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Araştırma/Research

Investigation of the effect of chlorpyrifos-ethyl and pendimethalin on *Desmodesmus communis* (E.Hegewald) E. Hegewald

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Abstract: This study was carried out to determine the effect of herbicides and insecticides on *Desmodesmus communis.* Pendimethalin and chlorpyrifos-ethyl were applied to isolate *D. communis* from microalgae collected from natural environment. The experimental was regulated control group (C), herbicide [H1 (19 mgL^{1}), H2 (110 mgL^{-1}) and H3 (280 mgL^{-1})] and insecticide [I1 (10 mg/l), I2 (30 mg/l) and I3 (115 mg/l) 30 mgL^{-1})] and each group 3 times was repeated. The highest biomass in herbicide application was determined on first day. The highest values according to the groups (H1, H2 and H3) was found 0.40±0.09 μgL^{-1}, 0.47±0.18 μgL^{-1} and 0.49±0.15 μgL^{-1} respectively. In the case of insecticide application, the highest value was calculated on the starting day in all groups. According to the groups (I1, I2 and I3) biomass values were determined as 0.33±0.01 μgL^{-1}, 0.37±0.00 μgL^{-1} and 0.38±0.01 μgL^{-1}, respectively. At the end of the experiment, it was observed that pesticide groups with nitrogenous compound in the development of *D. communis* were more active than the group of pesticides with phosphorous compound, and thus the decrease in cell numbers was less.

Keywords: Pendimethalin, chlorpyrifos-ethyl, biomass, *D. communis*

INTRODUCTION

Agriculture is the oldest and most well-known method of meeting the nutritional needs of the world. As a result of population growth, countries have started to search for ways to get more efficiency from the unit area in order to meet the need for quality and cheap food. Pesticides have been used to combat pests such as weeds and insects for a long time (Solmaz et al., 2010).

Intensive fertilization and pesticide activities are affected on freshwater ecosystems as a serious threat. Pendimethalin is the active ingredient of herbicide used in the control of weeds. It has been determined that 10-20% of this herbicide, which is used in terrestrial areas, evaporates in the first weeks after application. The half-maximum spread time or half-dose (LD50) of pendimethalin has been reported to last from a few days to 4200 days. As a result of the experiments carried out in the field and laboratory, it was determined that the concentration of pendimethalin reached 6 mgL^{-1} in fresh waters. The lethal concentration (LC_{10}) value for *Daphnia* was found to be 6 mgL^{-1}. It has been reported that soil microbiota is affected by pendimethalin for 4 weeks after application (Strandberg and Scott-Frodsmand, 2004). According to the Environmental Quality Standard, the limit value that can be found in rivers and lakes has been reported as 0.5 μgL^{-1} (Anonim, 2014).

According to the Environmental Impact Standard, the limit value of chlorpyrifos-ethyl, which can be found in rivers and lakes, is 0.5 $\mu g/L^{-1}$ (Anonim, 2014). In our country, pesticide derivatives used in plant protection, whose active ingredient is chlorpyrifos-ethyl, are prohibited in imported products according to the Veterinary Services Plant Health and Feed Law No. 5996 in 2016 (Anonim, 2016).

The aim of this research was isolate *D. communis* from lakes, and investigate of the effect pendimethalin and chlorpyrifos-ethyl active substances on this species.

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MATERIAL AND METOD

Phytoplankton samples were collected from Lake Tortum with a plankton net (55Ø) in June 2016 and October 2016. It was brought to the laboratory in 100 ml plastic sample storage containers. Then, the samples were incubated for 10 days in a 250 ml glass Erlenmeyer in Bold Basal 11 medium enriched with agar. The microalgae that grew most at the end of the incubation period were isolated under invert microscope (Zeiss) with 600X magnification, and *D. communis* was transferred to 10 ml glass tube using the method reported by Andersen (2005).

In the experiments the herbicide concentration, which is the trade name Herbimat 330 EC (active ingredient pendimethalinden), was prepared as 19 mgL^{-1}, 110 mgL^{-1} and 280 mgL^{-1}. The insecticide concentration, which is the trade name was Chlorfet 48 EC (active ingredient chlorpyrifos-ethyl), was prepared as 10 mgL^{-1}, 30 mgL^{-1} and 115 mgL^{-1}. Stock solutions were stored in the refrigerator at +4 °C until the experiment started. All of experiments was carry out in Atatürk University Faculty of Fisheries Algae Unit. It was carried out by applying 2% CO_2 every day to all groups and the room temperature was fixed to 26 °C. The research took a total of 32 days, with 7 days of the experimental phase and 25 days of the isolation, identification and production of *D. communis* before the experiment.

Experimental Procedure

In this research has been carried out in Laboratory of Atatürk University, Faculty of Fisheries, Basic Science Research Unit. Initially, microalgae were grown in 10 ml tubes. The microalgae that developed in the tubes were 250 ml Erlenmeyer flasks, and were grown at 26 ºC, in a 120 $\mu molm^{-2}s^{-}$ lighting and 110 rpm shaking incubator (JRS Lab 32 brand) in a 16:8-hour day-night photoperiod. For intensive production of microalgae, they were taken into 5L glass containers, where it was started to apply insecticide and herbicide with lids in the Algae Unit in the research unit.

In the experiment, modified Bold Bazal 11 Medium was used as nutrient medium. It is contained: $NaNO_3$ (1.5 gL^{-1}), $K_2HPO_4.3H_2O$ (40 gL^{-1}), $MgSO_4.7H_2O$ (75 gL^{-1}), $CaCl_2.2H_2O$ (36 gL^{-1}), Na_2CO_3 (20 gL^{-1}), $MgNa_2EDTA.H_2O$ (1.0 gL^{-1}), trace metal solution (1 ml) (Andersen, 2005).

Dry Biomass Analysis

Samples (50 ml) taken from all groups in the experiment were filtered using Whatman GF/C filter paper, then the filter papers were kept at 100 °C until they reached a constant weight. The samples were weighed by placing them in the tared petri dishes on a balance with a sensitivity of 0.001 g. The dry matter content was calculated by taking the differences between the wet and dry weights of the samples (Vonshak, 1997).

D. communis Cell Count

The number of *D. communis* cells in the experimental groups was counted daily under the Zeiss Primo Star AxioCam ERc 5s model binocular microscope (with 400X magnification).

Phytoplankton cell count (cell/ml) = N x DF x 10.000

In this;
N = *D. communis* cell numbers (cell),
DF = Dilution factor (mm^3)
10.000 = The coefficient used in converting the counting result in 0.1 mm^3 to the number in 1 ml

D. communis Biomass

D. communis biomass in the experimental groups were measured every day on spectrophotometer (Beckman Coulter DU730) at a wavelength of 680 nm. *D. communis* biomass was calculated from the following formula (Kang et al., 2005);

Biomass (μgL^{-1}) = $0.713 \times OD_{680}$

Statistical Analysis

The variation of *D. communis* biomass, cell count, pH, water temperate and dry biomass depending on groups and days was determined by One-Way (ANOVA) test using IBM SPSS 20. The significance of the differences was evaluated according to the DUNCAN test. Checks all of dates by Kesici and Kocabaş (2007).

RESULTS AND DISCUSSION

In this study was planned as 27 groups into control (C), pendimethalin – treated groups [H1 (19 mgL^{-1}), H2 (110 mgL^{-1}) and H3 (280 mgL^{-1})] and chlorpyrifos-ethyl - treated groups [I1 (10 mgL^{-1}), I2 (30 mgL^{-1}) l) and I3 (115 mgL^{-1})]. There were performed measurement of pH, temperature and light, with analysis of cell count, dry matter amount and biomass during the experiment.

Change of pH in Pendimethalin and Chlorpyrifos-Ethyl - Treated Groups

In this experiment, the change in pH value between the groups depending on the days was found to be statistically significant ($p<0.05$). In the control group, the highest pH value was measured on the 6th day (8.41±0.40), while the lowest value was determined on the 7th day (7.49±0.02). The mean pH value was 7.54±0.17 in the pendimethalin – treated groups, and 7.53±0.15 in the chlorpyrifos-ethyl - treated groups (Figure 1, Figure 2).

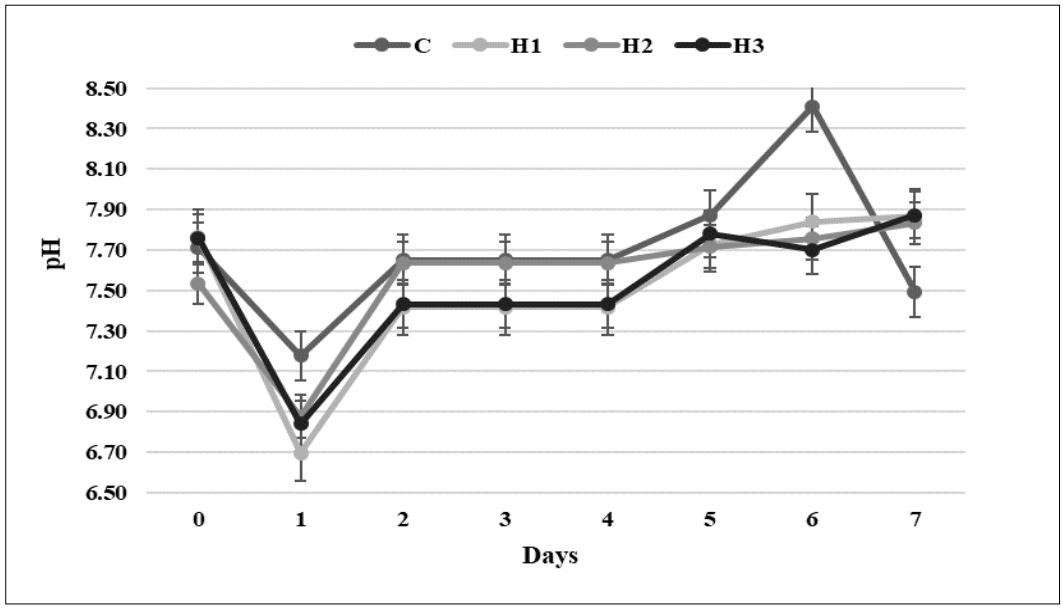


Figure 1. pH change depending on the day and groups in pendimethalin – treated groups.

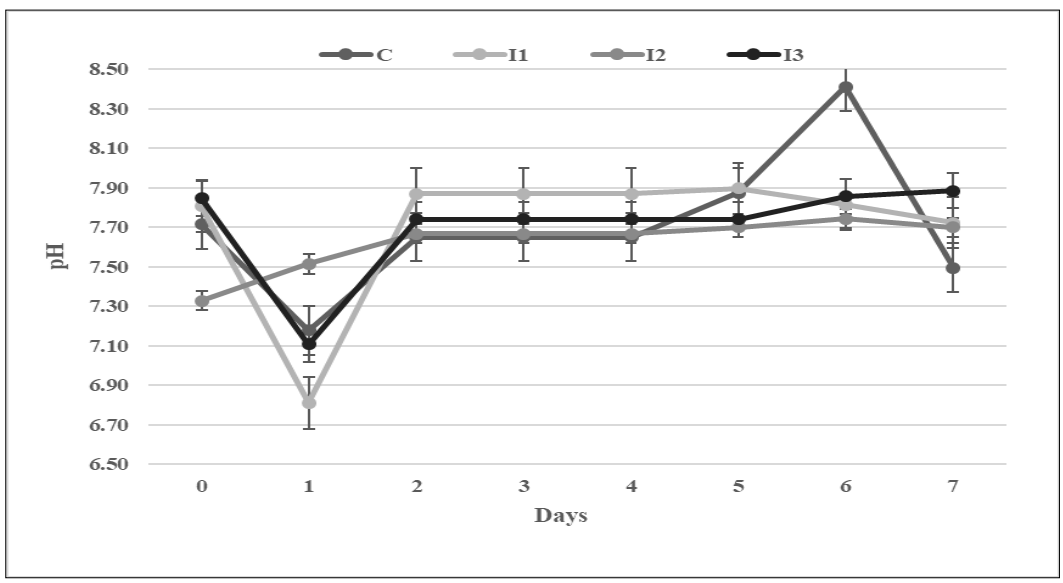


Figure 2. pH change depending on the day and insecticide (chlorpyrifos-ethyl) application groups

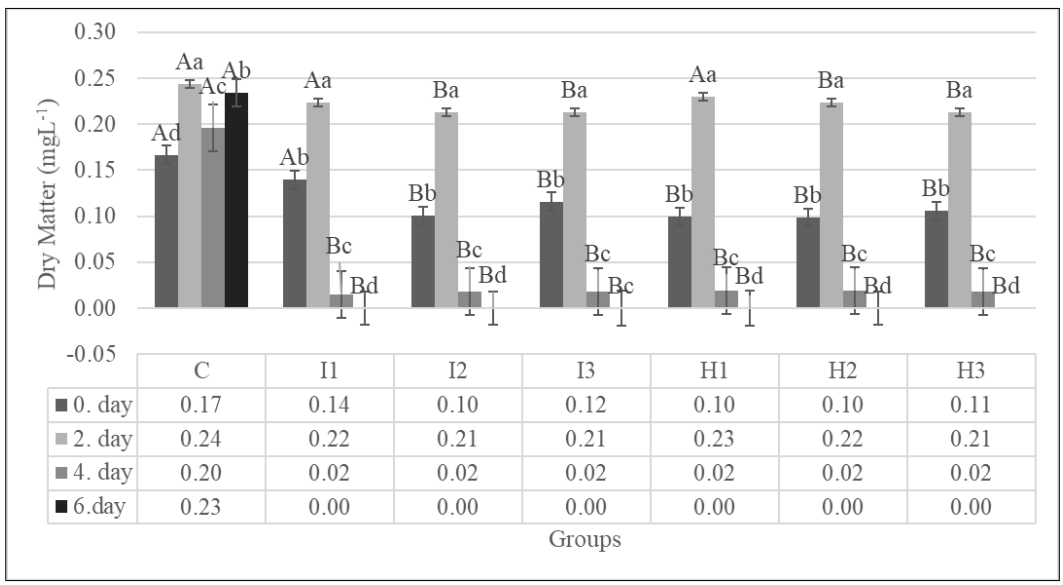
The changes in water temperature and light intensity of the pendimethalin – treated groups and chlorpyrifos-ethyl - treated groups and the control group were measured every morning at 9:00 am. The relationship between the untreated groups was found to be statistically insignificant ($p>0.05$).

The most important factor in the effects of pesticides on aquatic system is changed the pH value in a direction that increases or decreases. It was reported that the effects of dichlorodiphenyldichloroethane (DDD) and dichlorodiphenyldichloroethylene (DDE) change depending on the pH value, at the same time, cell uptake was highest when the pH value was 7 for both pesticides and cell death began after the 4th day (Luo et al., 2014). In this study was determined that the highest pH value was measured on the 6th day when *D. communis* cells died.

Dry Matter of Pendimethalin and Chlorpyrifos-Ethyl - Treated Groups

The variation of the dry matter amount depending on the days and the relationship between the groups were found to be

statistically significant ($p<0.05$). It was determined that the amount of dry matter increased suddenly in the weighing made on the 2nd day, and then a decrease was observed on the 4th day, although it increased again on the 6th day in the control group. It was calculated that dry matter values increased approximately two times on the 2nd day in the pendimethalin and chlorpyrifos-ethyl - treated groups. However, this groups were observed a sudden decrease on the 4th day, and the death of all cells on the 6th day (Figure 3).



	C	I1	I2	I3	H1	H2	H3
0. day	0.17	0.14	0.10	0.12	0.10	0.10	0.11
2. day	0.24	0.22	0.21	0.21	0.23	0.22	0.21
4. day	0.20	0.02	0.02	0.02	0.02	0.02	0.02
6.day	0.23	0.00	0.00	0.00	0.00	0.00	0.00

Figure 3. Changes of dry matter (mgL^{-1}) [ABCDE Capital letters indicate the difference of each group within days ($p<0.05$), and abc Lowercase letters indicate the difference between each group and the other groups ($p<0.05$)]

In this study, the amount of dry matter in the pendimethalin and chlorpyrifos-ethyl - treated groups increased approximately two times on the 2nd day, while it showed a sudden decrease on the 4th day. Change of dry matter values depend on days was observed similar with variation of *D. communis* biomass depend on days. This situation was found similar with *C. vulgaris* experiment, which was investigated effect of in waste water contaminated with organic substances on chlorophyll-a and cell count of *C. vulgaris* (Shelknanloymilan et al., 2012).

***D. communis* Cell Counting**

During the experiment, *D. communis* cell count between groups and days was found to be statistically significant ($p<0.05$). The highest *D. communis* numbers was found pendimethalin – treated groups. *D. communis* numbers (H1, H2 and H3) were calculated as 130.67±7.02 cell/ml, 133.67±28.10 cell/ml and 153.67±19.40 cell/ml, respectively (Table 1). The highest *D. communis* numbers were determined in chlorpyrifos-ethyl - treated groups (I1, I2 and I3) as 95.67±6.66 cell/ml, 134.67±14.57 cell/ml and 125.33±8.02 cell/ml, respectively (Table 2).

In this experiment, the least *D. communis* cell counts were determined on the 6th day. It was reported that *C. vulgaris* cell count was determined as 63.5±3.9 μgL^{-1}, and at the end of the 4th day, the cell number was 29.7±1.0 μgL^{-1}, and this situation was caused by abiotic factors such as sunlight and ventilation (Hultberg et al., 2016).

It has been reported that *Monoraphidium contortum* numbers was found to be affected by increasing the dose concentrations of herbicides [(l.1-dimethyl-4.4-bipydridyldiylium dichloride), 2.4-D (2.4-dichlorophenoxyacetic acid) and daminozide (N-dimethyl amino succinic acid)], for instance gradual decreases were observed in the amount of water up to the 96th hour. At the 96th hour, it was observed that the 1.0 ppm dose affected the number of organisms less than the 0.5 ppm dose (Dere and Sıvacı, 2003). In this study, *D. communis* cell numbers were decrease in pendimethalin – treat groups until the 3rd day, while the number of cells (20.00±3.61 cell/ml) in the H3 group (280 mgL^{-1}) was determined on the 3rd day. It was determined that the number of cells in the H1 group (19 mgL^{-1}) was higher (7.67±0.58 cell/ml). In the groups treated with chlorpyrifos-ethyl on the same day, the number of cells in group I1 (10 mgL^{-1}) (3.33±3.21 cell/ml) was higher than the number of cells in group I3 (115 mgL^{-1}).

Table 1. Change in the number of *D. communis* cells count (cell/ml) depending on the day and groups in pendimethalin - treated groups (n=3, Mean±SD)

Days	Groups			
	C	**H1**	**H2**	**H3**
0	142.00 ± 14.00^{Aa}	130.67 ± 7.02^{Ab}	133.67 ± 28.10^{Ab}	153.67 ± 19.40^{Aa}
1	156.00 ± 7.00^{Aa}	74.67 ± 11.93^{Bc}	101.00 ± 19.40^{Ab}	120.67 ± 7.00^{Ab}
2	78.67 ± 20.50^{Ba}	11.67 ± 3.79^{Cc}	16.00 ± 0.58^{Bc}	40.67 ± 5.29^{Ab}
3	45.00 ± 3.00^{Ca}	7.67 ± 0.58^{Dc}	11.00 ± 2.00^{Bb}	20.00 ± 3.61^{Bb}
4	45.00 ± 3.00^{Ca}	2.33 ± 0.58^{Ec}	3.67 ± 1.53^{Cc}	6.33 ± 1.53^{Cb}
5	23.00 ± 10.58^{Da}	1.33 ± 0.58^{Eb}	1.00 ± 0.58^{Cb}	1.33 ± 0.00^{Db}
6	25.67 ± 2.52^{Da}	0.67 ± 0.58^{Eb}	0.00 ± 0.58^{Db}	0.67 ± 0.00^{Eb}
7	12.67 ± 2.08^{Ea}	0.00 ± 0.00^{Eb}	0.00 ± 0.00^{Db}	0.00 ± 0.00^{Eb}

*ABCDE Capital letters indicate the difference of each group within days, and the difference between means with different capital letters in the same column is statistically significant ($p<0.05$).
**abc Lowercase letters show the difference between each group and the other groups, and the difference between means with different lowercase letters in the same row is statistically significant ($p<0.05$).

Table 2. Change in *D. communis* cell count (cell/ml) depending on the day and groups in chlorpyrifos-ethyl - treated groups (n=3, Mean±SD)

Days	Groups			
	C	**I1**	**I2**	**I3**
0	142.00 ± 14.00^{Aa}	95.67 ± 6.66^{Ab}	134.67 ± 14.57^{Aa}	125.33 ± 8.02^{Aa}
1	156.00 ± 7.00^{Aa}	79.67 ± 13.01^{Ab}	106.00 ± 13.45^{Aa}	92.00 ± 5.29^{Bb}
2	78.67 ± 20.50^{Ba}	10.33 ± 6.66^{Bc}	27.67 ± 8.50^{Bb}	19.67 ± 0.58^{Cb}
3	45.00 ± 3.00^{Ca}	3.33 ± 3.21^{Cb}	1.33 ± 0.58^{Cc}	1.00 ± 0.00^{Dc}
4	45.00 ± 3.00^{Ca}	3.33 ± 3.21^{Cb}	1.33 ± 0.58^{Cc}	1.00 ± 0.00^{Dc}
5	23.00 ± 10.58^{Da}	1.67 ± 0.58^{Cb}	1.00 ± 0.00^{Cb}	1.33 ± 0.58^{Db}
6	25.67 ± 2.52^{Da}	0.33 ± 0.5^{Db}	0.67 ± 0.58^{Db}	1.00 ± 0.00^{Db}
7	12.67 ± 2.08^{Ea}	0.00 ± 0.00^{Db}	0.00 ± 0.00^{Db}	0.00 ± 0.00^{Eb}

*ABCDE Capital letters indicate the difference of each group within days, and the difference between means with different capital letters in the same column is statistically significant ($p<0.05$).
**abc Lowercase letters show the difference between each group and the other groups, and the difference between means with different lowercase letters in the same row is statistically significant ($p<0.05$).

D. communis Biomass

During the experiment, *D. communis* biomass between groups and days was found to be statistically significant ($p<0.05$). The lowest biomass value was measured on the 5^{th}, 6^{th} and 7^{th} days (0 μgL^{-1}) between the pendimethalin – treated groups; However, the highest biomass value was calculated as 0.40 ± 0.09 μgL^{-1} on the 1^{st} day. In H2 group, the lowest and highest values were determined as 0 μgL^{-1} on the 5^{th} day and 0.47 ± 0.18 μgL^{-1} on the 1^{st} day, respectively in H3 group, the lowest value was 0 μgL^{-1} on the 5^{th} day, and the highest value was 0.49 ± 0.15 μgL^{-1} on the 1^{st} day (Table 3).

Table 3. Change of *D. communis* biomass (μgL^{-1}) depending on the day and the groups in the pendimethalin – treated groups (n=3, Mean±SD)

Days	Groups			
	C	H1	H2	H3
0	0.33±0.01^{aA}	0.37±0.01^{aA}	0.35±0.01^{aA}	0.34±0.00^{aA}
1	0.40±0.01^{aA}	0.40±0.09^{aA}	0.47±0.18^{aA}	0.49±0.15^{aA}
2	0.38±0.02^{aA}	0.34±0.25^{aA}	0.11±0.01^{bB}	0.14±0.03^{bB}
3	0.38±0.02^{aA}	0.01±0.01^{bB}	0.03±0.01^{bC}	0.01±0.00^{bC}
4	0.38±0.01^{aA}	0.01±0.01^{bB}	0.01±0.00^{bC}	0.00±0.00^{cD}
5	0.22±0.02^{aB}	0.00±0.00^{bC}	0.00±0.00^{bD}	0.00±0.00^{bD}
6	0.22±0.02^{aB}	0.00±0.00^{bC}	0.00±0.00^{bD}	0.00±0.00^{bD}
7	0.02±0.01^{aC}	0.00±0.00^{bC}	0.00±0.00^{bD}	0.00±0.00^{bD}

*ABCDE Capital letters indicate the difference of each group within days, and the difference between means with different capital letters in the same column is statistically significant ($p<0.05$).
**abc Lowercase letters show the difference between each group and the other groups, and the difference between means with different lowercase letters in the same row is statistically significant ($p<0.05$).

The lowest *D. communis* biomass were determined as 0 μgL^{-1} on the 6th day in all chlorpyrifos-ethyl – treated groups. The highest value of *D. communis* biomass was calculated on the first day in all groups. According to the groups (I1, I2 and I3), the *D. communis* biomass were 0.33±0.01 μg/l, 0.37±0.00 μg/l and 0.38±0.01 μg/l, respectively (Table 4).

Table 4. Change of *D. communis* biomass (μgL^{-1}) depending on the day and groups in chlorpyrifos-ethyl – treated groups (n=3, Mean±SD)

Days	Groups			
	C	I1	I2	I3
0	0.33±0.01^{aA}	0.33±0.01^{aA}	0.37±0.00^{aA}	0.38±0.01^{aA}
1	0.40±0.01^{aA}	0.31±0.01^{aA}	0.37±0.05^{aA}	0.33±0.01^{aA}
2	0.38±0.02^{aA}	0.10±0.16^{bB}	0.16±0.04^{bB}	0.21±0.11^{bB}
3	0.38±0.02^{aA}	0.02±0.02^{bC}	0.02±0.01^{bC}	0.01±0.01^{bC}
4	0.38±0.01^{aA}	0.02±0.00^{bC}	0.03±0.00^{bC}	0.03±0.03^{bC}
5	0.22±0.02^{aB}	0.00±0.00^{cD}	0.01±0.01^{bC}	0.02±0.00^{bC}
6	0.22±0.02^{aB}	0.00±0.00^{bD}	0.00±0.00^{bD}	0.00±0.00^{bD}
7	0.02±0.01^{aC}	0.00±0.00^{bD}	0.00±0.00^{bD}	0.00±0.00^{bD}

*ABCDE Capital letters indicate the difference of each group within days, and the difference between means with different capital letters in the same column is statistically significant ($p<0.05$).
**abc Lowercase letters show the difference between each group and the other groups, and the difference between means with different lowercase letters in the same row is statistically significant ($p<0.05$).

In this study was carried out under the laboratory condition. The *D. communis* biomass was determinate lower in the pendimethalin – treated groups than in the chlorpyrifos-ethyl – treated groups. The lowest *D. communis* biomass was found on the 4th day in the pendimethalin – treated groups, while the lowest *D. communis* biomass was calculated on the 5th day in the chlorpyrifos-ethyl – treated. It was reported that galactopyranoside-treated on *Pseudokirchneriella subcapitata*, *Scenedesmus acutus*, *Scenedesmus quadricauda* and *Coelastrum reticulatum* had no effect on this species growth up to concentration of 5 mgL^{-1}, but inhibited the growth of *P. subcapitata* at a concentration of 10 mgL^{-1} (Nakajima vd., 2007).

It was determinate that TRIA (triacontanol chloroform) affected increase in chlorophyll and biomass of *Chlorella vulgaris*, and basic physiological mechanisms (Aminfarzaneh, 2010). It was found that the growth phase of pesticide-treated *C. vulgaris* and *Desmodesmus communis* were short period, but the stagnation and collapse phases take longer, and the cultures collapse more slowly (Öterler and Albay, 2010). In this study, *D. communis* biomass was detected suddenly increase in all groups within one day. However, the stationary phase and collapse phases were realized within two days contrary to the expectations. It is thought that the applied active substances accelerate the biomass increase, but reach the saturation in a short time and damage the cells.

Herbicides and insecticides are important chemicals for increase production quality used to agricultural field. However, the mixing of these chemicals into aquatic ecosystems without decomposition, except for the pests

they affect, harms many living things living in these areas. These chemicals cause accumulation in aquatic organisms. Therefore, depending on the concentration of the chemical and the water quality values of the environment, it causes cell deformation and/or death in these organisms.

As a result of this study, both pesticide derivatives increased the cell numbers of *D. communis* until the 3rd day, but decreased it rapidly from the 4th day. It was observed that *D. communis biomass* was affected more rapidly in the groups exposure with pendimethalin than in the groups exposure with chlorpyrifos-ethyl. In our opinion that *D. communis* bimass is more affected by pesticide groups containing nitrogen compounds (Pendimethalin) rather than the pesticide group containing phosphorus compounds (Chlorpyrifos-Ethyl). For this reason, the correct adjustment of the application doses and methods of pesticides used in agricultural areas is important in order to preserve all of organism in the aquatic ecosystem.

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REFENCES

Aminfarzaneh, H. (2010). Siyanobakterilerde Bitki Büyüme Düzenleyicilerin Biyokütle Üretimi ve Pestisit Giderimi Üzerine Etkisi. (Doktora Tezi) Ankara Üniversitesi Fen Bilimleri Enstitüsü Ankara, Türkiye.

Andersen, R.A. (2005). *Algal Culturing Techniques*. Elsevier Academic Press,578 p, California, USA.

Anonim. (2014). Yüzeysel sular ve yeraltı sularının izlenmesine dair yönetmelik. www.resmigazete.com. Erişim Tarihi: 27.07.2018.

Anonim. (2016). 5996 sayılı veteriner hizmetleri bitki sağlığı ve yem kanunu. www.resmigazete.com. Erişim Tarihi: 25.07.2018.

Dere, Ş., & Sıvacı, E.R. (2003). Bazı pestisitlerin farklı dozlarının (monoraphidium contortum (thur) komârk-legn.) türünün populasyon yoğunluğuna etkisi. *Anadolu Üniversitesi Bilim ve Teknoloji Dergisi*. 4(1), 93-100.

Hultberg, M., Bodin, H., Ardal, E., & Asp, H. (2016). Effect of microalgal treatments on pesticides in water. *Environmental Technology*, 37(7), 893-898. https://doi.org/10.1080/09593330.2015.1089944

Kang, C.D., Lee, J.S., Park, T.H., & Sim, S.J. (2005). Comparison of heterotrophic and photoautotrophic induction on astaxanthin production by *Haematococcus pluvialis*. *Applied Microbiology and Biotechnology*, 68(2), 237-241. https://doi.org/10.1007/s00253-005-1889-2

Kesici, T., & Kocabaş, Z. (2007). *Biyoistatistik*. Ankara Üniversitesi Eczacılık Fakültesi Biyoistatistik Yayın No:94, 369 s, Ankara, Türkiye.

Luo, L., Wang, P., Lin, L., Luan, T., Ke, L. & Tam, N.F.Y. (2014). Removal and transformation of high molecular weight polycyclic aromatic hydrocarbons in water by live and dead microalgae. *Process Biochemistry*, 49(10), 1723-1732. https://doi.org/10.1016/j.procbio.2014.06.026

Nakajima, N., Teramoto, T., Kasai, F., Sano, T., Tamaoki, M., Aono, M., Akihiro, K., Hiroshi, K., Yoshitaka, A., & Hikaru, S. (2007). Glycosylation of bisphenol A by freshwater microalgae. *Chemosphere*, 69, 934-941. https://doi.org/10.1016/j.chemosphere.2007.05.088

Öterler, B., & Albay, M. (2010). The effect of 5 organophosphate pesticides on the growth of *Chlorella vulgaris* Beyerinck [Beijerinck] 1890. *International Journal of Research Studies in Biosciences*, 4, 26-33. doi:dx.doi.org/10.20431/2349-0365.0404003

Shelknanloymilan, L., Atici, T., & Obal, O. (2012). Removal of nitrogen and phosphate by using Chlorella vulgaris on synthetic and organic materials waste water. *Biological Diversity and Conservation*, 5(2), 89-94.

Solmaz, S. K., Hilal, A.Z.A.K., Üstün, G.E., & Morsünbül, T. (2010). Pestisit gideriminde fenton proseslerinin kullanımına yönelik bir envanter çalışması. *Uludağ University Journal of The Faculty of Engineering*, 15(1):179-194.

Strandberg, M., & Scott-Fordsmand J.J. (2004). Effects of pendimethalin at lower trophic levels-a review. *Ecotoxicology and Environmental Safety*, 57(2), 190-201. doi:10.1016/j.ecoenv.2003.07.010.

Vonshak, A. (1997). Outdoor mass production of spirulina: the basic consept. *Spirulina platensis (Arthrospira) Physiology. Cell- Biology and Biotechnology.* Great Britain,79-99 p.