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COMBINED EFFECTS OF TEMPERATURE AND LIGHT ON HERBICIDES, DIURON AND THIOBENCARB SENSITIVITY IN THE GROWTH OF *ACHNANTHIDIUM MINUTISSIMUM* (Kützing) NIES-71

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ABSTRACT

Diatom plays an important role at the base of the trophic food chain, primary producers and represent a source of food for numerous other organisms. Diatom with other aquatic organism may be seriously affected by herbicide exposure. Present study found that the growth rate of diatom, *Achnanthidium minutissimum* was significantly affected by both diuron and thiobencarb concentration and temperature. Diuron toxicity was significantly affected by high light intensity but there is no significant effect of temperature and light intensity to thiobencarb toxicity on growth inhibition of *A. minutissimum* other than its concentration.

Keywords: *Diatom, diuron, thiobencarb, toxicity, temperature, light intensity*

Introduction

Pesticides used in agriculture in our country contaminate rivers via surface runoff or spray drift. Herbicides which might be particularly toxic to algae, constitute the most important pesticide class that contaminate rivers, owing basically to the big amounts used in agriculture and on city regions and every now and then as a result of their environmental persistence (Gilliom 2007, Bashir *et al.* 2020, Rani *et al.* 2021). Therefore, herbicide pollution may additionally represent a main threat to the health and productivity of aquatic ecosystems. However, diatoms are primary producers, base of the trophic food chain, which represent a source of food for numerous organisms, may be seriously affected by herbicide or other pesticides exposure. It's already established that such pollutants may significantly disturb the equilibrium of the trophic food chain (Stevenson and Pan 1999). Diatoms are commonly used as bioindicators of trophic and saprobic pollution in rivers. However, diatoms sensitivity to toxicants such as agricultural herbicides at different environmental condition are still unknown in many cases. Numerous studies have been performed to study the effects of pesticides on individual species and on species communities. Indeed, if diatom communities are recognized as useful bioindicators of herbicide-contaminated

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water, their efficiency to detect such toxic pollutants has yet to be demonstrated (Dorigo *et al.* 2004). Diatom communities that have species capable of switching from autotrophic to heterotrophic modes, when photosynthesis is inhibited (e.g., after pesticide exposure), can continue to grow, even in the presence of high pesticide pollution. Therefore, it is necessary to know toxicant impact on diatoms an actual condition.

Achnanthidium minutissimum is common and widespread diatom in a range of ecological conditions but does best in clean, low conductivity streams. There are some studies are available about toxicity test of diatom, *A. minutissimum* with heavy metals or herbicide. Environmental parameters (light exposure, nutrient concentrations, and hydraulic conditions) affect and often interfere with the response of diatoms to pesticides. So, the complexity of aquatic ecosystems and the complexity of pesticide to easily detect the effects of such pollutants on diatoms with real scenario. Therefore, more research may be required to deal with this problem in future. Thus it is necessary to know the effects of environmental parameters on chemical toxicity. The abundance and persistency of herbicide diuron and thiobencarb described in previous article and it was observed toxicity behavior of these two herbicides was different specially sensitivity of water temperatures to green algae, *Raphidocelis subcapitata* (Tasmin *et al.* 2013, Tasmin *et al.* 2018). So, the present study was carried out to know the effect of two environmental parameters temperature and light on herbicides diuron and thiobencarb toxicity to diatom, *Achnanthidium minutissimum*.

Materials and Methods

Acute Toxicity Test

Diatom, *A. minutissimum* strain NIES-71 was obtained from the National Institute for Environmental Science (NIES), Japan. The diatom was cultured in C (*Closterium*) medium (Ichimura *et al.* 1971). In this present experiment diatom, *A. minutissimum* strain NIES-71 was failed to grow at low nutrient condition (C media with 1% nitrate and phosphate). The nominal diuron and thiobencarb concentration in test medium was adjusted to 16 and 125 µg L^{-1} respectively (n = 3 for each group) using ethanol (0.01% in all test tubes) as a solubilizing agent, and this concentration was selected based on a previous toxicity test at different water temperatures, some review and environmental concentrations (Tasmin *et al.* 2014). Therefore, only standard C media was used to check diuron and thiobencarb toxicity at three different temperatures (10, 20 and 30 °C) and two light intensities (100 and 1000 µmol photons m^{-2} s^{-1}) for 72h and 144h.

Data analysis

Generalized linear model (GLM) was used to analyze the effects of diuron and thiobencarb treatment and other factors (temperature and light intensity) on growth rate and effective concentrations (EC) for growth using the statistical software R (R Development Core Team 2011).

Results and Discussion

Effects of water temperature and light on growth inhibition in diatom, *A. minutissimum* exposed to duiron (16 µg L^{-1}) are summarized in fig. 1. It was observed that, percentage of growth

inhibition by diuron treatment reached more severe condition (100%) under high light for all temperature condition and it was 3.1 and 1.15 times higher for temperature 20 and 30°C respectively than that of low light after 72h. Interestingly, no inhibition found at temperature 10°C and low light condition until 72h but inhibition was appeared after 144h of exposure period.

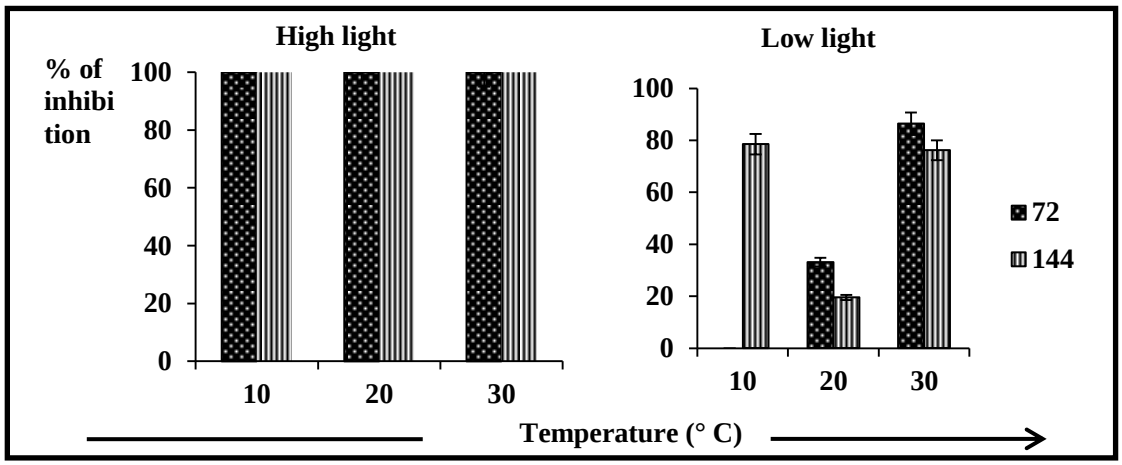

Fig. 1. Percentage of growth inhibition to diatom, *Achnanthidium minutissimum* exposed to duiron (16 µg L^{-1}) at different light and temperature (10, 20 and 30°C) condition.

According to the GLM analysis, growth rate was significantly increased by suppressed temperature ($p < 0.05$) and diuron treatment ($p < 0.01$). On the other hand, growth inhibition rate was significantly decreased by increased light intensity (Table 1). The model for growth inhibition was described by the following equation: Growth Inhibition (GI) = $e^{\wedge}$ [(8.584+ (1.050 × temperature) ─ (0.127× time) + (0.056× light).

Table 1. Analysis result of generalized linear model (GLM) on growth inhibition by diuron (16 µg L^{-1}) treated diatom, *Achnanthidium minutissimum* at different light and temperature (10, 20 and 30°C) condition.

	Growth inhibition			
	Estimate	Std. Error	*t* value	*p* value
(Intercept)	8.584	31.362	0.274	0.791
Temp	1.050	0.9183	1.144	0.285
Time	0.127	0.2082	0.610	0.558
Light	0.056	0.0166	3.402	<0.05

Effects of water temperature and light on growth inhibition of diatom, *Achnanthidium minutissimum* to thiobencarb (125 µg L^{-1}) are summarized in fig. 2. Percentage of growth inhibition by thiobencarb treatment found complete inhibition (100%) under all temperature, high light intensity condition until 72h but decreasing of inhibition rate was observed at higher temperature (20 and 30°C) at 144h. Interestingly, no inhibition was observed at temperature 10°C and low light condition at both 72h and 144h.

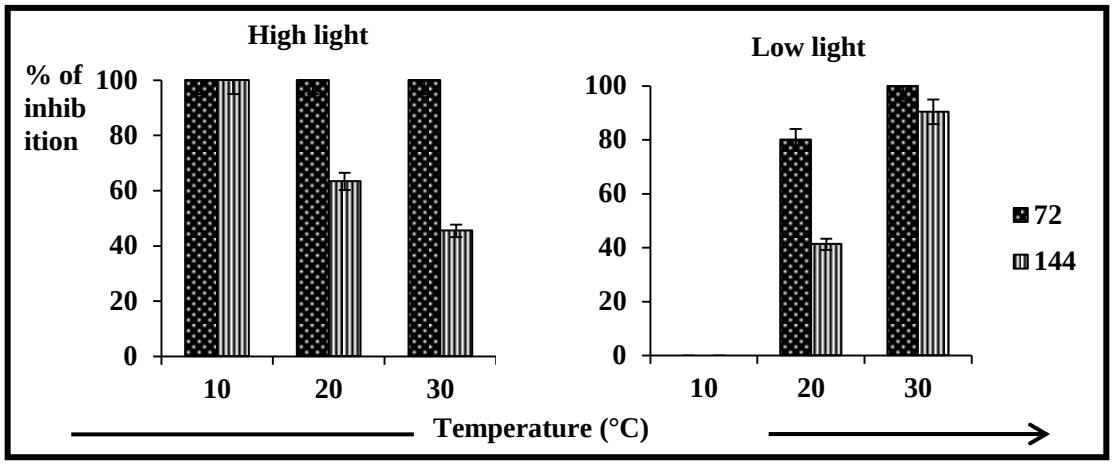

Fig. 2. Percentage of growth inhibition to diatom, *Achnanthidium minutissimum* exposed to thiobencarb (125 µg L^{-1}) at different light and temperature (10, 20 and 30 °C) condition.

According to the GLM analysis, growth rate was significantly increased by temperature ($p<0.05$) and suppressed by thiobencarb treatment ($p<0.001$). On the other hand, growth inhibition rate was not significantly affected by temperature or light intensity (Table 2). The model for growth inhibition was described by the following equation: Growth Inhibition (GI) = $e^{\wedge}$ [(49.173 + (1.698 × temperature) ─ (0.322 × time) + (0.036× light)].

Table 2. Analysis result of generalized linear model (GLM) on growth inhibition by thiobencarb (125 µg L^{-1}) treated diatom, *Achnanthidium minutissimum* at different light and temperature (10, 20 and 30°C) condition.

	Growth inhibition			
	Estimate	Std. Error	*t* value	*p* value
(Intercept)	49.173	40.685	1.209	0.261
Temp	1.698	1.191	1.426	0.192
Time	-0.322	0.270	-1.194	0.267
Light	0.036	0.021	1.689	0.130

Present study revealed that the growth rate of diatom, *A. minutissimum* was significantly affected by both diuron and thiobencarb concentration and temperature. Diuron toxicity was significantly affected by high light intensity but there is no significant effect of temperature and light intensity to thiobencarb toxicity on growth inhibition of *A. minutissimum* other than its concentration.

Previous study also found environmental parameters (light exposure, nutrient concentrations etc.) also interfere in the responses of algal communities to herbicides (Guasch *et al.* 1997, Guasch and Sabater, 1998, Berard and Benninghoff, 2001, Navarro *et al.* 2002). Among them temperature sensitivity for pollutant toxicity is well established. In this present study, it was observed that the growth of diatom, *Achnanthidium minutissimum* was completely suppressed by all temperature at high light condition after 72h for diuron and thiobencarb but, after 144h thiobencarb treated diatom found little resistance at 20 and 30°C. Navarro *et al.* 2002 noted lower tolerance to atrazine for periphytic communities during the summer. The opposite results were obtained in phytoplankton communities exposed to the same herbicide (Berard and Pelte, 1996). Therefore, toxicity could be varied with species and chemical differences and there is no report about temperature effects on diuron and thiobencarb to this diatom species. However, present study found that the growth inhibition was more severe at high light condition compare to low. Some previous study found toxicity of atrazine to diatoms in natural communities was greater high-light conditions than for those that are adapted to low-light conditions (Guasch *et al.* 1997, Guasch and Sabater, 1998). Though nutrient concentrations also affect the sensitivity of algae to herbicides (Lin *et al.* 2005) but in present study diatom was failed to grow at low nutrient condition. Therefore, temperature and light intensity sensitivity should consider for risk assessment of diatom because toxic effect at different environmental condition could be helpful to know their actual impact in field condition.

Conclusion

Diatoms are sensitive to toxicant and their sensitivity could be varied with species differences. On the other hand, that toxicity could be more affected (synergistic/antagonistic) along with environmental parameters also. The results of the present study showed that water temperature and high light influences the toxicity of two herbicides, diuron and thiobencarb on diatom growth which was complex with exposure duration. Therefore, temperature, light intensity and exposure period plays important roles in toxic evaluation of herbicides and that should be considered in estimation of the actual risk of pollutants.

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