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Effects of DDT and dicofol on population growth of *Brachionus calyciflorus* under different algal (*Scenedesmus obliquus*) densities

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Abstract

A number of organochlorine pesticides, including DDT and dicofol, used to be important in crop protection and management. Their residues may reach water bodies and eventually affect the non-target organisms such as rotifers. In the present study, we evaluated the effects of DDT (0.05, 0.1, 0.2 and 0.4 mg l^{-1}) and dicofol (0.1, 0.2, 0.4 and 0.8 mg l^{-1}) on the population growth of rotifer *Brachionus calyciflorus* under two levels of *Scenedesmus obliquus* (1.0×10^6 and 3.0×10^6 cell ml^{-1}). Regardless of the food level, DDT was more toxic than dicofol to *B. calyciflorus*. Under low food level, DDT at 0.1 and 0.2 mg l^{-1} decreased the population growth rate (r), and DDT at 0.05-0.4 mg l^{-1} decreased the maximum population density (*K*). Dicofol at 0.4 and 0.8 mg l^{-1} decreased r and *K*, but dicofol at 0.2 mg l^{-1} increased *K*. Under high food level, DDT at 0.05-0.2 mg l^{-1} increased *K*, whereas DDT at 0.4 mg l^{-1} as well as dicofol at 0.4 and 0.8 mg l^{-1} decreased r and *K*. Increase in food level increased r exposed to DDT at 0.05-0.2 mg l^{-1} as well as dicofol at 0.8 mg l^{-1}, and *K* exposed to DDT at 0.05-0.2 mg l^{-1} as well as dicofol at 0.1 and 0.2 mg l^{-1}. DDT concentration, algal density and their interaction affected r and *K* of *B. calyciflorus*. Both dicofol concentration and algal density affected r. Dicofol concentration, algal density and their interaction affected *K*. Both r and *K* were suitable endpoints for assessing the effects of DDT and dicofol on the rotifers population dynamics under two algal densities, and the latter was more sensitive.

Key words

Algal density, *Brachionus calyciflorus*, DDT, Dicofol, Population growth rate

Introduction

Organochlorine pesticides, such as DDT and dicofol, were used with great success in the past century to control pests and diseases, e.g. malaria and typhus, but the extensive use of these pesticides also brings about serious ecological problems because they or their metabolites may eventually reach water bodies and pose toxicity to non-target organisms, including zooplankton. In the 12th Stockholm Convention, DDTs and other eight organochlorine pesticides were proposed to be controlled as persistent organic pollutants (POPs), a variety of organic chemicals that have lasting harmful effects to the environment and the ecosystem.

Due to hydrophobic and lipophilic properties, DDT and its metabolites in aquatic ecosystems are absorbed by aquatic organisms and on suspended particles, leaving little DDT dissolved in water itself. Its breakdown products and metabolites, DDE and DDD, are also highly persistent and have similar chemical and physical properties (Barnhoorn *et al.,* 2009). Therefore, although banned in numerous countries and areas, DDT has been detected in many water bodies and its environmental impact was not completely eliminated (Guo *et al.*, 2011). Dicofol is the substitute of DDT and used widely as acaricide of plants (e.g. fruit trees, cottons, vegetables) in lots of countries including China at present. Dicofol is usually synthesized from technical DDT. In 1986, the US Environmental

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Protection Agency (EPA) temporarily canceled the use of dicofol because relatively high levels of DDT contamination were ending up in the final product. Some evidence showed that the content of DDT in environment has been rising again in recent years, which was considered mainly to be caused by use of dicofol though the modern processes can produce technical grade dicofol containing less than 0.1% DDT (Yu *et al.*, 2003; Zhao *et al.*, 2004).

Several studies have found that DDT and dicofol have potential of endocrine disrupting effects on invertebrates, vertebrates and humans. For example, DDT through its metabolite DDE caused eggshell thinning, resulting in severe population declines in multiple North American and European bird of prey species (Vos *et al.*, 2002). A positive, monotonic, exposure-response association was found between preconception serums total DDT and the risk of subsequent early pregnancy losses (Venners *et al.*, 2005). Dicofol significantly increased the number of male neonates and affected the sex ratio in *Daphnia magna* during the sub-chronic assay (Maher *et al.*, 2008). However, few surveys of their chronic toxicities on rotifers have been conducted (Xi *et al.*, 2007).

Rotifers are among the most common and generally small (<200 um) invertebrates, that play a key role in aquatic ecosystem (Wallace, 2002). The freshwater rotifer, *Brachionus calyciflorus*, is useful as one of models in aquatic ecotoxicology because of their global distribution, easy cultivation, sensitivity to vast number of toxic substances and predominantly parthenogenetic reproduction (providing genetically and phenologically identical clones for testing) (Snell and Janssen, 1995; Snell and Célia, 2007; Dahms *et al.*, 2011). It is well known that the fitness and population abundance of rotifers depend mainly on the levels of food availability. Therefore, strong seasonal variations in the phytoplankton abundance may also affect the toxicity of herbicides and pesticides to rotifers (Jak *et al.*, 1998). For this reason, there has been an increasing number of studies evaluating the combined effect of toxicant and food level on rotifers in the past decade (Luna-Andrade *et al.*, 2002; Gama-Flores *et al.*, 2004; Teresa *et al.*, 2004; Saraí and Roberto, 2006; Shi *et al.*, 2010).

Here, the interaction between the concentrations of pesticides (DDT and dicofol) and food (*Scenedesmus obliquus*) levels on the population of growth of rotifer *B. calyciflorus* were studied. The present study aimed to obtain a better understanding of how the sublethal concentrations of DDT and dicofol affect the dynamics of *B. calyciflorus* under different algal densities, and how the algal density affected the toxicity of DDT and dicofol to rotifers.

Materials and Methods

Test organisms : The test rotifer, *B. calyciflorus,* originally isolated from Lake Jinghu (31°33'N, 118°37'E), located in the center of Wuhu city, Anhui Province, China, was clonally cultured in laboratory for more than 1 year. Stock rotifer cultures were kept at 25±1ºC in natural light, using green algae *S. obliquus* as exclusive food. Algae were grown in a semi-continuous culture using HB-4 medium (Li *et al.*, 1959) was renewed daily at 20%. In exponential growth, algae were centrifuged and then re-suspended in EPA medium, which was prepared by dissolving 96 mg $NaHCO_3$, 60 mg $CaSO_4$, 60 mg $MgSO_4$ and 4 mg KCl in one litre of distilled water (USEPA, 1985). For experiments, we chose two algal densities viz. low (1.0×10^6 cell ml^{-1}) and high (3.0×10^6 cell ml^{-1}) of *S. obliquus*. Before experiment commenced, rotifers were daily fed on 1.0×10^6 and 3.0×10^6 cell ml^{-1} of *S. obliquus* for two weeks, respectively.

Toxicants : DDT and dicofol (standard grade, Sigma-Aldrich, Germany) were used as toxicants. Stock solutions were prepared by dissolving DDT and dicofol in 100% acetone, then diluted to the test concentrations using EPA medium (final concentration of acetone in each test solution was less than 0.1% v/v). All the solutions were stored at 4 ºC.

Based on the preliminary findings, four concentrations of DDT (0.05, 0.1, 0.2 and 0.4 mg l^{-1}) and dicofol (0.1, 0.2, 0.4 and 0.8 mg l^{-1}), including a blank control and a solvent control were selected.

Experiments design : Population growth experiments were conducted in 12 ml test tube containing 10 ml of the medium with one toxicant concentration at the chosen algal density. Two algal densities for each toxicant concentration, and 4 replicates for each toxicant-algae combination were selected. Thus, in all, 80 test tubes (10 toxicant concentrations x 2 algal densities x 4 replicates) were used. The experiments were initiated with an initial density of 3 ind. ml^{-1} of neonate (<12 hr old) from the stock cultures and kept at 25±1 ºC in darkness. Following inoculation, every day the density of living rotifers in each replicate, for the first few days using whole-counts and later using aliquots (three of 1 or 0.5 ml each), was counted. After counting, the rotifers were transferred to fresh tubes containing appropriate toxicant-algae mixture. Tests were terminated after 11 days by which time population densities in most replicates began to decline.

From data, the population growth rate (r) was calculated by the equation: $r = (\ln N_t - \ln N_o) / t$, where N_o = the initial density of rotifers, N_t = the final density, and t = time in days (Krebs, 1985). In general, 4-6 data points were taken from the exponential phase of the population growth curve. The mean value of calculated from four replicates of each treatment (Dumont *et al.*, 1995). Data on the population growth rates and the maximum population density (*K*) were subjected to two-way analysis of variance (ANOVA) and multiple comparison using standard statistical methods (Sokal and Rohlf, 1981).

Results and Discussion

As a tool for evaluating the effect of toxicants on *B. calyciflorus* and *B. rubens*, population growth experiment was

first used by Halbach (1984). Later, several researchers used this approach to estimate the effect of toxicants on *B. patulus*, *B. plicatilis* and *B. angularis* (Rao and Sarma, 1986; Sarma *et al.*, 2001a,b; Luna-Andrade *et al.*, 2002; Gama-Flores *et al.*, 2004). The endpoints, such as population growth rate, maximum population density as well as the frequency and amplitude of population oscillations, could be derived from this way. Because of longer exposure, the effect of toxicants on rotifers were multi-generational, comprehensive and close to field.

In the present study, the population growth curves of *B. calyciflorus* in relation to DDT and dicofol concentration at algal food levels of 1.0×10^6 and 3.0×10^6 cell ml^{-1} are shown in Fig. 1 and 2, respectively. Under low food level, DDT at all concentrations inhibited rotifer population growth. Compared to controls, maximum population density of the rotifers exposed to 0.05 and 0.1 mg l^{-1} DDT increased to lower than 12 ind. ml^{-1}, and the rotifers exposed to 0.2 and 0.4 mg l^{-1} DDT survived for less than 2 days. Under high food level, however, DDT at all concentrations, except 0.4 mg l^{-1}, increased rotifer population densities. The maximum population density in control was lower than 360 ind. ml^{-1}, whereas the values in 0.05, 0.1 and 0.2 mg l^{-1} of DDT treatments were all more than 500 ind. ml^{-1}.

Under low food levels, dicofol at 0.1 and 0.2 mg l^{-1} did not inhibit population growth of the rotifers, but increase in algal food level supported better population growth. Dicofol at both 0.4 and 0.8 mg l^{-1} greatly inhibited the population growth of rotifers under two food levels. Compared to control, under low food level, the maximum population density of the rotifers exposed to 0.4 mg l^{-1} dicifol increased to lower than 40 ind. ml^{-1}. Under high food level, the rotifer population exposed to 0.4 mg l^{-1} dicofol increased to about 90 ind. ml^{-1} till it drastically declined at 7-8th day for unknown reason. Regardless of food level, the rotifers exposed to 0.8 mg l^{-1} dicofol survived for less than 7 days.

Compared to control, under low food level, DDT at 0.1 and 0.2 mg l^{-1} decreased the population growth rate by 148.78% and 807.32%, respectively ($p < 0.05$); and DDT at 0.05-0.4 mg l^{-1} caused 89.64%, 93.75%, 97.32% and 97.32% respectively reduction of the maximum population density ($p < 0.05$). Under high food level, DDT at 0.05-0.2 mg l^{-1} increased maximum population density by 56.53%, 44.32% and 46.59% respectively, whereas DDT at 0.4 mg l^{-1} decreased the population growth rate and the maximum population density by 258.33% and 99.15%, respectively ($p < 0.05$). Increase in food level increased the population growth rate and the maximum population density of rotifers exposed to DDT at 0.05-0.2 mg l^{-1} ($p < 0.05$) (Fig. 3).

Compared to control, under low food level, dicofol at 0.4 and 0.8 mg l^{-1} decreased the population growth rate by 34.15% and 165.85%, respectively ($p < 0.05$), and caused 66.07% and 97.32% reduction in the maximum population density ($p < 0.05$). But dicofol at 0.2 mg l^{-1} increased the maximum population density by 51.78% as compared to control ($p < 0.05$). Under high food level, dicofol at 0.4 and 0.8 mg l^{-1} decreased the population growth rate by 25% and 118.75% ($p < 0.05$), and caused 73.86% and 91.48% reduction in the maximum population density of the control ($p < 0.05$). Increase in food level increased the population growth rate of rotifers exposed to dicofol at 0.8 mg l^{-1}, and the maximum population density of rotifers exposed to dicofol at 0.1 and 0.2 mg l^{-1} ($p < 0.05$) (Fig. 4).

Both the population growth rate and maximum population density were sensitive to most tested toxicants (Snell *et al.*, 2001; Gama-Flores *et al.*, 2004; Marcial *et al.*, 2005; Sarma *et al.*, 2010). Similar results were also obtained in the present study. A clear dose-response relationship existed between the population growth rate as well as the maximum population density and log toxicant concentration expect the maximum population density in DDT under low food level (Table 1). Furthermore, with lower LOEC, *K* was more sensitive than r to increasing food level and toxicant concentration, which is in agreement with the results obtained by Halbach (1984). The no observed effect concentration (NOEC), the lowest observed effect concentration (LOEC) and the median effective concentration (EC_{50}) of both the population growth rate and maximum population density of rotifers, exposed to DDT were lower than those of rotifers exposed to dicofol, indicated that DDT was more toxic than dicofol to *B. calyciflorus* (Table 2).

The adverse influence of chronic toxicant stress on *B. calyciflorus*, such as growth inhibition, reproduction suppression, and abnormal feeding and swimming behavior have been frequently studied (Ferrando *et al.*, 1993; Janssen *et al.*, 1994; Snell and Janssen, 1995; Xi and Hu, 2003; Teresa *et al.*, 2004; Xu *et al.*, 2005; Saraí and Roberto, 2006; Xu *et al.*, 2011). However, the effect of stimulation on *B. calyciflorus* population growth were found for some pollutants, including aldrin, chlordecone, di-*n*-butyl phthalate (DBP) and butyl benzyl phthalate (BBP) (Zha *et al.*, 2007; Huang *et al.*, 2007; Zhao *et al.*, 2009). Similarly, in the present study, DDT (0.05, 0.1 and 0.2 mg l^{-1}) and dicofol (0.2 mg l^{-1}) significantly increased *K* at high and low algal density, respectively.

Because both DDT and dicofol have the characteristics of endocrine disruptors (ED), the stimulation effects mentioned above were in general associated with the endocrine disruption. Some researchers have suggested that rotifers have similar endocrine-mediated mechanisms as other invertebrates and may be affected by chemicals via endocrine disruption (Gallardo *et al.*, 1997; Preston *et al.*, 2000; Radix *et al.*, 2002; Snell *et al.*, 2006). For example, Preston *et al*. (2000) observed depression of fertilization rate in *B. calyciflorus* upon exposure to nonylphenol (estrogen agonist), testosterone (androgen agonist) and flutamide (androgen antagonist). However, the knowledge of endocrine systems of aquatic invertebrates was mainly based on arthropod, and endocrine disruption responses

in rotifers were still seldom known. Thus, the effect observed might be mediated through non-endocrine mechanisms and may be a general toxic response (Snell and Joaquim-Justo, 2007). Further, research determined whether *B. calyciflorus* could be affected by DDT and dicofol *via* endocrine disruption or not.

The pattern of responding in *K* and r to increasing DDT and dicofol concentrations was also identical to that of hormesis,

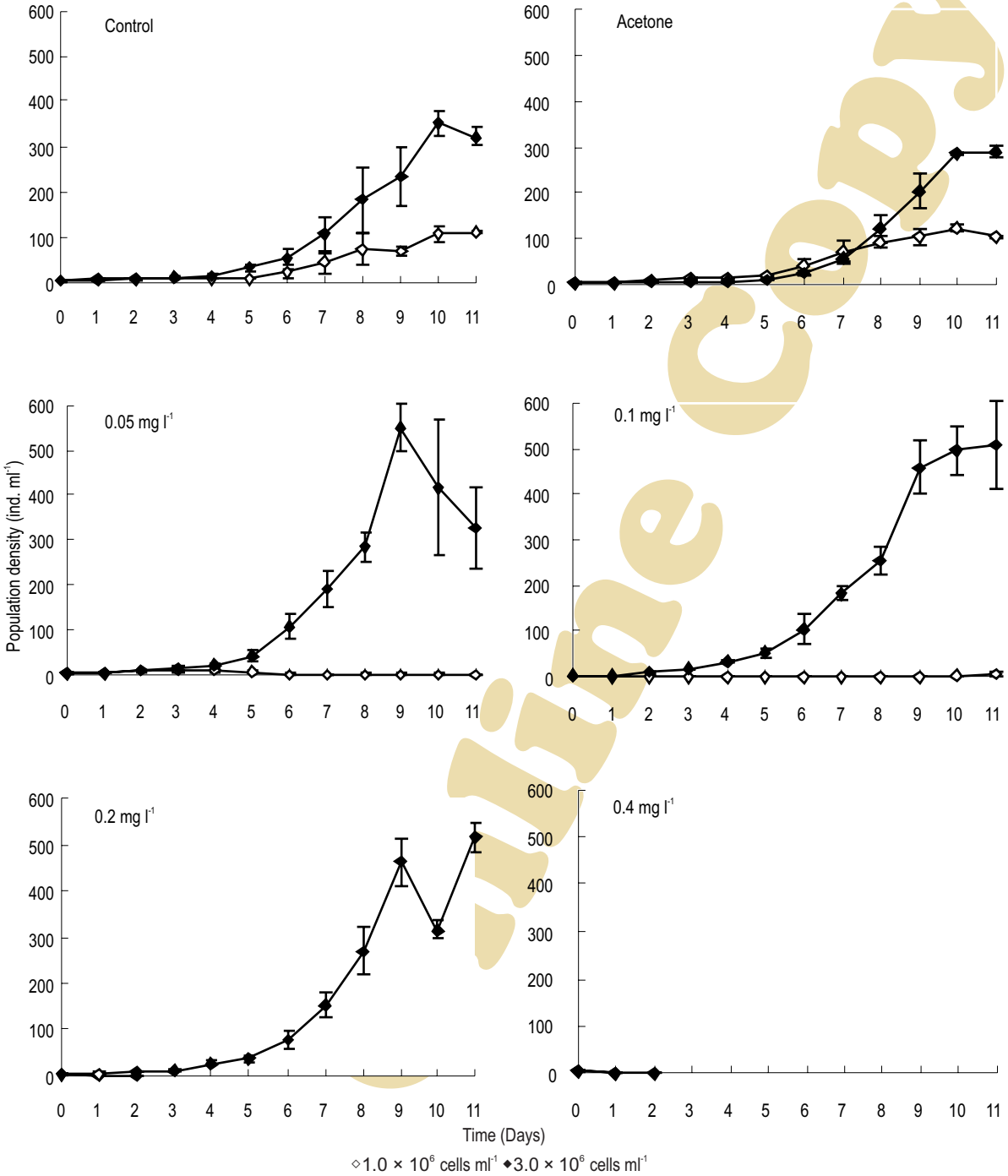

Fig. 1 : Population growth of *Brachionus calyciflorus* under four DDT concentrations and two *Scenedesmus obliquus* densities

which is characterized as a low-dose stimulation and a high-dose inhibition. This phenomenon has been documented widely in toxicological studies (Calabrese, 2008), and has been observed in some latest studies of rotifer ecotoxicology (De Schamphelaere and Janssen, 2010; Garaventa *et al.*, 2010; Lv *et al.*, 2010; Guo *et al.*, 2012). Costantini (2010) thought that energy intake could be slightly increased when the animal is exposed to intermediate levels of a stressor, and its use could be optimized in terms of energetic trade-offs between self-maintenance (i.e. longevity) and other activities (e.g. reproduction). According to this view, there is a shift in the allocation of assimilation energy from reproduction to longevity in the present study, which resulted in significant increase for *K* but r. However, this speculation still deserve further investigation with more species before

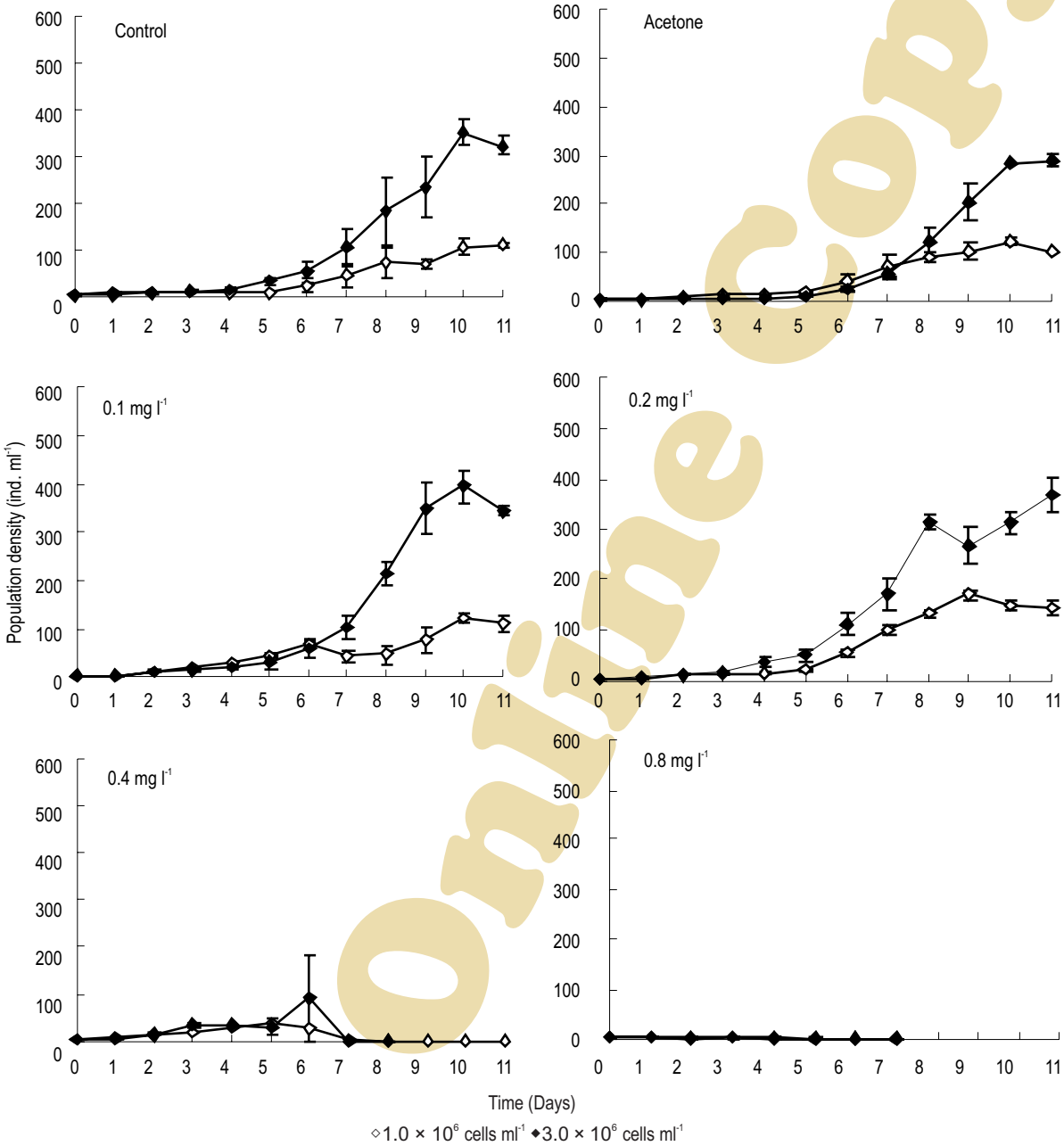

Fig. 2 : Population growth of *Brachionus calyciflorus* under four dicofol concentrations and two *Scenedesmus obliquus* densities

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generalization. Several studies have shown that mild exposure to stressors can simultaneously increase both longevity and reproduction (Cardoso *et al.,* 2002; Calabrese and Baldwin, 2003; Calabrese and Blain, 2005; Cutler *et al.,* 2009).

Earlier several studies have reported that food availability had great impact on the results of ecotoxicological test with rotifers (Sarma *et al.*, 2001a, b; Luna-Andrade *et al.*, 2002; Gama-Flores *et al.*, 2004; Teresa *et al.*, 2004; Saraí and Roberto, 2006). In general, increase in food level could reduce toxicity of pollutants and enhance the toxicant-resistance of rotifers. Similar results were obtained in the present study. Our results indicated that algal density, DDT concentration, DDT concentration × algal density and dicofol concentration affected the population growth rate of the rotifers and the maximum population density ($p<0.01$), dicofol concentration × algal density affected the maximum population density ($p<0.01$) (Table 3). In addition, the food level had a great influence on toxicity of DDT even at the lowest DDT concentration (0.05 mg l^{-1}) (Fig. 1), which was similar to the result obtained by Rao and Sarma (1986).

The phenomenon that toxicants may be degraded or accumulated in algal cells has been confirmed by several researchers (Lavoie *et al.*, 2009; Liu *et al.*, 2010; Zhang *et al.*, 2011; Jin *et al.*, 2012). Further work is needed to make clear mode of action of the toxicants to a given algal species. In the present study, solution and algae were daily renewed and the problem of bioaccumulation of DDT and dicofol in alga might be negligible. In nature, algal density in water bodies varies considerably through seasons. Therefore, the effect of toxicants could be expected to become intense in the oligotrophic ponds, but diminish in the eutrophic ponds. Roex *et al.* (2000) reported that if the concentration of a toxicant was above certain value, its effect was independent of algal leve. This trend was also observed in the present study.

Most toxicity tests performed with rotifers were conducted under ideal nutritional conditions (Cecchine and

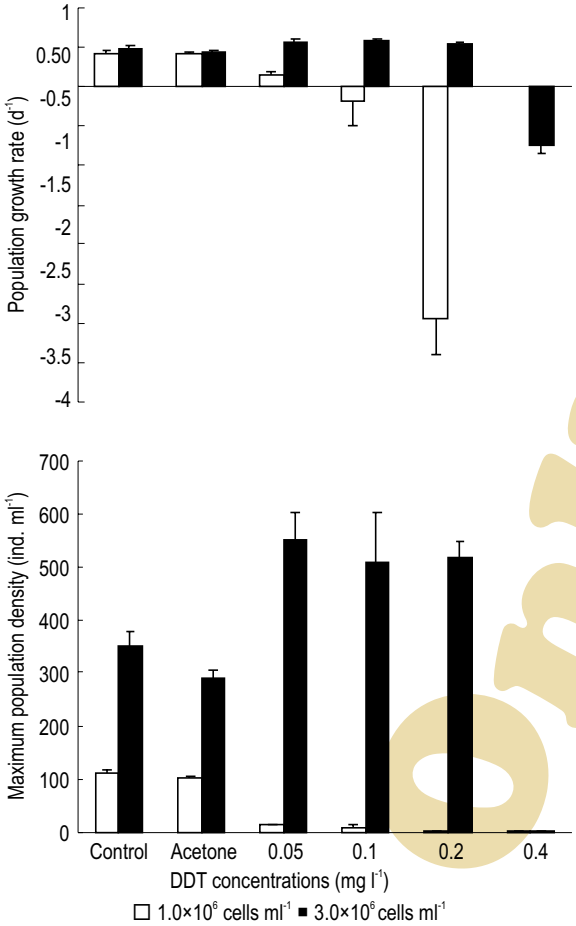

Fig. 3 : Population growth rate and maximal population density of *Brachionus calyciflorus* under four DDT concentrations and two *Scenedesmus obliquus* densities

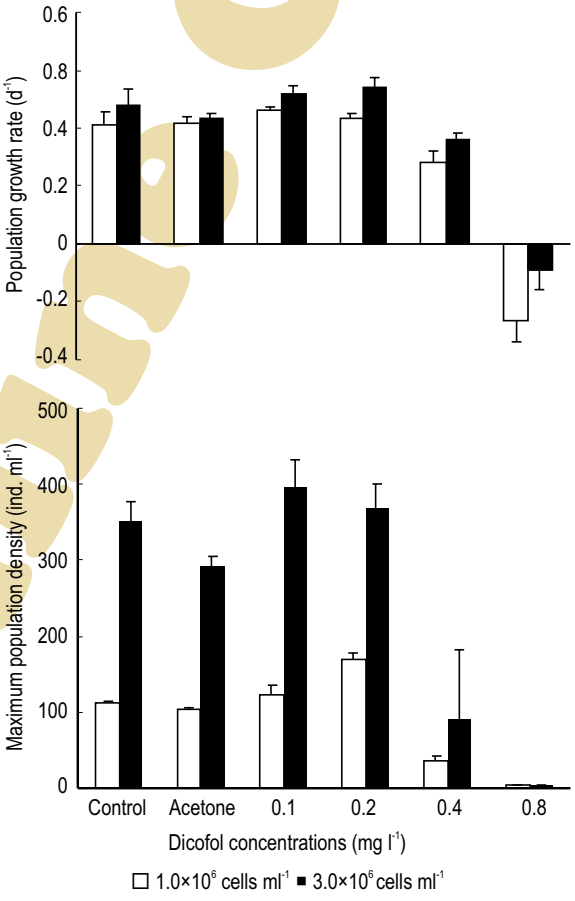

Fig. 4 : Population growth rate and maximal population density of *Brachionus calyciflorus* under four dicofol concentrations and two *Scenedesmus obliquus* densities

Snell, 1999). These conditions represent an abundant unlimited food supply and therefore, artificially inflate population health and fecundity. Normal field conditions for rotifers would more likely be food limiting, such as those present in oligotrophic waters. Hence, the effects observed in the present study probably will not occur in natural freshwater ecosystems because of high algal density being used. But considering that experimental outcome could be strongly influenced by the nutritional state of test animals (Cecchine and Snell, 1999; Wheelock *et al*., 2002), the higher food levels were still used frequently in the standard rotifer chronic toxicity tests (Xi *et al*., 2007; Zhao *et al*., 2009; Lv *et al*., 2010; Sarma *et al*., 2010; Huang *et al*, 2012). In other words, the high food levels were used in order to eliminate the effects of starvation, debility, and infecundity caused by food limitation on the toxicity evaluation.

Table 1 : Relationships between the population growth rate (y, r) as well as the maximal population density (y, K) of *Brachionus calyciflorus* and the log toxicant concentration (x) of DDT and dicofol under two *Scenedesmus obliquus* densities

Toxicants	Food levels (cell ml^{-1})	Parameters	Regression equation	Significant tests
DDT	1.0×10^6	r	y=-13.21x^2-31.54x-18.53	r^2=0.911, p<0.01
	3.0×10^6	r	y=-3.64x^2-7.5x-3.15	r^2=0.921, p<0.01
		K	y=-1.33E3x^2-2.79E3x-867.85	r^2=0.782, p<0.01
Dicofol	1.0×10^6	r	y=-1.44x^2-2.36x-0.47	r^2=0.925, p<0.01
		K	y=-225.19x^2-410.39x-48.42	r^2=0.752, p<0.01
	3.0×10^6	r	y=-1.29x^2-2.09x-0.28	r^2=0.926, p<0.01
		K	y=-222.4x^2-720.88x-83.59	r^2=0.789, p<0.01

Table 2 : Estimates of chronic toxicity of DDT and dicofol on the population growth rate (r) and the maximal population density (*K*) *Brachionus calyciflorus* under two *Scenedesmus obliquus* densities

		DDT			Dicofol		
Food levels (cell ml^{-1})	Parameters	NOEC	LOEC	EC50	NOEC	LOEC	EC50
1.0×10^6	r	0.05	0.1	0.08	0.2	0.4	0.39
	K	-	0.05	-	0.1	0.2	0.44
3.0×10^6	r	0.2	0.4	0.19	0.2	0.4	0.46
	K	-	0.05	0.27	0.2	0.4	0.28

NOEC: no-observed effect concentration; LOEC: lowest-observed-effect concentration; EC50: median effective concentration

Table 3 : Two-way analysis of variance on the population growth rate and the maximal population density of *Brachionus calyciflorus* under different toxicants concentrations and two *Scenedesmus obliquus* densities

Source	SS	DF	MS	F	p
Population growth rate					
DDT concentration (A)	18.702	4	4.676	53.385	0.000
Algal density (B)	7.905	1	7.905	90.772	0.000
A×B	13.800	4	4.600	52.522	0.000
Error	2.041	23	0.088		
Dicofol concentration (A)	2.448	4	0.622	74.392	0.000
Algal density (B)	0.095	1	0.048	5.709	0.009
A×B	0.015	4	0.004	0.454	0.769
Error	0.226	27	0.008		
Maximal population density					
DDT concentration (A)	413812.730	4	103453.183	18.763	0.000
Algal density (B)	1170536.081	1	117053.081	212.299	0.000
A×B	419469.686	4	104867.421	19.020	0.000
Error	148867.948	27	5513.628		
Dicofol concentration (A)	467396.597	4	116849.149	36.609	0.000
Algal density (B)	220277.730	1	220277.730	69.013	0.000
A×B	109492.212	4	27373.053	8.576	0.000
Error	89371.673	28	3191.845		

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In the absence of toxicant stress, population growth rate of *Brachionus,* in general, varied from 0.1 to 1.0 d^{-1} depending on the food concentration (Sarma *et al.,* 2001c). Xi *et al.* (2001) found that the optimal *S. obliquus* density for survival and reproduction of *B. calyciflorus* was 2.0×10^6 cell ml^{-1}, which was the basis of selection of "low" (1.0×10^6 cell ml^{-1}) and "high" (3.0×10^6 cell ml^{-1}) fool level. In the present study, r values in control were 0.41 and 0.48 d^{-1} under low and high food level, respectively. Although, the results indicated that rotifer in control did not perceive the food levels as different, the responses of rotifers to DDT and dicofol were significantly affected by the food level, which was main aim of this study. It is not difficult to infer that rotifer could be more sensitive to these toxicants under lower food level, but the dose response should be evaluated further because it could alter how risk assessment are conducted and implemented.

In conclusion, the present results showed that DDT and dicofol had significant effect on the population dynamics of *B. calyciflorus* under two algal densities. Certain concentrations of DDT and dicofol increased r and *K* value. Algal density played an important role in influencing the toxicity of DDT and dicofol to *B. calyciflorus*.

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