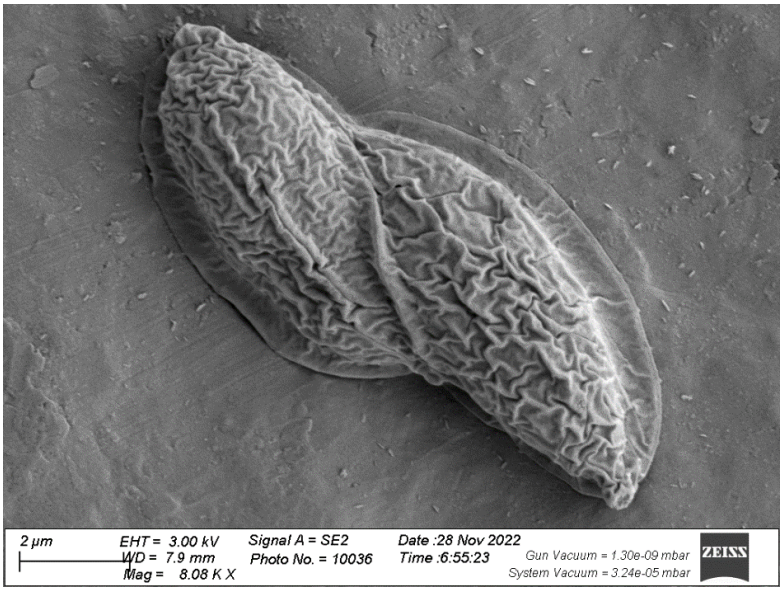

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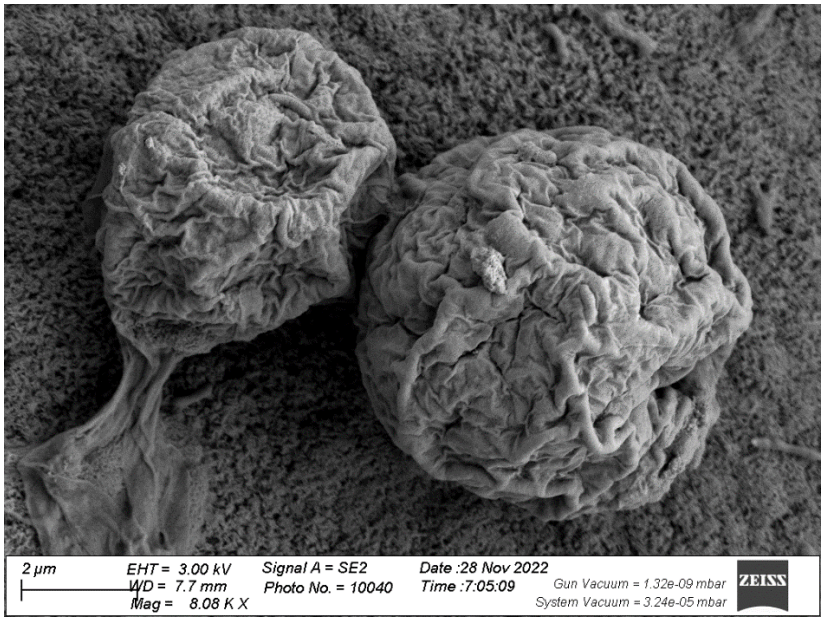
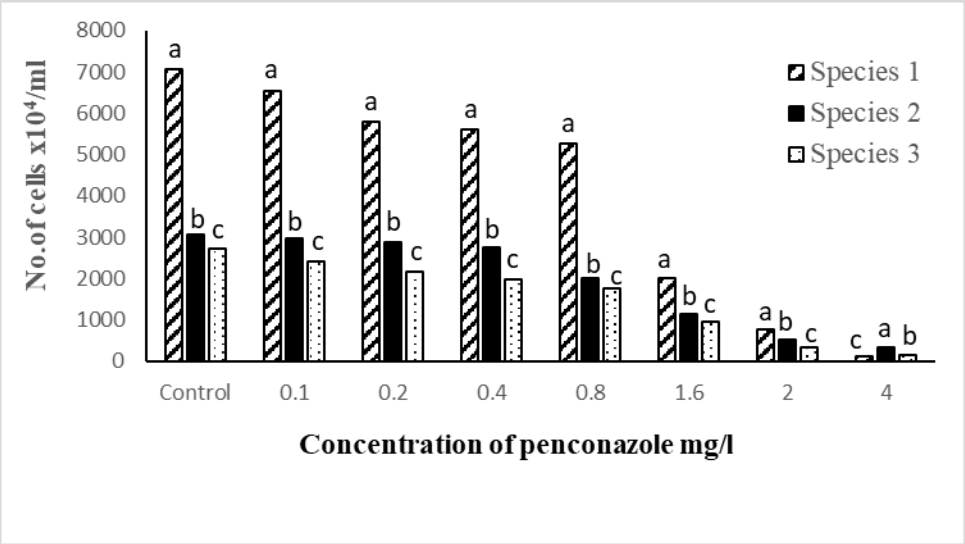


C

Fig. 6. (A) *Scenedesmus dimorphs* under the light microscope at the control phase, **(B)** *Scenedesmus dimorphs* under the scanning electron microscope at the control phase, and **(C)** *Scenedesmus dimorphs* under the scanning electron microscope at 1.6mg/ l concentration of penconazole pesticide showing deformation of the cell shape



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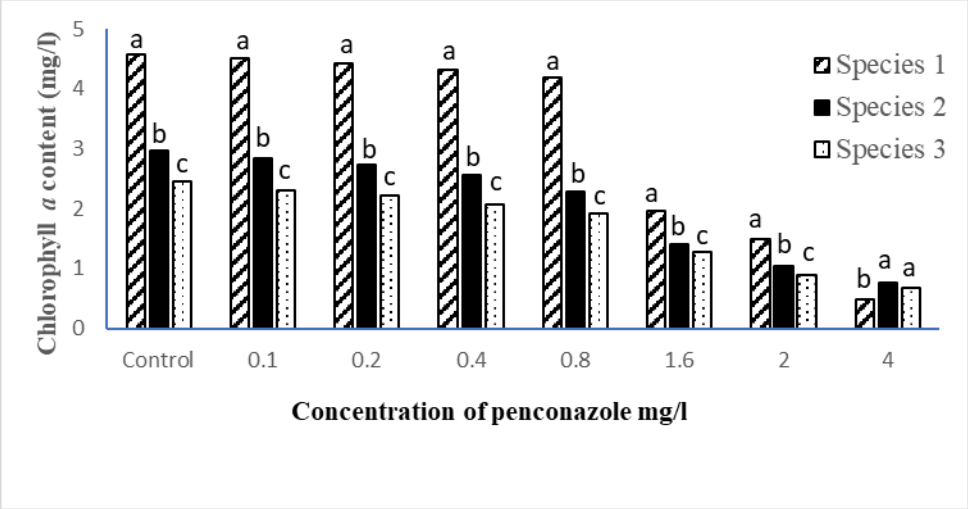
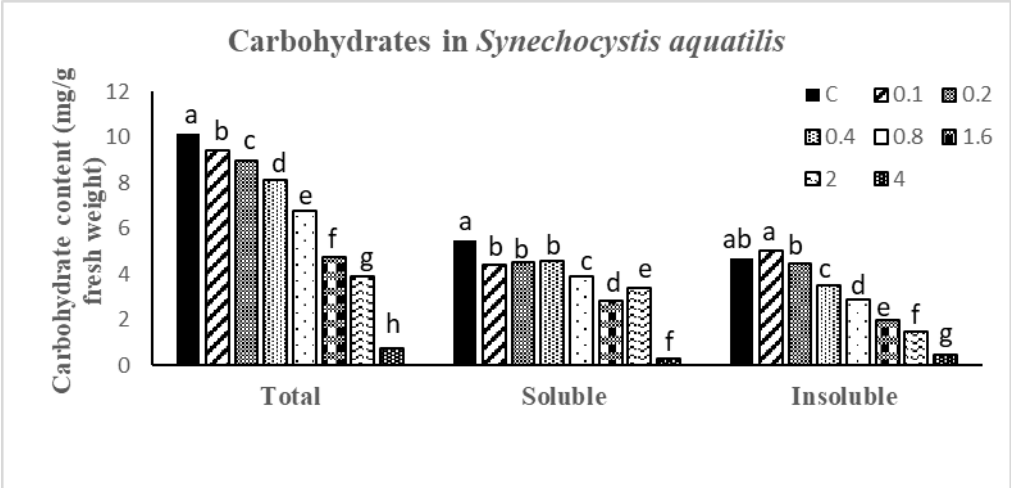
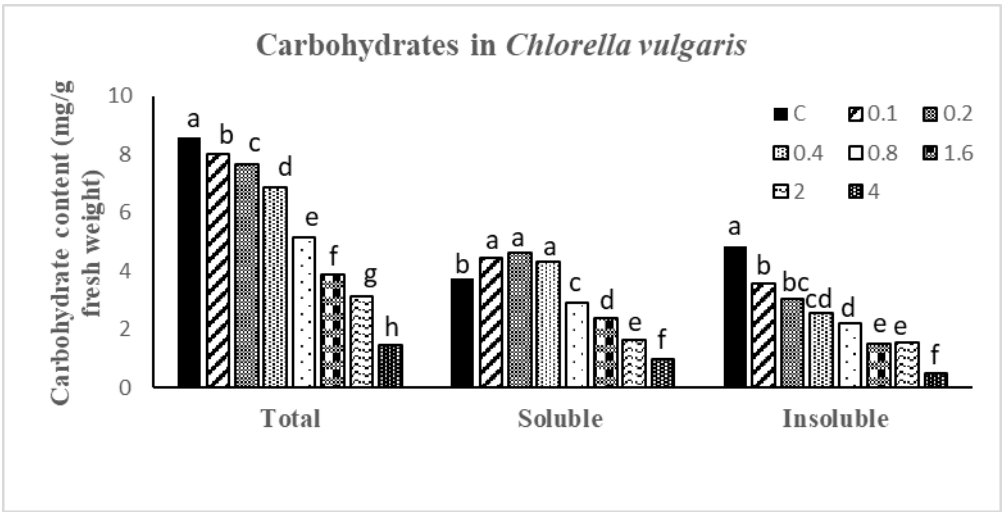


B

Fig. 7. Duncan multiple range test illustrated differences in the growth of the selected species after 24 days of the experiment showing: **A)** Cell count; **B)** Chlorophyll *a*, and species 1: *Synechocystis aquatilis*, species 2: *Chlorella vulgaris* in addition to species 3: *Scenedesmus dimorphs.* **a, b, c)** Average in having different superscripts are significantly ($P \leq 0.05$) different





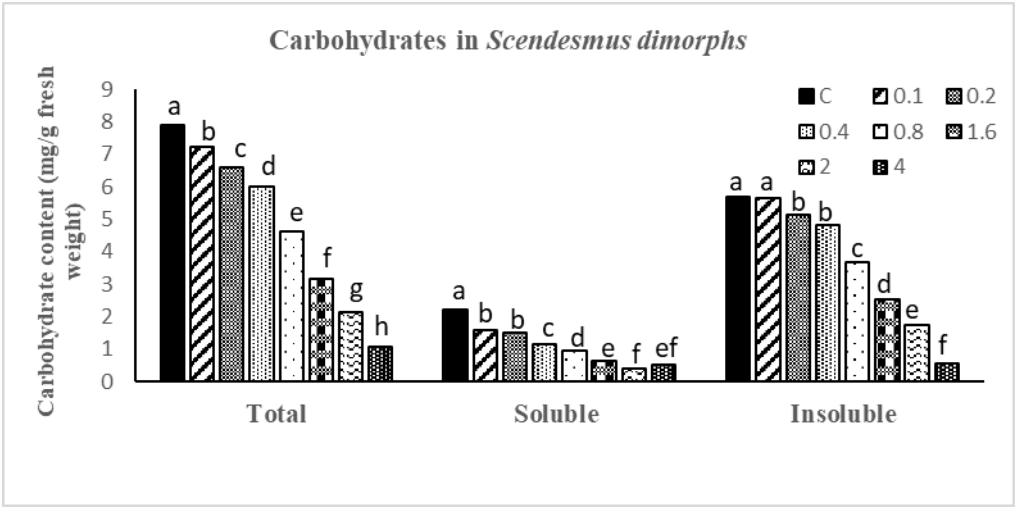


Fig. 8. Effect of different concentrations of penconazole pesticide on the carbohydrate contents of the tested species; **a, b, c** show that average in having different superscripts are different significantly ($P \leq 0.05$)

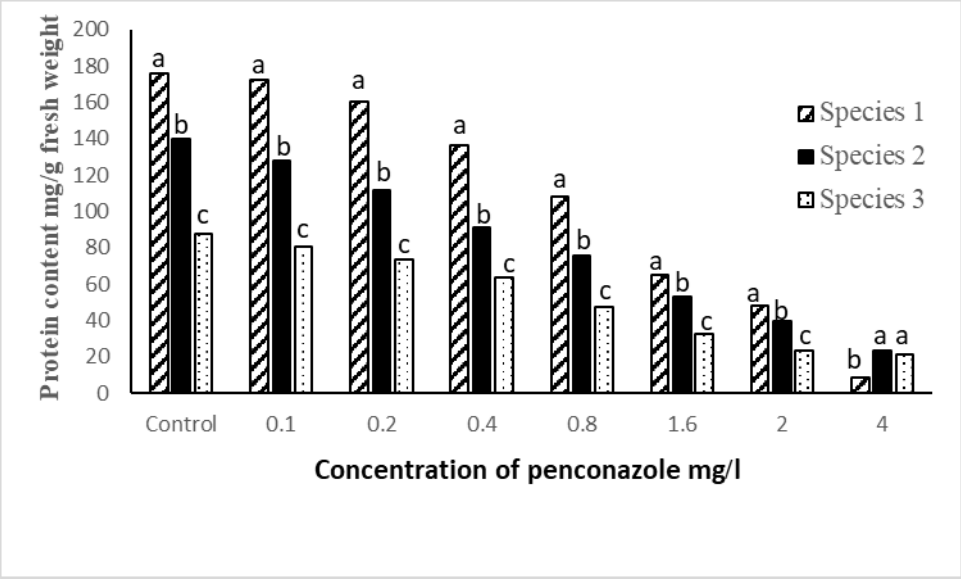


Fig. 9. Effect of different concentrations of penconazole pesticide on the protein contents of the tested species where **a, b, c)** average in having different superscripts differ significantly ($P \leq 0.05$)

DISCUSSION

The present results approved that it was difficult for tested algal species to survive under the high concentrations of PEN. The growth of the tested species was decreased by increasing the PEN concentration. This is due to the damaging influence of the pesticide on the pigment synthesis, as approved by **Mengwei *et al*. (2022)**, who illustrated that the high concentrations of some triazoles could cause the inhibition of microalgal growth, while low concentrations may promote it. Pesticide contamination disrupts the functioning of aquatic ecosystems by harming aquatic organisms and negatively affecting water quality. Specifically, pesticides can reduce dissolved oxygen (DO) levels, increase biochemical oxygen demand (BOD), deplete food sources, and decrease biodiversity **(Ravindra & Haq, 2019; Singh *et al*., 2020)**. Additionally, these contaminants can lead to significant ecological imbalances, as noted by **Adhikari and Mandal (2019)**. They may cause direct or indirect ecotoxicity to microalgae species and cyanobacteria, as well as inducing changes through ecological relations **(Tao *et al*., 2021)**.

Synechocystis aquatilis was more tolerant to different concentrations of PEN than *Chlorella vulgaris* and *Scenedesmus dimorphs*. This observation coincides with that of **Burcin and Kadri (2017)**, who mentioned that the number of algal cells varied significantly depending on the fungicide concentrations and the exposure time, as the cells number decreased when the fungicide concentrations increased. Additionally, at the same time, the penconazole fungicide had significant impacts on the population growth of

Chlorella vulgaris in liquid cultures. As the number of cells in the control cultures increased from the first day of inoculation until the end of the experiment, while in cultures subjected to penconazole pesticide, the cells number decreased in parallel to the fungicide concentrations. **Agirman *et al*. (2015)** elucidated that the influence of penconazole on the algal growth decreases upon increasing fungicide concentrations, and they added that the impact of the toxicity of fungicides on *Scenedesmus acutus* decreases the chlorophyll *a* content as penconazole affects the population growth in liquid cultures.

The study highlights the significant impact of pesticide contamination, particularly penconazole (PEN), on the biochemical content of microalgae. It was observed that PEN significantly affected the carbohydrate content of the tested algae. This finding aligns with previous research indicating that environmental conditions, such as nutrient limitation, salt stress, light intensity, and temperature substantially influence carbohydrate content in microalgae **(Carrieri *et al.*, 2010; Branyikova *et al.*, 2011)**.

In addition, the study of **Mei *et al.* (2014)** supports these observations, showing that the triazole fungicide uniconazole can notably inhibit algal growth, dry weight, chlorophyll a, and carbohydrate content at high concentrations. This effect is linked to a reduction in superoxide dismutase (SOD) and catalase (CAT) enzymatic levels, alongside an increase in lipid peroxidation.

In contrast, the present study found that *Synechocystis aquatilis* had the highest protein content, followed by *Chlorella vulgaris* and *Scenedesmus dimorphus* at various concentrations of PEN. However, at the highest concentration (4mg/ L), *Chlorella vulgaris* exhibited the highest protein content, surpassing both those of *Scenedesmus dimorphus* and *Synechocystis aquatilis*. Lower PEN concentrations (0.1 to 0.8mg/ L) resulted in higher protein content, while higher concentrations (1.6, 2, and 4mg/ L) led to a decrease in protein content.

These results are consistent with previous studies indicating that pesticide exposure can reduce protein synthesis in microalgae. **Cetin and Mert (2005, 2006)** and **Agirman *et al.* (2014, 2015)** highlighted decreased protein quantities in *Scenedesmus acutus* and *Chlorella vulgaris* cultures due to pesticide exposure. This decrease is likely due to the inhibition of enzymes and structural proteins crucial for growth **(Battah *et al.*, 2001)**.

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