THE TOXICITY OF TWO NEWLY-SYNTHESIZED COMPOUNDS ON *SCENEDESMUS OBLIQUUS* KÜTZ

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Abstract: LY-04 and WSQIF are newly-synthesized compounds which were presented by the Institute of Pesticides Chemistry, Central China Normal University. LY-04 belongs to α-oxophosphonic acid derivatives, while WSQIF is an aromatic acetyl oxophosphonic acid derivative. In this study, the toxicity of LY-04 and WSQIF on *Scenedesmus obliquus* Kütz was examined. The EC_{50} of LY-04 and WSQIF were determined in *S. obliquus* cells at 24h, 48h, 72h, 96h represented by cell density. Our data showed that the 24h, 48h, 72h, 96h EC_{50} of LY-04 were 1983mg/L, 2943mg/L, 240mg/L and 65.0mg/L acetone, respectively, and the 24h, 48h, 72h, 96h EC_{50} of WSQIF were 15.9mg/L, 53.0mg/L, 98.0mg/L and 28.8mg/L respectively. LY-04 displayed a higher insecticidal activity, while WSQIF showed a higher phytocidal activity. As LY-04 and WSQIF were designed to act as insecticide and herbicide, respectively, the results were in contrast to the anticipated ones. According to the toxicant classification standard, EC_{50} of both substances was far higher than 3mg/L, while the toxicity of LY-04 and WSQIF to aquatic algae were fairly low, indicating that these two pesticides are rather safe to the aquatic ecosystem.

Key words: LY-04 and WSQIF; Scenedesmus obliquus Kütz; Toxicity
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With the development of modern agriculture, environmental contamination by a variety of organic toxic agents has been detected in many freshwater systems. These include herbicides, insecticides, surfactants and other organic compounds, which have, been reported by Stratton[1] and many other authors to affect both their target and non-target organisms whenever discharged into the environment, thus impairing heavily on the structure and function of ecosystems. Therefore, it is necessary to develop pesticides which are highly effective but low toxicity, low in residue built-up and highly selective in order to minimize their environmental impact.

Organo-phosphate pesticides have been widely used since the 1980s, and from that time they became the most critical elements in agriculture-caused pollution. Tang & Li[2] has reported the toxicity of organo-phosphates on the physiology of marine species. LY-04 (yellow crystal) and WSQIF (white powder) are two newly synthesized organophosphates. The toxicity of these two new pesticides is until now very unclear and requires indepth studies.

Certainly, a noticeable decrease in algae density and species number as primary producer within a food web, affects the aquatic ecosystem directly by reducing their primary productivity and biodiversity. Therefore, unicellular algae are frequently used in various bioassays[3]. *Scenedesmus obliquus* was chosen in this study because it is easy to cultivate, sensitive to toxicants and its response is highly reproducible[4]. Toxic compounds may affect micro-algal photosynthesis, growth, enzyme activity and respiration. The effective concentration of toxicants that inhibits 50% micro-algal growth at 96h (96h EC_{50}) is widely used as an index of toxicity[5]. In this study, the EC_{50} was determined in order to evaluate the toxicity of LY-40 and WSQIF on *S. obliquus* and to provide scien-

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tifically sound information for the regulatory authorities on how to manage these pesticides with minimized environmental impact.

1 Material and methods

1.1 Test material The *S. obliquus* FACHB39 was obtained from the Freshwater Algae Culture Collection, Institute of Hydrobiology, the Chinese Academy of Sciences, Wuhan, Hubei Province.

1.2 Main reagents LY-04 and WSQIF were presented by the Institute of Pesticides Chemistry, Central China Normal University. Acetone, produced by the Shanghai Chemical Reagent Factory, was used as a solvent. Liquid HB-4 was used as the medium[6].

1.3 Main instrumentation Bio-incubator, freezing centrifuge (4℃, 5000rpm), autoclave, 721-spectrophotometer (650nm), microscope (40×).

1.4 Culture The algae were cultivated in 100ml of liquid HB-4 medium[6] in 250ml flasks and illuminated with cool-white fluorescent lights (70μE·m^{-2}·s^{-1}) at 12∶12 LD cycle[7]. Temperature was maintained by using an air-conditioned growth chamber at 28℃. pH was kept at 7—8. Cells in the exponential phase of growth were collected from stock cultures and used as the inocula for the experiments. All operations were carried out under sterile conditions in order to avoid contamination from bacteria or other algae.

1.5 NOEC (No Observed Effect Concentration) of solvent's Acetone was chosen as their solvents since LY-04 and WSQIF are only slightly dissolved in water. However, Stratton[8] reported that acetone was also toxic to *S. obliquus*. So, it was necessary to evaluate the NOEC of acetone. Firstly, *S. obliquus* was cultivated in a series concentration of acetone: 0.1%, 0.15%, 0.20%, 0.50% and 1% respectively. After 96 hours, compared with the control group, the treatment of 0.1% stimulated the algal growth, while the treatment of 0.50% to 1%, the growth of *S. obliquus* had been inhibited significantly. Using Student's t-tests, analysis of significant differences showed that the NOEC of acetone was 0.15%. Therefore, the concentration of acetone should be lower than 0.15% in order to avoid the side-effect of solvent.

1.6 Test process At the beginning of the experiment, LY-04 and WSQIF were added to the culture medium and sterilized at 121℃ for 20min. Five concentrations were prepared for each pesticide. Algal cells were treated with each pesticide for 96 hrs, and each experiment was repeated 3 times. The data presented here were the average values of three parallel samples, and their relative standard deviations were less than 10%. From day 0 to day 4, 0.5ml algal cells were taken daily and cell numbers were counted with a Petroff-Hausser counting chamber under a microscope to determine the growth rate and inhibition percentage.

The growth rate was calculated according to the following equation[9]:

$$U = (\mathrm{Ln}N_t - \mathrm{Ln}N_0)/(t - t_0) \quad (1)$$

Where U = growth rate

N_t = the cell number at t time

N_0 = the cell number at 0 time

t = sample time for counting cell number

t_0 = origin time of the treatment

The effects of each pesticide on the growth rate of algae were calculated as follows:

$$\%\,\mathrm{Inhibition} = (U_{ck} - U_{tox})/U_{ck} * 100 \quad (2)$$

Where U_{tox} = growth rate in the presence of each pesticide

U_{ck} = growth rate in the control

The percentages of growth inhibition were compared with the concentration data in a regression analysis "logarithmic-probit" from a computer program (Statistica software), and the exact value of 96h EC_{50} was determined. The dose response equation was X^2 tested with 95% coefficient.

2 Results

2.1 The effect of WSQIF on *S. obliquus* The algal population growth was consistently inhibited by different WSQIF concentrations (Fig. 1), and presenting a significant "dose-response" relationship by comparing the data obtained for EC50 with WSQIF concentrations——with the increase of the concentrations of WSQIF, the growth rate of the algal cells dropped gradually. Moreover, treated with high concentrations of WSQIF, morphological changes of the algal cells were observed under the optical microscope. Some cells were swollen and cell divisions appeared abnormal. During the process of maternal cell division, the descendant cells remained attached. This indicated that WSQIF is potentially mutagenic.

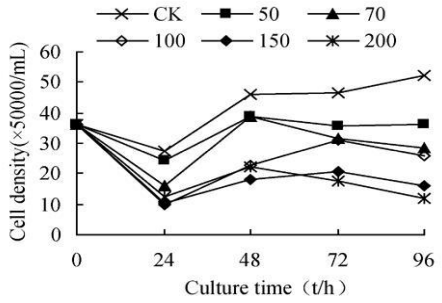

Fig. 1 Effects of different concentrations of WSQIF on growth of *S. obliquus*

By using the statistic program described above, the EC_{50} of different intervals (24, 48, 72 and 96hrs) was calculated (Tab. 1).

Tab. 1 The toxicity of WSQIF to *S. obliquus* Kütz

Time (hr)	Concentration effect curve equation	Correlation coefficient (r)	$\rho_t(EC_{50})$/mgL^{-1}
24	y=0.171x+4.527	0.7476	15.9
48	y=0.237x+4.059	0.7348	53.0
72	y=0.366x+3.322	0.9727	98.0
96	y=0.468x+3.428	0.9623	28.8

With the prolongation of the exposure, the EC_{50} value increased at the beginning (short-term exposure), however; when exposure time expanded, a decreasing trend was observed. This demonstrated that *S. obliquus* recovered partly, but the long-term toxicity of WSQIF was high when dissolved in acetone.

2.2 The effect of LY-04 on *S. obliquus* Impacted by different concentrations of LY-04, the growth curve of *S. obliquus* obtained by the same method is depicted in Fig. 2. The growth of the algal cells was inhibited to various degrees. Like the phenomena observed for WSQIF, the dose-effect relationship was obvious. With the increase of the concentration of LY-04, the growth rate of the algal cells declined gradually.

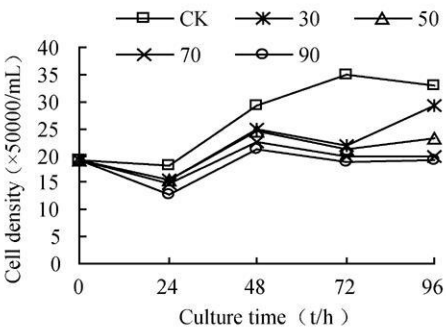

Fig. 2 Effects of different concentrations of LY-04 on growth of *S. obliquus*

The EC_{50} values and correlation coefficients obtained for different exposure intervals (24, 48, 72 and 96hrs) are shown in Tab. 2.

Tab. 2 The toxicity of LY-04 to *S. obliquus* Kütz

Time (hr)	Concentration effect curve equation	Correlation coefficient (r)	$\rho_t(EC_{50})$/mgL^{-1}
24	y=0.904x+0.2697	0.9793	1982.5
48	y=0.154x+3.77	0.9728	2942.5
72	y=0.073x+4.6	0.9812	239.7
96	y=0.321x+3.66	0.9192	65.0

With the progression of the test, the EC_{50} values decreased sharply, which indicated that the tolerance of *S. obliquus* to LY-04 was low. It was likely that the effective concentrations were lower than the theoretical ones in that LY-04 test amounts were only slightly and perhaps not completely dissolved in water.

2.3 Comparison of the toxicity between LY-04 and WSQIF on *S. obliquus* The 96h EC_{50} values of LY-04 (Tab. 1) and WSQIF (Tab. 2) on *S. obliquus* were 65.0mg/L and 28.8mg/L respectively. The EC_{50} of WSQIF was lower than that of LY-04, indicating that the toxicity of WSQIF in *S. obliquus* is higher than that of LY-04. Similar pattern was also observed at 48, 72hrs EC_{50}. However, the patterns of the alterations of their 48, 72 and 96hrs EC_{50} values may suggest that when dissolved in acetone, the tolerance of algae cells to these two new pesticides was both still relatively low, while the algae cells were uneasy to recover, indicating that the long-term toxicity was rather high.

3 Discussion

Many studies on pesticide toxicity of *S. obliquus* have been reported in the scientific literature. The primary impacts of pesticides on algal cells are to kill the algae and to inhibit algae cell growth. The EC_{50} is an important bio-technological index to evaluate the environmental safety of any substance in a comparative manner. Generally, the 96h EC_{50} was used as a reference criterion to classify the toxicity[10]: low level (> 3.0mg/L), mid-toxic (0.3—3.0mg/L), high toxicity (< 0.3mg/L). According to the previous reports, the EC_{50} of tested pesticides to *S. obliquus* were rather low, for example, the 96h EC_{50} of lindan and DMA (N'-(2,4-Dimethylphenyl)-N-Methyl-

formamidine) were about 2.5 and 6.5mg/L, respectively, the 72h EC_{50} of parathion methyl has been determined with 15 mg/L[11]. The impact of pesticides on *S. obliquus* assumed an obviously clear dose-response relationship. Moohapatra & Mohanty[12] considered that the toxicity of pesticides decreased with time prolonging, mostly because of (1) the bio-degradation of pesticides, (2) the auto-degradation of pesticides, (3) bio-adaptability, and (4) the reduction of pesticides that could enter into the algae cells. Another reason could be that the algae do find themselves confronted with gradually declining nutrients in the culture medium during the exposure period, which may affect their growth rate ones nutrients reach a critically low concentration.

According to our study, the 96h EC_{50} of LY-04, WSQIF on *S. obliquus* were 65.0mg/L and 28.8mg/L, respectively. Compared with the 96h EC_{50} (112.45mg/L) of cypermethrin[13], cypermethrin > LY-04 > WSQIF. In other words, the sensitive order of *S. obliquus* to them was WSQIF > LY-04 > cypermethrin.

A series of in vivo studies showed that LY-04 had a higher insecticidal activity, while WSQIF had a higher phytocidal activity. The target organisms of herbicides and insecticides were plants and pests respectively, so theoretically, the toxicity of WSQIF on algae must be larger than LY-04. The results of our study, however, were in contrast with the anticipated ones. According to the toxicant classification standards, EC_{50} of LY-04 and WSQIF are all far higher than 3mg/L; therefore the toxicity of LY-04 and WSQIF to the aquatic algae was low, indicating that these two pesticides were rather safe to the aquatic ecosystem in terms of toxicity to unicellular green algae.

Both LY-04 and WSQIF, occurring in high concentrations, would cause a rather high toxic to algae. Released directly into the environment, they would enter the water body by run-off and affect the aquatic ecosystem near the place of origin. A decrease in algae density and species as primary producers in food webs will affect the aquatic ecosystem directly by reducing their primary productivity and species diversity, the food chain might be severely altered and the balance of energy and nutrient flow in the ecosystem will certainly be disturbed. Hence, the concentrations of WSQIF and LY-04 must be controlled tightly during their application. In addition, the tolerance of *S. obliquus* to WSQIF and LY-04 were both relatively low, and the algae cells were uneasy to recover, indicating that the long-term toxicity of these new pesticides must be taken into consideration.

This study provides a preliminary reference on the toxicity of these pesticides to aquatic micro-algae. Further studies are needed to fully evaluate their toxicity and hence their potential method for safe application with minimized effects on the whole environment.

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