



GROWTH INHIBITORY EFFECT OF POTASSIUM DICHROMATE ON *PSEUDOKIRCHNERIELLA SUBCAPITATA* (KORSHIKOV) HINDAK

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ABSTRACT

In the present study, Potassium dichromate was evaluated against *Pseudokirchneriella subcapitata* (average specific growth rate, growth rate inhibition and yield reduction) at different concentrations. The average specific growth rate of control was 1.90, 1.93 and 0.60 for 1st, 2nd and 3rd day respectively. At 4 mg/l of Potassium dichromate exhibited average specific growth rate of 0.12, -0.07 and -0.06 for 1st, 2nd and 3rd day respectively. Growth rate inhibition and yield reduction of 101.67% and 98.90% respectively were recorded in 4 mg/l concentration of potassium dichromate. An increased concentration from 1, 2, 3 and 4 mg/l, growth rate was drastically decreased.

Keywords: Potassium dichromate, Growth inhibition, Alga

INTRODUCTION

In fast growing world populations, there is need to provide sufficient and adequate food and pharmaceutical products for human beings and livestock's so that many chemicals were used. In this connection all the chemicals enter into the aquatic system; it affect the alga. About half of the atmospheric oxygen was delivered by algae and it is the well-known primary producer for many zooplanktons. It is the significant part of the food chains in aquatic ecosystems which serve directly as a food source for invertebrates, which are then consumed by fish or birds. Any modifications in the quality of the

water will affect the algal population. This will directly or indirectly affect the rest of the ecosystem [1]. Algae are sensitive, fast growing and easily maintained under laboratory conditions and this owed their importance for environmental toxicity study against different chemicals. *Pseudokirchneriella subcapitata* is commonly used as a bioindicator to assess the toxic substances in freshwater ecosystem.

The species was first defined as *Selenastrum capricornutum* [2], then Korschikov in 1990 classified as *Pseudokirchneriella subcapitata* [3]. The ecotoxicological effect of pharmaceutical mixtures was studied against *P. subcapitata* and found that it affected the expression of chloroplast protein [4]. Among the different algal species *P. subcapitata* is the sensitive organism. The bioaccumulation of Cadmium and Copper was studied against three different alga and found robust effect on *P. subcapitata* [5]. Naik and Wanganeo [6] stated that different pesticides inhibit the growth of the phytoplankton. The toxicity of Zinc sulphate, Copper sulphate, Potassium dichromate and some pesticides were tested with seven algal species and the result showed that *P. subcapitata* was most sensitive [7]. Hence *P. subcapitata* is the suitable organism for environmental safety studies.

MATERIALS AND METHODS

Alga



Pseudokirchneriella subcapitata formerly known as *Selenastrum capricornutum*, maintained at Bioscience Research Foundation (BRF), Chennai, India, was used for the growth inhibition test and the experiment was performed according to OECD test guideline 201[8].

Preparation of OECD TG 201 medium

The OECD TG 201 medium was used as growth and test medium. It was prepared by adding stock solution I (macro nutrient): 10 ml (ammonium chloride 1.5 g/l, magnesium chloride hexahydrate 1.2 g/l, calcium chloride hexahydrate 1.8 g/l, Magnesium sulfate heptahydrate 1.5 g/l and Potassium dihydrogen phosphate 0.16 g/l). Stock solution II (iron): 1 ml (Ferric chloride hexahydrate 64 mg/l and Disodium Ethylene diamine tetraacetic acid 100 mg/l). Stock solution III (trace elements): 1 ml (Boric acid 185 mg/l, Manganese chloride 415 mg/l, Zinc chloride 3 mg/l, Cobalt chloride hexahydrate 1.5 mg/l, Copper chloride dihydrate 0.01 mg/l and Sodium Molybdate dihydrate 7 mg/l) and Stock solution IV (bicarbonate): 1 ml (Sodium bicarbonate 50 g/l) into 500 ml of sterile distilled water and 8.1 was initial pH of the medium. Then sterile distilled water was added to the prepared solution to attain final volume 1 liter of growth medium. The prepared growth medium was sterilized by membrane filtration (0.2µm pore sized membrane) [8].

Alga Culture

A known quantity of algal cells was inoculated into the 100 ml of filter sterilized medium in the 250 ml conical flask and incubated under the light intensity of 6500 lux and 21-22°C. The exponentially growing phase of the culture was known by the growth curve of the culture. It is most important to know the exponential growth phase of the culture in which the cells are healthier and multiplies actively. This stage of the culture was used for the growth inhibition test [8].

Growth Inhibition Test

The different concentrations of potassium dichromate were prepared in the growth medium. An amount of 4 mg Potassium dichromate was dissolved in 100 ml of growth medium, considered as initial stock. Further the concentrated stock solution was diluted to attain concentrations of 4 mg/l, 3 mg/l, 2 mg/l and 1mg/l concentration. Growth medium without potassium dichromate concentrations are considered as control. The each 100 ml of control and the test medium was taken in the 250 ml conical flask in three replicates. The control and test flasks of three replicates are inoculated with exponentially growing *P. subcapitata* culture at an initial concentration of 1×10^5 cells/ml. The cultures were incubated in shaker with 100 rpm for continuous exposure of test medium to the organism for 3rd day. The room temperature was maintained at 21-22° C and the light intensity was maintained at 6500 lux as same as during

culturing. The culture flasks were repositioned on each day for even distribution of light intensity to the culture. The incubated cultures were subjected to counting using Neubaur's chamber and a microscope to measure the biomass in each test concentration and control for 1st, 2nd and 3rd day. The pH of the medium was measured on initial and final day of the test and it should not deviate by ± 1.5 units [8].

Average growth rate, growth inhibition and yield

The average specific growth rate for a particular period was calculated for each replicates of controls and treatments as given below,

$$\mu_{i-j} = \frac{\ln X_j - \ln X_i}{t_j - t_i} \quad (\text{day}^{-1})$$

Where,

μ_{i-j} - the average specific growth rate from time i to j

X_i - the biomass at time i

X_j - the biomass at time j

The percent inhibition of growth rate for each treatment replicate was calculated as given below,

$$\% \text{Ir} = \frac{\mu_C - \mu_T}{\mu_C} \times 100$$

Where,

% Ir - percent inhibition in average specific growth rate
 μ_C - mean value for average specific growth rate (μ) in the control group

μ_T - average specific growth rate for the treatment replicate.

The percent inhibition in yield (%Iy) was calculated for each treatment replicate as follows,

$$\% \text{Iy} = \frac{(Y_C - Y_T)}{Y_C} \times 100$$

Where,

% Iy - percent inhibition of yield

Y_C - mean value for yield in the control group

Y_T - mean value for yield for the treatment replicate.

Statistical analysis

The mean and average were calculated for average specific growth rate, growth inhibition and yield reduction. The EC_{50} value was calculated by using NCSS.

RESULTS AND DISCUSSION

The pH of the medium was 8.0 – 8.1 on initial and 8.0 - 8.3 on final day. Hence there was no deviation observed in the pH of the medium during the test period. The maximum average specific growth rate of 1.90, 1.93 and 0.60 for 1st, 2nd and 3rd day observations respectively, in control. Over all the maximum average specific growth of 1.48 was observed in control for 0-3 day. The minimum average specific growth rate of 0.12, -0.07 and 0.06 was



observed for 1st, 2nd, and 3rd day respectively at 4 mg/l concentration (Figure 1). The present study, coincide with earlier findings of Nyholm [9] who reported that different concentrations of copper exhibited the relative growth rate inhibition of *Selenastrum capricornutum* in concentration dependent. Satyavani et al. [10] reported that pyrethroids, herbicides and fungicides exhibited similar activity against *P. subcapitata*. Perfluorooctane sulfonate inhibits the cell density of *P. subcapitata* during the test period [11]. In the present study potassium dichromate inhibit cell concentration of *P. subcapitata*. The present findings coincide with findings of Pendashte et al. [12] who reported that Zinc Oxide Nanoparticles inhibit the cell concentration of *Chlorella vulgaris*. Similarly, Ebenezer and Ki [13] reported that endosulfan reduced the cell count of treated of diatom *Ditylum brightwellii*, *Prorocentrum minimum* and alga *Tetraselmis suecica*.

At 4 mg/l concentration inhibit more than 90% growth rate on 1st, 2nd and 3rd day of treatments. The inhibition of growth rate was increased while concentration of potassium dichromate increases (Figure 2). The present finding supported with earlier findings Satyavani et al. [10] reported that Pyrethroids of Lambda-cyhalothrin 5% EC, Fenvalerate 20% EC and Alphacypermethrin 10% SC inhibited 100% growth rate against *P. subcapitata*. Similarly, atrazine inhibit growth rate (%) of *Raphidocelis subcapitata* with increasing concentration [14].

The potassium dichromate exhibited yield reduction of *P. subcapitata* with concentration dependent manner. Maximum yield reduction of 97.66, 100.33 and 100.07% in 4 mg/l concentration for 1st, 2nd and 3rd day after treatments was observed (Figure 3). The present finding supported with earlier findings of Kamaya et al.[15] reported that 4-HBA, BA, 2-HBA, and 3-HBA exhibited concentration dependent growth rate inhibition against *P. subcapitata*. Satyavani et al. [10] also reported that herbicide Fenaxaprop-p-ethyl 9.3% EC and 2,4 D sodium salt 80% WP exhibited 100% yield reduction of *P. subcapitata*. The EC₅₀ value of 2.40 mg/l concentration (lower limit 2.3 mg/l and upper limit 2.5 mg/l) was noticed for 50% growth rate inhibition on 0-3 day. Similarly, Mischke and Avery [16] reported that imazosulfuron, imazapyr, imazosulfuron and mesotrion exhibited LC₅₀ was less than 10 ppm against *Pseudokirchneriella* sp. The study conclude that Potassium dichromate was toxic to alga and suggested that reduce the usage for protect environmental.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

Figure 1. Effect of potassium dichromate on average specific growth rate of *Pseudokirchneriella subcapitata*

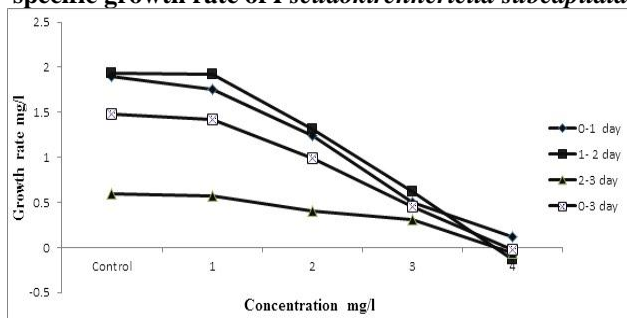


Figure 2. Effect of Potassium dichromate on growth rate inhibition of *Pseudokirchneriella subcapitata*

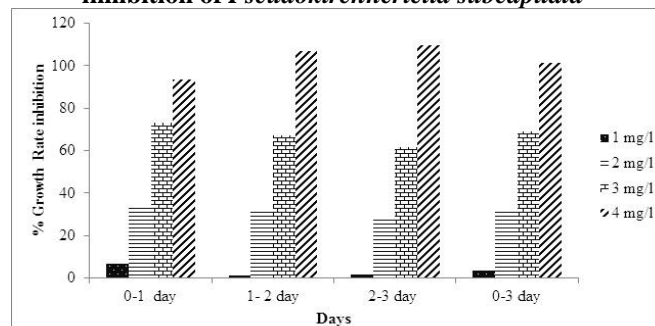
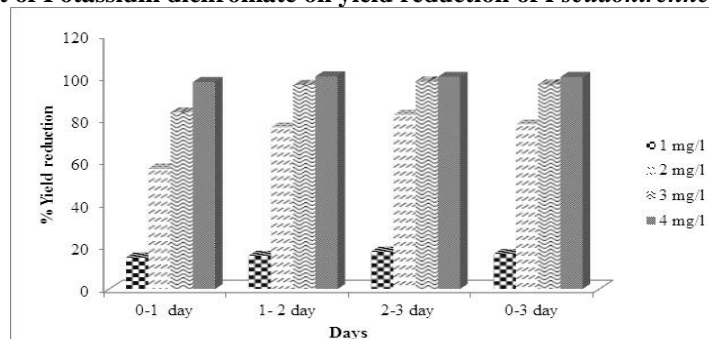


Figure 3. Effect of Potassium dichromate on yield reduction of *Pseudokirchneriella subcapitata*



REFERENCES

1. Nyholm N, Petersen HG. (1997). Laboratory bioassays with microalgae. In: Wang W, Gorsuch JW, Hughes JS. eds. Plants for Environmental Studies, Lewis Publishers, Boca Raton, FL.
2. Printz H. (1914). Kristianiatraktens Protococcoideer. Videnskabselskapets skrifter. I. Den Matematisk-naturvitenskapelige Klasse 1913, 6 (in Norwegian).
3. Hindák F. (1990). Studies on the Chlorococcal algae (Chlorophyceae). V. Biologické práce, 36, 1-228.
4. Backhaus T. (2014). Medicines, shaken and stirred: a critical review on the ecotoxicology of pharmaceutical mixtures. Phil Trans R Soc B, 369: 20130585. <http://dx.doi.org/10.1098/rstb.2013.0585>
5. Girotti S, Bolelli L, Ferri E, Carpené E, Isani G. (2015). Bioindicators in environmental monitoring: Bioluminescent bacteria, algae and honeybees. Proceedings of the 14th International Conference on Environmental Science and Technology Rhodes, Greece, 3-5 September.
6. Naik AA, Wangane A. (2014). Occurrence of some pesticides in Bhoj wetland Bhopal and their effect on phytoplankton community: An ecological perspective. J Toxicol Environ Health Sci, 6, 170-180.
7. Rojíčková, R, Maršálek B. (1999). Selection and sensitivity comparisons of algal species for toxicity testing. Chemosphere, 38, 3329-3338.
8. OECD (2006). Freshwater alga and cyanobacteria, Growth Inhibition Test. Guidelines for the testing of chemicals (Adopted: 23 March 2006 Annex 5 corrected: 28 July 2011)
9. Nyholm (1990). Expression of results from growth inhibition toxicity tests with algae. Archives Environ Contam Toxicol, 19, 518-522.
10. Satyavani G, Chandrasehar G, Varma KK, Goparaju A, Ayyappan S, Reddy PN, Murthy PB. (2012). Toxicity assessment of expired pesticides to green algae *Pseudokirchneriella subcapitata*. ISRN Toxicol, ID 247072, doi:10.5402/2012/247072.
11. Boudreau TM, Sibley PK, Mabury SA, Muir DGC, Solomon KR. (2003). Laboratory evaluation of the toxicity of Perfluorooctane Sulfonate (PFOS) on *Selenastrum capricornutum*, *Chlorella vulgaris*, *Lemna gibba*, *Daphnia magna*, and *Daphnia pulex*. Archives Environ Contam Toxicol, 44, 307-313.
12. Pendashte H, Shariati F, Keshavarz A, Ramzanpour Z. (2013). Toxicity of zinc oxide nanoparticles to *Chlorella vulgaris* and *Scenedesmus dimorphus* algae species. World J Fish Marine Sci, 5, 563-570.
13. Ebenezer V, Ki, JS. (2014). Quantification of toxic effect of the organochlorine insecticides endosulphan on marine green algae, diatom and dinoflagellate. Indian J Geo-Marine Sci, 43, 393-399.
14. Klementova S, Rabova-Tousova Z, Blaha L, Kahoun D, Simek P, Keltnerova L, Zlamal M. (2015). Photodegradation of atrazine on TiO₂—products toxicity assessment. Open J Appl Sci, 5, 14-21.
15. Kamaya Y, Tsuboi S, Takada T, Suzuki K. (2006). Growth stimulation and inhibition effects of 4-Hydroxybenzoic acid and some related compounds on the freshwater green alga *Pseudokirchneriella subcapitata*. Archives Environ Contam Toxicol, 51, 537-541.
16. Mischke C, Avery J. (2013). Toxicities of agricultural pesticides to selected aquatic organisms. Southern Regional Aquaculture Center (SRAC) Publication No. 4600.

