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EFFECTS OF METHAMIDOPHOS AND ACEPHATE ON EXPERIMENTAL POPULATION DYNAMICS OF ROTIFER *BRACHIONUS CALYCIFLORUS*

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Abstract: Effects of sublethal levels (0. 01, 0. 1, 1. 0, 10. 0, 100. 0, 1000. 0 and 10000. 0 μg/L) of organophosphorus insecticides including methamidophos (O, S-Dimethyl phosphoramidothioate, CAS No. 10265-92-6) and acephate (O, S-Dimethyl acetylphosphoramidothioate, CAS No. 30560-19-1) on population dynamics of rotifer *Brachionus calyciflorus* were studied by 3-day population growth test at 25 ± 1℃, in dark, and with 3.0×10^6 cells/mL of *S. obliquus* as the rotifers' food. The results showed that both methamidophos and acephate influenced significantly the population growth rate, the ratio mictic females/amictic females and the mictic rate of the rotifers. Methamidophos influenced markedly the carrying-egg females/non-carrying-egg females of the rotifers, but acephate did not. Compared to the controls, 100. 0 μg/L methamidophos and 1. 0—10000. 0 μg/L acephate increased significantly the population growth rate, but the reverse was true for 1, 000. 0 and 10, 000. 0 μg/L methamidophos. 1, 000. 0 μg/L methamidophos increased significantly the ratio carrying-egg females/non-carrying-egg females. 0. 1 μg/L methamidophos and 10. 0—10000. 0 μg/L acephate increased significantly the ratio mictic females/amictic females, and both 0. 1 μg/L methamidophos and 10 μg/L acephate increased significantly the mictic rate. 10. 0—10000. 0 μg/L acephate significantly increased the resting egg production. The above-stated results indicated that sublethal levels of methamidophos and acephate influenced significantly the population dynamics of *B. calyciflorus*.

Key words: Organophosphorous pesticide; Methamidophos; Acephate; *Brachionus calyciflorus*; Population dynamics

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Rotifers, especially *Brachionus calyciflorus* and *B. plicatilis*, are frequently used species for aquatic toxicology due to their feasibility of cultures, availability of cysts, cosmopolitan distribution, well-established taxonomic characters, planktonic mode of life, and professional endorsement of toxicological evaluations [1]. Most planktonic rotifers have a cyclically parthenogenetic life cycle where asexual reproduction predominates, but there are periods where both asexual and sexual reproductions occur simultaneously. Since rotifers have been used as bioassay organisms for ecotoxicological studies, toxicity tests utilizing rotifers incorporate mainly the asexual phase of the life cycle. In 1995, Snell and Carmona [2] compared the toxicant sensitivity of asexual and sexual reproductions in freshwater rotifer *B. calyciflorus*. Preston, *et al*. [3] investigated the effects of potential endocrine disruptors on the sexual reproduction of *B. calyciflorus*. Radix, *et al*. [4] and Xi and Feng[5] studied the effects of some environmental pollutants on asexual and sexual reproductions of *B. calyciflorus*. Because the studies on only a portion of the rotifer life cycles often underestimate the true vulnerability of rotifer life cycles to toxicants [6], more complete life-cycle tests should be made to investigate the ecological impact of pollutants on rotifers now.

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Organophosphorous pesticides are agriculturally important pesticides and usually used in China. During the last two decades, their production and consumption have greatly increased [7]. Large quantities of these organophosphorous pesticides enter increasingly the water bodies [8], and affect aquatic organisms. Some researchers investigated the effects of organophosphorous pesticides on aquatic organism[9], and Sarma, *et al.* [10] and Gama-Flores [11] studied the acute and chronic toxicity of the pesticide methyl parathion to the rotifer *B. patulus* and *B. angularis*, but the effects of methamidophos and acephate on the population dynamics of rotifer remains unknown. Methamidophos and acephate are important organophophorous pesticides widely used in China. Up to now, studies on methamidophos and acephate in water environment have mainly concentrated on its residues determination[12], but their influence on the population dynamics of rotifers has not been reported.

The main objective of the present study was to assess the effects of sublethal concentrations of methamidophos and acephate on the population parameters including the population growth rate, the ratio of ovigerous females to non-ovigerous females, the ratio mictic females to amictic females, and the resting egg production of rotifer *B. calyciflorus*.

1 Materials and methods

The rotifer *B. calyciflorus* used as the test animal in the present study was obtained by hatching resting eggs collected from sediments of Lake Jinghu and thereafter clonal culturing. Stock rotifer cultures were kept at 25 ± 1℃ on natural light and daily fed on 3.0×10^6 cells/mL of *Scenedesmus obliquus*. For mass cultures as well as for experiments, moderately hard synthetic freshwater (EPA medium) [13] was used as the medium. Algae were grown in a semi-continuous culture using HB-4 medium [14] renewed daily at 20%. Algae in exponential growth were centrifuged, counted and resuspended in EPA medium with the cell density of 3.0×10^7 cells/mL, then stored in a refrigerator (3—4℃) and used for 3 days.

Methamidophos and acephate (technical grade, 99.9%, Sigma Co.) were used as toxicants. Stock solutions of 100 mg/L were prepared using distilled water. At seven concentrations (0.1, 1.0, 2.0, 5.0, 10.0, 20.0 and 50.0 mg/L) of methamidophos and acephate, preliminary tests were conducted in 6 mL glass cups and started by placing 10 neonates (< 2h old) into each cup which contained 5 mL of test solution with 3.0×10^6 cells/mL of *S. obliquus*, and the results showed that the LC_{50} values of *B. calyciflorus* for both methamidophos and acephate were higher than 50mg/L. Considering the nominal concentrations of methamidophos and acephate in natural water bodies and the special significance of low-dose study [15], seven concentrations (0.01, 0.1, 1.0, 10.0, 100.0, 1000.0 and 10000.0 μg/L) of methamidophos and acephate, and a control were chosen and prepared through serial dilution with EPA medium from the 100 mg/L stock solution. 3-day population growth tests were conducted in 10 mL test tubes and started by introducing 5 females which began to produce an amictic egg into each test tubes which contained 5 mL of test solution with 3.0×10^6 cells/mL of *S. obliquus*. Four replicates were set for each concentration of methamidophos and acephate, and the control. The test tubes were covered and maintained in a thermostat-controlled water bath at 25 ± 1℃, in dark. During the experimental period, all the test tubes were manually rocked gently at 4—6h intervals to minimize the algal sedimentation. After 72h of culture, the number of mictic females, amictic females, non-carrying-egg females and resting eggs in each test tube were counted. Mictic and amictic females were identified by the size and morphology of their eggs. Thereafter, the population growth rate for the rotifers at each concentration was calculated using the equation $r = (\ln N_t - \ln N_0)/t$, where N_t = density of females at time t, $N_0 = 1$ female/mL, and $t = 3$ days. The ratio carrying-egg females/non-carrying-egg females (OF/NOF) and the ratio mictic females/amictic females (MF/AF) were calculated according to Radix, *et al.* [4], the mictic rate was calculated for each test tube as the proportion of mictic females in all the females.

One-way analysis of variance (ANOVA), with concentration as the independent variable, and *r*, mictic rate, fertilization rate, ratio OF/NOF or the ratio MF/AF as the dependent variable, followed by Dunnett's test was conducted for pairwise comparisons of each pesticide concentration relative to the control.

2 Results

Methamidophos and acephate both significantly influenced the population growth rate of the rotifers ($p < 0.01$). Compared to the controls, 100.0 μg/L methamidophos and 1.0—10,000.0μg/L acephate increased the population growth rate, but the reverse was true for 1,000.0 and 10,000.0 μg/L methamidophos (Fig. 1).

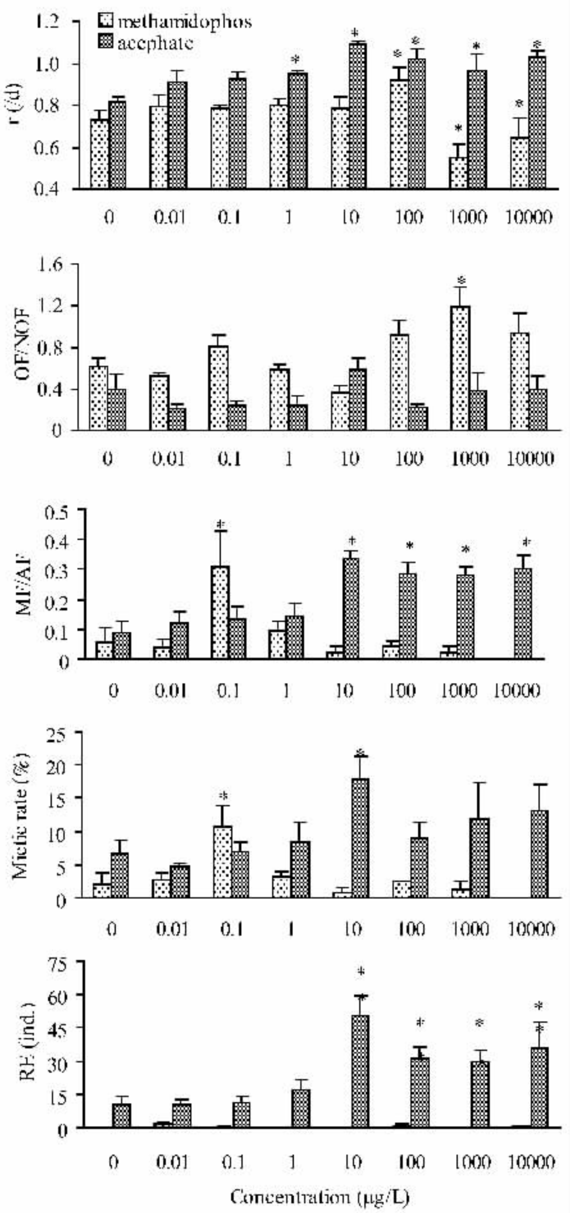

Fig. 1　The effects of methamidophos and acephate on the population growth rate (r), the ratio ovigerous amictic female/non-ovigerous females (OF/NOF), the ratio mictic females/amictic females (MF/AF), the mictic rate (%) and the number of resting eggs (RE) of *Brachionus calyciflorus* (The columns represent the mean calues of 4 replicates and the bars are the standard deviation derived from these replicates, and these data reflect the results obtained at the end of the test period. * significantly different from the controls, $p < 0.05$)

Methamidophos significantly influenced the ratio ovigerous females/non-ovigerous females of the rotifers ($p < 0.01$), but the reverse was also true for acephate ($p > 0.05$). Compared to the controls, 1,000.0μg/L methamidophos increased significantly the ratio ovigerous females/non-ovigerous females (Fig. 1).

Both methamidophos and acephate significantly influenced the ratio mictic females/amictic females and the mictic rate of the rotifers ($p < 0.01$). Compared to the controls, 0.1μg/L methamidophos and 10.0—10,000.0μg/L acephate increased significantly the ratio mictic females/amictic females, and both 0.1μg/L methamidophos and 10μg/L acephate increased significantly the mictic rate (Fig. 1).

Methamidophos did not significantly influence the resting egg production of the rotifers ($p > 0.05$), but the reverse was also true for acephate ($p < 0.05$). Compared to the controls, 10.0—10, 000.0μg/L acephate significantly increased the resting egg production (Fig. 1).

3 Discussion

Up to now, and in all the chronic toxicity tests with rotifers as test animals, the concentrations near to the LC50s were chosen to be the highest concentrations for all the chemical pollutants [16]. Organophorus pesticides were thought having low toxicity [9], but whether methamidophos and acephate entering natural water bodies after they had been used in agriculture affect the population dynamics of rotifers remains unknown. Considering the nominal concentrations of methamidophos and acephate in natural water bodies, we chosen sublethal concentrations of them for test in this study, and the results indicated that sublethal levels of methamidophos and acephate influenced significantly the population dynamics of *B. calyciflorus.*

The population growth rate is a measure on the rate of parthnogenetic reproduction in rotifers, and is usually used to assess the effect of toxicants on asexual reproduction. In the present study, 100.0 μg/L methamidophos, and 1.0—10,000.0μg/ acephate increased significantly the population growth rate, which were similar to the effects of 50mg/L gamma-aminobutyric acid, 0.0025 and 0.025 IU/mL growth hormone, 0.25 and 2.5 IU/mL human chorionic gonadotropin, 5.0 mg/L 5-hydroxyl-tryptamine, and 4.0—8.0 mg/L glyphosate on the population growth rates of *B. plicatilis* and *B. calyciflorus*[17,5]. However, 1,000.0 and 10,000.0 μg/L methamidophos decreased significantly the population growth rate of rotifers, which was identical to the effects of all the other tested chemical pollutants on the population growth rates of *B. plicatilis* and *B. calyciflorus* [4,17—21]. The reason for 100.0 μg/L methamidophos, and 1.0—10,000.0μg/ acephate increased significantly the population growth rate might be that they stimulate the asexual reproduction of the rotifers which is worthy of further researching, or they enhance the growth of the algae, because sublethal levels of organophosphorus insecticides such as monocrotophos at concentrations lower than 0.5 and 1.0 mg/L increased the relative growth of marine microalgae *Phaeodactylum tricornutum* and *Dunaliella* sp., respectively [22], and subinglin, profenofos and phoxin all at concentrations lower than 1.0 mg/L increased the Chl-a content in *Platymonas* [23].

As usual, the increase in the ratio carrying-egg females/non-carrying-egg females in a rotifer population indicates a positive growth of the population in the future. In the present study, 1,000.0 μg/L methamidophos increased significantly the ratio carrying-egg females/non-carrying-egg females, but decreased significantly the population growth rate. Considering the higher ratio carrying-egg females/non-carrying-egg females in the rotifer population exposed to 1,000.0 μg/L methamidophos, a positive growth of the population might be expected in the future. Radix, *et al.* [4] found that the ratio carrying-egg females/non-carrying-egg females in *B. calyciflorus* population was a suitable endpoint for assessing the effects of ethinylestradiol and nonylphenol. However, in the present study, the ratio carrying-egg females/non-carrying-egg females was not significantly correlated with the concentration of methamidophos, indicating that it was not a suitable endpoint for quantitatively monitoring the effects of sublethal concentrations of methamidophos on population dynamics of rotifers, so was the ratio mictic females/amictic females.

The mictic rate was commonly used in toxicity tests to assess the effects of toxicants on sexual reproduction of rotifers. Xi and Feng[5] found that the population growth rate of *B. calyciflorus* increased significantly when the concentration of herbicide glyphosate was 4.0 mg/L, but the mictic rate increased markedly when the concentration of glyphosate was 2.0 mg/L. Similarly, the population growth rate of *B. calyciflorus* decreased significantly when the concentration of pesticide thiophanate-methyl was 0.4 mg/L, but the mictic rate decreased markedly when the concentration of thiophanate-methyl was 0.1 mg/L. Similar results were obtained by Preston and Snell [6], Marcial, *et al.* [24], and Snell and Carmona[6] in their studies. The results stated above showed that sexual reproduction was more sensitive than asexual reproduction to all the tested chemicals, but not to sublethal concentrations of methamidophos and acephate (in the present study).

Resting egg is the product of sexual reproduction of rotifers, and can be used as another endpoint to assess the effects of toxicants on sexual reproduction of rotifers[5,6]. In the present study, methamidophos did not significantly influence the resting egg production of the rotifers, which was similar to the effect of thiophanate-methyl [5]. However, acephate influenced significantly the resting egg production of the rotifers, which was identical to the effects of pentachlorophenol and glyphosate.

Rotifers are extremely important in freshwater ecosystem. They can convert primary (algal and bacterial) production into a form usable for second consumer (eg., insect larvae, fish fry) and achieve this transformation with remarkable efficiency, producing up to 30% of the total plankton biomass [25]. In the present study, although each of the population parameters was not correlated with the concentrations of methamidophos and acephate, and each of them could not be used as endpoints to quantitatively assess the effects of sublethal concentrations of methamidophos and acephate on the population parameters of rotifers, the present results indicated that sublethal levels near to nominal concentrations in natural water bodies of methamidophos and acephate influenced significantly the population dynamics of *B. calyciflorus*, and changed the structures and functions of freshwater ecosystems. For large-scale agriculture use, the final concentration of less than 0.1μg/L for methamidophos or 1.0μg/L acephate after they entered into freshwater bodies should be required.

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甲胺磷和乙酰甲胺磷对萼花臂尾轮虫实验种群动态的影响

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摘要：应用3天种群增长实验方法，在(25±1)℃、无光照、以 3.0×10^6 cells/mL 的斜生栅藻为轮虫的食物等条件下，研究了亚致死浓度(0.01、0.1、1.0、10.0、100.0、1000.0 和 10000.0 μg/L)的甲胺磷和乙酰甲胺磷对萼花臂尾轮虫实验种群动态的影响。结果显示，甲胺磷和乙酰甲胺磷显著地影响萼花臂尾轮虫的种群增长率、混交雌体数与非混交雌体数的比值和混交率。甲胺磷显著地影响萼花臂尾轮虫种群中的带卵雌体数与不带卵雌体数的比值，但乙酰甲胺磷对其无显著的影响。和对照组相比，浓度为 100.0 μg/L 的甲胺磷和浓度为 1.0—10,000.0 μg/L 的乙酰甲胺磷均使轮虫种群增长率显著增大，而浓度为 1000.0 μg/L 和 10000.0 μg/L 的甲胺磷却使之显著减小；1000.0 μg/L 的甲胺磷使轮虫种群中的带卵雌体数与不带卵雌体数的比值显著上升，0.1 μg/L 的甲胺磷和 10.0—10000.0 μg/L 的乙酰甲胺磷均使轮虫种群中的混交雌体数与非混交雌体数的比值显著上升，0.1 μg/L 的甲胺磷和 10 μg/L 的乙酰甲胺磷均使轮虫的混交率显著增大，10.0—10000.0 μg/L 的乙酰甲胺磷使轮虫休眠卵产量显著提高。上述结果表明，亚致死浓度的甲胺磷和乙酰甲胺磷对萼花臂尾轮虫实验种群动态具有显著的影响。

关键词：有机磷杀虫剂；甲胺磷；乙酰甲胺磷；萼花臂尾轮虫；种群动态