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A study on tartrazine-induced biochemical changes in aquatic macrophyte *Salvinia natans*

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Abstract
The present study aims to find out the effects of tartrazine in aquatic weeds *Salvinia natans* in cultural conditions. For the study, the fresh plant samples were exposed to 1 and 2.5 mg/L of tartrazine in glass jars. Tartrazine is a well-known azo dye that also has weedicide properties. For the study, the different concentrations (1 mg/L-T1) and (2.5 mg/L-T2) of tartrazine were treated for 7 days. The biochemical composition such as total proteins, total lipids, total carbohydrates, and pigments like total chlorophyll were estimated at the end of exposure experiments. The dye was accumulated in the plant materials and imparted a yellowish colour to the leaves. The biochemical attributes showed significant ($p<0.001$) alterations with respect to the control. There was no germination in the test plants and which also showed a reduction in total biomass. The study confirmed the weedicide property of tartrazine and the indirect toxicity to other aquatic fauna such as herbivorous fishes.

Keywords: tartrazine, salvinia, aquatic weeds, biochemical changes

Introduction
Aquatic weeds are the producers in the aquatic environments and form the aquatic ecosystem functions, however, they are known as unwanted and undesirable plants which grow and reproduce immensely in eutrophic water bodies [1]. Untreated industrial effluents containing azo dyes, artificial lake colours and weedicides with azo dyes can destruct the aquatic macrophytes and related aquatic fauna. The azo dye tartrazine, also known as acid yellow 23 is very extensively used in garments, synthetic food items and artificial lake colours. The product "aquashade" artificial lake colour and weedicide contain tartrazine in combination with acid blue 9, sometimes with brilliant blue FCF and direct blue [2]. The toxicity studies of tartrazine on rats [3], humans [4] and fishes [5] were also documented and there is not much evidence about the phytotoxicity of tartrazine.
The dye effluents are capable to make the water bright coloured when released into the environment. The colour itself is a pollutant which reduces the light penetration and surface oxygen diffusion and leads to reducing the photosynthetic activity [6]. Reduction in photosynthesis ultimately causes low productivity and diminishes ecosystem health. The biochemical composition and quantity of pigments can indicate plant physiology and overall well-being. In view of biomonitoring, it is a promising aspect of the health assessment of the aquatic ecosystem. For the study, the aquatic macrophyte *salvinia natans* commonly called floating fern is selected which is widely distributed in natural streams as well as in lakes worldwide. Sometimes, it covered the entire water body and makes disturbances for other living beings, even though some species' biomass can be commercially used for fertiliser production, fish feed production and ornamental plants [7]. The present study aims to evaluate the specific effect of azo dye tartrazine on aquatic macrophyte *salvinia natans*, in laboratory conditions.

Materials and Methods
Plant Collection and experimental design
The plant materials were collected from culture farms associated with fish hatcheries in Thiruvanathapuram, Kerala. The whole materials were collected and washed thoroughly and transported to the laboratory in aerated polythene bags. The plants were kept in the culture tanks for 5 days with chlorine-free water and optimum water quality parameters [8]. After the acclimatization, the plants were grouped into three with equal biomass which is 10 g FW each. The plant groups included were Control (C), Test 1 (T1- 1mg/L of tartrazine), and Test 2 (T2- 2.5 mg/L) and the tests were done in 20L capacity glass jars. The tartrazine concentration was fixed after the range-finding test. The macrophytes were exposed to tartrazine for 7 days. During the experiments, the water quality was checked at regular intervals and morphological observations of plants were also done. After the experiments, the plants were subjected to biochemical analysis. All the analyses were done in triplicates for accuracy. The water quality parameters, such as temperature, pH, colour and dissolved oxygen were also estimated. The statistical analysis was also done using Microsoft Excel 2016 and ANOVA for windows 16.

Chemical used

Tartrazine- 95% (C.I. 19140, CAS No 1934-21-0, Mw 534,37, synonyms: E 102, Food Yellow 4, Acid Yellow 23; IUPAC Name: 4,5-Dihydro-5-oxo-1-(4-sulfophenyl)-4-((4sulfophenyl) azo)-1H-pyrazole-3-carboxylic acid and the trisodium salt) and other chemicals were of AR grade purchased from TCI chemicals (Tokyo Chemical Industries), and E Merck India.

Biochemical Analysis

The biochemical analyses were done using standard procedures. The total protein content of macrophytes was analysed spectrophotometrically by the method of [9]. The total carbohydrate content of macrophytes was done colourimetrically by the phenol-H2 SO4 method after extraction into a buffer solution of pH 7 [10]. The total lipid content of macrophytes was estimated by the Sulphophosphovanillin method after extraction in a buffer solution of pH 7 [11]. The total chlorophyll is also determined by using a spectrophotometer [12]

Results and Discussion

The water quality parameters were changed in the test and control water and it is shown in table 1. Temperature, pH, and dissolved oxygen (DO) were altered in test waters, especially in the last days of the experiment. Tartrazine itself causes severe changes in the water even in low concentrations also. However, in the field conditions, there may be a chance of dilution and other hydrological factors can affect the tartrazine-induced changes in water quality. Here, the plant decays in test jars are also responsible for the changes in water quality parameters. From the morphological observations, it was found that the macrophytes in control were healthy and started to develop just one or two leaves while the test plants were with decayed parts and no signs of further development. The plants also showed deep yellow colour in the leaves of the test plants, which can affect the photosynthetic activities and other processes in the leaves.

The results of the biochemical analyses are given in table 2 and figure 1. In the present study, the total proteins show a significant reduction in test groups (T1 and T2) with respect to control. Generally, the protein content in the aquatic macrophytes can vary according to the species, growth stage, locality, and water quality. Here, the same species in similar laboratory conditions show the variation is due to the presence of tartrazine in test water. The variation in protein content of the same species can be the influence of the Physico-chemical attributes of water as suggested by Rather and Nazir (2015) [13]. In the study, the total carbohydrates also significantly ($P<0.001$) diminished in test plants with respect to control which may be due to the toxicity-induced change in the carbohydrate accumulation in tissues. Certain parts of the test plants became shredded and decayed at the end of the exposure experiments and which leads to the damage-induced loss of carbohydrates. The lipid content was increased significantly ($P<0.001$) in test macrophytes with respect to control. Generally, the macrophyte *Salvinia sps* is characterised by low lipid contents, which was reported in studies by Ganie *et al* (2019) [14]. The fluctuations noticed in the total lipid content were also due to the presence of azo dye and induced changes in water quality. In test plants, the lipid content was increased may be due to the plants' losing the capacity of lipid utilisation for growth and development under stress conditions. SO, the lipids remain as accumulated while in control the lipids were used for further metabolism and growth. Lipid content has an important role in metabolism and under stressful conditions, there may be a demand for high lipid content. This need can be met by producing high lipid content by adjusting the plant's physiological as well as biochemical processes [15].

The results of the total chlorophyll are shown in fig.1. There is a significant ($P<0.001$) reduction in total chlorophyll content by increasing the concentration of tartrazine. Due to the stress induced by the presence of tartrazine in test jars, the macrophytes were not growing. The constant unfavourable environment in the culture medium affects chlorophyll production. Similar studies also reported that the toxicity of heavy metals can reduce chlorophyll production in aquatic weeds [16]. Most of the water-born contaminants can affect the weeds by reducing the chlorophyll production of plants [17] [18]. The studies by Ng and Chan (2018) [19] reported that *Salvinia sps* are highly tolerable to certain contaminants like hydrocarbons and heavy metals and studies also suggested that weed can be used for bioremediation. In the present study, there are not many tolerance and remediation properties for tartrazine dye in normal conditions. However, there is a possibility for further studies using the same species for phytoremediation of tartrazine-containing effluents in modified conditions.

Table 1: Water quality parameters in the control and test jars

	Group	1st day	7th day
Temp (^{0}C)	Control	28.2	27.4
	T1	28.3	30.2
	T2	28.1	29.9
pH	Control	6.68	7.15
	T1	6.99	8.01
	T2	6.79	7.98
DO (mg/L)	Control	7	6.03
	T1	6.5	4.9
	T2	6.4	5.0

Table 2: Biochemical Analysis of S. *natans* control and test groups

Biochemical constituents	Control	T1	T2
Total Proteins	145.43±0.66	128±0.91	121±0.83
Total Carbohydrates	163.76±0.34	152.49±0.37	145.32±0.54
Total Lipids	9.8±0.97	14.94±0.48	19.41±0.47
All values are Mean ± SD (Unit: mg/g FW).			

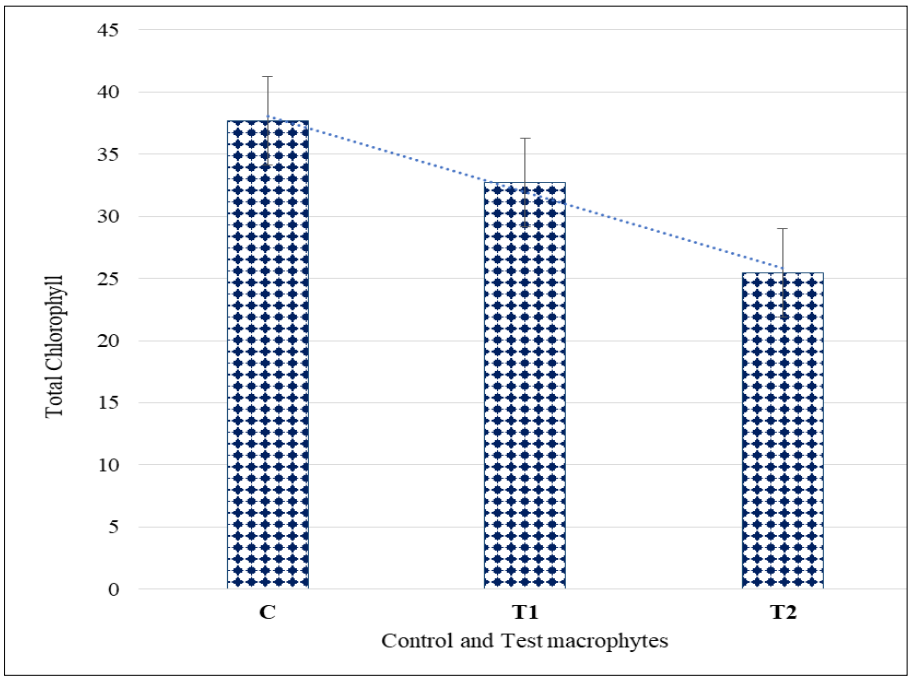

Fig 1: Total Chlorophyll (mg/g FW) in control and test macrophytes

Conclusion

From the study, it was found that tartrazine affected the macrophyte's biochemical composition, and the test plants T1 and T2 shows a significant reduction in total proteins, carbohydrates, and chlorophylls, while the lipids increased in the test plants. It was confirmed that the herbicide property of tartrazine is associated with its phytotoxicity-induced biochemical alterations. So, there should be limited use of aquatic weedicides and artificial lake colours containing dyes in the environment. The toxicity of the dye may vary with time and plant species, and it is recommended that there should be a detailed study before introducing such synthetic chemicals into the environment. In the view of phytoremediation of tartrazine-containing effluents, there should be a detailed study on modified conditions.

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Conflict of Interest

The authors declare that there are no conflicts of interests and no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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