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Effect of 2,4-Dichlorophenoxyacetic acid on growth, protein and chlorophyll-*a* content of *Chlorella vulgaris* and *Spirulina platensis* cells

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Abstract: *In this study, effect of different 2,4 -dichlorophenoxyacetic acid (2,4-D) concentrations (0.0, 9.10^{-5}, 9.10^{-4}, 9.10^{-3} and 9.10^{-2} mM) on growth rate, content of protein and chlorophyll-a in Chlorella vulgaris and Spirulina platensis cells was investigated. The most stimulatory effect on growth rate, protein and pigment ratio of C. vulgaris and S. platensis was observed at 9.10^{-4} mM concentrations of 2,4-D. The results show that low concentrations of 2,4-D have hormonal effect due to being a synthetic auxin. Cell number, protein and pigment rates were inhibited at 9.10^{-2} mM concentration in C. vulgaris. Such parameters were inhibited in S. platensis, both at 9.10^{-3} and 9.10^{-2} mM 2,4-D concentrations. This is due to herbicidal effect of high concentrations of 2,4-D. S. platensis was found to be more sensitive than S. vulgaris to 2,4-D applications. The use of algae as bio-indicators in herbicide contaminated fresh water habitats, was discussed.*

Key words: *Chlorella vulgaris, Spirulina platensis, Growth, Protein, Chlorophyll-a*
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Introduction

Pesticides are being used to protect the agricultural crops against many pests. However, excessive use of such chemicals without any restrictions causes pollution in soil and water and they reach to human body *via.* food chain, or pesticide contaminated fruits and vegetables (Toros and Maden, 1985; Sarnaik *et al.,* 2006). Herbicides are used to control weeds within agricultural areas. Applied herbicides reach aquatic environment *via.* rain fall, and this causes pollution in aquatic areas that damages microorganisms, plankton, fish and indirectly human beings through the food chain (Ozturk and Ozge, 1978; Budak and Budak, 2006).

Many reports have indicated that herbicides are toxic to different algae (Brown and Lean, 1995; Okay and Gaines, 1996, Kobraei and White, 1996 ; Yong *et al.,* 2006). According to Irmer *et al.* (1986), *Chlorella* sp is the most studied within the single celled green algae, and it is more resistant to chemical applications compared to other green algae, such as *Chlamydomonas* sp. Many researches have been done on effect of various organic and inorganic compounds on *Scenedesmus quadricauda.* Some of these works are on herbicide toxicity. Ibrahim (1990) reported the effect of paraquat on algal growth, and Fargasova (1994) revealed effect of some plant growth regulators on algal growth. Peterson *et al.* (1994) reported the effect of 23 different pesticides on the ^{14}C uptake, whereas Wong (2000) reported the effect of 2,4-D, glifosate and paraquat on growth, photosynthesis and chlorophyll synthesis in *S. quadricauda* .

The present work was undertaken to study hormonal and herbicidal effect of different 2,4-D concentrations on growth rate, content of protein and chlorophyll-*a* on *Chlorella vulgaris* (eukaryote) and *Spirulina platensis* (prokaryote).

Materials and Methods

Algal species, *Chlorella vulgaris* (Chlorophyta) and *S. platensis* (Cyanophyta), used in this study, were provided by University of Cukurova, Faculty of Fisheries (Adana, Turkey). These were cultured in 1000 ml of glass bottles for 9 days under controlled conditions of temperatures 25 ± 2^{o}C and 35^{o}C for *C. vulgaris* and *S. platensis,* respectively. The culture media was Jaworsky (Humpage *et al.*, 1994) for *C. vulgaris* and *Spirulina* media (Schlosser, 1982) for *S. platensis.* The algae were cultured in 5 different 2,4-D (Aldrich) concentrations that were 0.0 , 9.10^{-5}, 9.10^{-4}, 9.10^{-3} and 9.10^{-2} mM. Similar 2,4-D concentrations were applied by Wedding *et al.* (1959). The illumination system was 36 μmol (m^{2}.s) and the cultures were exposed to 16 hr light and 8 hr dark regime. The cultures were agitated 3 times a day to prevent cell sinking. All experimental cultures, including control, were set up in triplicate.

The protein analyses of the cultures were done with Lowry method (Lowry *et al.*, 1951). The pigment analyses were done according to Parsons and Strickland (1963). All analyses were carried out in triplicate. The growth rate in the culture media was calculated using the following formula (Gulliard, 1973):

$$\mu = \log (N_1/N_0) \times (3.332/t)$$

Where μ is specific growth rate, N_0 starting cell density (cell/ml), N_1 cell density at *t* time (cell/ml) and *t* is time (day).

For statistical analyses variance (ANOVA) in statistical analysis system (SPSS 11.0 for windows) was employed. The significance of differences between mean values was determined by a multiple range test (LSD; least significant difference). For this reason, p value was preferred to be 0.05, which corresponds to a confidence level of 95%.

Results and Discussion

Cell countings: Cell numbers of *C. vulgaris,* cultured in different concentrations of 2,4-D are given in Table 1. Time dependent increases in all tested 2,4-D concentrations were counted in cell number of the green algae cultures. Cell numbers increased significantly (p<0.05) at 9.10^{-5} and 9.10^{-4} mM 2,4-D concentrations in 3rd, 5th, 7th and 9th days, when compared to control (p<0.05). There was significant decrease in cell numbers at 9.10^{-3} and 9.10^{-2} mM 2,4-D concentrations in same harvest time (p<0.05). Similar results were generally observed for *S. platensis* (Table 2). As similar to be *C. vularis,* time dependent increases in all tested 2,4-D concentrations were also counted in cell number of the blue-green algae cultures.

Growth rate: Growth rate values of *C. vulgaris* were insignificantly changed at the low concentrations of 9.10^{-5} to 9.10^{-3} mM 2,4-D (p>0.05) (Fig. 1). However, the minimum rate, 0.43, was calculated at 9.10^{-2} mM (p<0.05). Growth rate values of *S. platensis* increased in the low 2,4-D concentrations (9.10^{-5}-9.10^{-4} mM), but decreased at 9.10^{-2} mM 2,4-D (Fig. 1). According to the findings, the maximum rate was calculated to be at 9.10^{-5} mM 2,4-D as 0.68 (p<0.05). The minimum rate was found at 9.10^{-2} mM as 0.40 (p<0.05).

Pollution of surfacewaters by organic pesticides and herbicides is expected to cause serious damage to the existing water resources and leads to significant changes in the aquatic ecosystem. The impact of herbicides on algal populations may be considered from various environmental points of view. Herbicides are liable to exert algistatic, inhibitory or lethal effects on algae. The magnitude of effects will depend on the concentration and chemical properties of the herbicide as well as its selective effects on the

Table 1. Changes of cell number of *C. vulgaris* in response to the treatment of 2,4-D concentrations

2,4-D (mM)	Cell number (cell / ml)					
	Counting time (d)					
	0	1	3	5	7	9
0.0	12.7±1.2	24.0±0.7	88.0±0.6	355.6±0.6	542.0±1.5	656.3±0.9
9.10^{-5}	12.0±1.4	26.3±0.3	105.0±0.6*	393.0±1.5*	615.0±0.6*	746.0±1.5*
9.10^{-4}	14.7±1.2	27.3±0.3*	103.7±0.9*	434.7±0.9*	676.7±0.9*	794.0±1.5*
9.10^{-3}	13.0±0.7	25.3±0.3	92.0±0.6*	314.0±1.2*	531.3±0.9*	616.0±0.6*
9.10^{-2}	14.0±0.5	22.7±0.3	56.7±0.9*	126.3±0.9*	267.3±1.2*	393.7±1.9*

Values expressed as mean±SE, * = indicates significantly difference from control according to LSD test (p<0.05)

Table 2: Changes of cell numbers of *S. platensis* in 2,4-D concentrations

2,4-D (mM)	Cell number (cell / ml)					
	Counting time (d)					
	0	1	3	5	7	9
0.0	12.0±0.6	27.7±0.3	130.0±0.6	416.7±0.7	596.7±0.9	687.0±1.5
9.10^{-5}	10.3±0.3	30.0±0.6	135.7±1.5*	584.3±0.9*	687.7±1.5*	811.3±2.4*
9.10^{-4}	11.3±0.9	32.0±0.6	162.7±1.2*	632.0±2.1*	707.0±1.5*	856.3±1.5*
9.10^{-3}	12.3±1.9	25.3±2.1	114.3±2.3*	394.3±2.3*	589.7±0.9	659.7±3.3*
9.10^{-2}	10.7±1.5	24.0±1.5	46.0±1.5*	86.0±2.5*	124.3±1.2*	171.3±1.5*

Values expressed as mean±SE, * = indicates significantly difference from control according to LSD test (p<0.05)

Table - 3: Changes of chlorophyll-*a* contents of *C. vulgaris* in 2,4-D concentrations

2,4-D (mM)	Chlorophyll-*a* (mg / l FW)					
	Harvest time (d)					
	0	1	3	5	7	9
0.0	52.9±3.1	73.8±2.3	123.4±2.1	146.5±2.0	177.7±1.5	336.4±4.2
9.10^{-5}	56.8±2.6	84.5±1.5	132.0±2.0*	152.6±1.9	191.1±4.1	377.7±2.0*
9.10^{-4}	62.9±1.8	87.0±2.5	162.3±4.0*	182.9±3.0*	263.5±31.8*	441.9±2.0*
9.10^{-3}	59.9±4.7	76.0±2.5	85.9±1.9*	137.5±3.7	175.5±3.3	278.1±4.1*
9.10^{-2}	50.7±0.5	70.6±1.0	81.4±3.3*	113.1±4.0*	149.2±3.7*	224.8±2.0*

Values expressed as mean±SE, * = indicates significantly difference from control according to LSD test (p<0.05)

Table - 4: Changes of chlorophyll-*a* contents of *S. platensis* in 2,4-D concentrations

2,4-D (mM)	**Chlorophyll-*a* (mg/l FW)**					
	Harvest time (d)					
	0	**1**	**3**	**5**	**7**	**9**
0.0	68.2±0.8	104.1±0.5	175.4±2.2	409.3±2.0	469.9±0.8	538.0±1.9
9.10^{-5}	67.4±0.7	103.8±1.9	176.3±2.3	442.0±2.2*	499.0±1.4*	563.0±1.8*
9.10^{-4}	69.5±3.4	105.5±0.9	202.2±3.7*	462.6±1.9*	521.2±1.9*	607.2±3.4*
9.10^{-3}	63.0±2.9	91.1±1.9	146.5±1.8*	367.9±1.4*	446.5±1.8*	492.2±1.6*
9.10^{-2}	63.1±1.7	81.3±0.9*	97.2±1.4*	189.9±1.0*	276.9±0.8*	353.9±1.5*

Values expressed as mean±SE, * = indicates significantly difference from control according to LSD test (p<0.05)

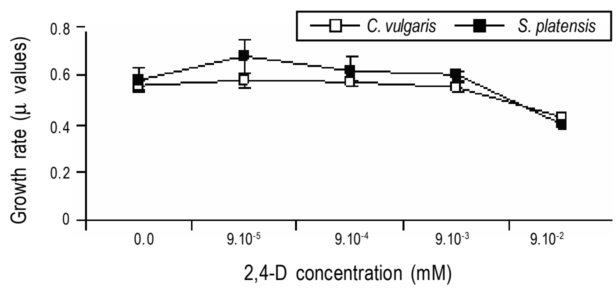

Fig. 1. Growth rates of *C. vulgaris* and *S. platensis* in different 2,4-D concentrations after nine days of treatment

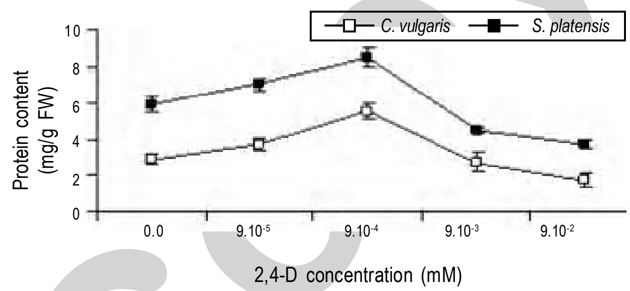

Fig. 2: Protein contents of *C. vulgaris* and *S. platensis* in different 2,4-D concentrations after nine days of treatment

various species exposed to it (Maly and Ruber, 1983). *Nostoc* growth was inhibited at 10 mg/l 2,4-D concentration (Gangawane *et al.*, 1980). Bednarz (1981) reported that low concentrations of 2,4-D stimulated the growth of most species of algae including green and blue-green algae, whereas high concentrations inhibited growth. In our study, algal species were adopted to cultural conditions during first three days. Increase in cell number in *C. vulgaris* and *S. platensis* was observed at 3th day. 9.10^{-5} 9.10^{-4} and 9.10^{-3} mM concentrations of 2,4-D no toxic effect on algae growth, however at 9.10^{-2} mM 2,4 D concentrations, growth of the algae species was inhibited. This could be due to toxic effect of 2,4-D at high concentrations.

Protein content: Protein contents of the *C. vulgaris* and *S. platensis* are given in Fig. 2. Protein content of the *C. vulgaris* gradually increased up to 9.10^{-3} mM (p<0.05). However sharp decline was observed at 9.10^{-2} mM 2,4-D after 9-days of application (p<0.05). Although protein content of *S. platensis* also significantly increased in 9.10^{-5} and 9.10^{-4} mM 2,4-D after 9-days of treatment (p<0.05), however, reductions was measured in 9.10^{-3} and 9.10^{-2} mM concentration (Fig. 2).

Protein content of *C. ellipsoidea* increased with the treatment of 2,4-D at 22 mgl^{-1} concentration. However, the content was decreased in the effect of 2,4-D at 88 mgl^{-1} oncentration (Chai and Chung, 1975). Protein content in *C. vulgaris* at low concentrations of 2,4-D increased and decrease was observed at high concentrations. The maximum increase in protein content was observed at 9.10^{-4} mM 2,4-D concentration. In *S. platensis*, the protein content increased at low concentrations, and decreased at high concentrations. The highest increase occured at 9.10^{-4} mM 2,4-D concentration. *S. platensis* was found to be more sensitive to high concentrations of 2,4-D than of *C. vulgaris.*

Chlorophyll-*a* content: Chlorophyll-*a* content of *C. vulgaris* cultured in different concentrations of 2,4-D is given in Table 3.The content of chlorophyll-*a* at 9.10^{-5} and 9.10^{-4} mM concentrations increased whereas at 9.10^{-3} and 9.10^{-2} mM decrease and change in both cases was statistically significant. Chlorophyll-*a* values of *S. platensis* exposed to different concentrations of 2,4-D are given in Table 4. Increase in chlorophyll-*a* content at 9.10^{-5} and 9.10^{-4} mM 2,4-D concentrations and decrease at 9.10^{-3} and 9.10^{-2} mM concentrations were found to be significant in 5th, 7th and 9th days (p<0.05).

Photosynthesis was inhibited with high concentrations of 2,4-D in *Chlorella elipsoidea* cultures, and low concentrations stimulated the parameter (Chai and Chung, 1975). Effect of 2,4-D concentrations on photosynthesis and chlorophyll-*a* synthesis in *Scenedesmus quadricauda* was investigated by Womg (2000). Chlorophyll-*a* content of the algae increased at 0.02 and 0.2 mgl^{-1} 2,4-D, but the content was decreased at 20and 200 mgl^{-1}In our study, in *C. vulgaris* and *S. platensis*, increase in chlorophyll-*a* was detected at 9.10^{-5} and 9.10^{-4} mM 2,4-D concentrations, however decrease occured at 9.10^{-3} and 9.10^{-2} mM 2,4-D concentrations. The highest increase was detected at 9.10^{-4} mM 2,4-D concentrations, and the maximum decrease was observed at 9.10^{-2} mM 2,4-D concentration.

High dosage applications of 2,4-D in agricultural practices have inhibitory effect on *C. vulgaris* and *S. platensis*. Stimulatory effect of low dosage of 2,4-D indicates that these algae could be used as bio-indicators in aquatic environments contaminated with this herbicide.

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