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Population growth of *Brachionus calyciflorus* affected by deltamethrin and imidacloprid insecticides

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Abstract

Rotifers due to their relatively short lifespan, high fecundity and high rate of population growth are ideal for chronic toxicity tests. The aim of this research was to determine the lethal concentrations (LC_{50} 24 h) of deltamethrin and imidaclopride and their impacts on the reproduction and growth of the freshwater rotifer, *Brachionus calyciflorus*. Experiments were carried out based on the guidelines of the standard methods of OECD. Based on LC_{50} levels of pesticides, different concentration treatment groups designated and rotifer population responses in the five different concentrations of deltamethrin (0.00, 0.05, 0.10, 0.21 and 0.53 mg L^{-1}) and imidacloprid (0.00, 6.22, 12.45, 24.91 and 62.27 mg L^{-1}) were studied during ten days. The LC_{50} 24h of deltamethrin and imidacloprid for freshwater rotifer determined as 1.06 mg L^{-1} and 124.54 mg L^{-1}, respectively. The density of rotifers in all treatment groups of pesticides decreased significantly compared to the control group at tenth day ($p<0.05$). The ratio of ovigerous females to nonovigerous females (OF/NOF) and the ratio of mictic females to amictic females (mic/amic) were significantly affected ($p<0.05$) in all concentrations of both insecticides. The results of this study suggested that *B. calyciflorus* are severely sensitive to deltamethrin rather than to imidacloprid pesticide.

Keywords: *Brachionus calyciflorus*, Pesticide toxicity, Population growth, Lethal concentration.

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Introduction

Life-cycle toxicity tests are important components for realistic evaluation of ecological risks of pesticides. The life-cycle tests of fish provides excellent data on toxicity (Jafar Nodeh and Hosseini, 2013), but these tests are costly and time consuming. Recently, researchers sought ways for shortening the time of toxicity tests, while critical stages, sensitivity and ecological originality are to be considered. Therefore, use of the early life stages of fish proposed (McKim, 1985; Norberg-King, 1989; Kalbassi *et al*., 2013). On the other hand, aquatic invertebrates appear to be a good representative, because their life cycle is much shorter than fish and their small size decreases the test volume (Snell and Persoone, 1989).

In recent decades, industrial progress has led to increasing the use of detrimental bio-chemicals which ultimately have led to the accumulation of toxicants in our environment and living organisms (Sánchez-Bayo, 2012; Torre *et al*., 2013; Faggio *et al*., 2016; Pagano *et al*., 2016). Potential risk of toxins in aquatic ecosystems can be investigated by modelling their study in laboratory or field (Aliko *et al*., 2015; Savorelli *et al*., 2017). The information of toxicology experiment in ecotoxicology science can point out the effects of toxicants on population of aquatic animals in freshwater resources. Laboratory data are used to assess potential environmental impact of poisons and to limit using of toxic materials. So, the aim of pollutant toxicity measurement is to access their acceptable standard level in order to protect aquatic resources (Handy and Penrice, 1993).

Among the multicellular organisms, rotifers have short life cycle (Wallace and Snell, 1991) and they are very important in freshwater and coastal marine environments, as the main food of many invertebrates and larval period in fishes. Many chemicals, such as heavy metals, nanoparticles, pesticides, fly ashes, and perfluorinated compounds, have been assessed using rotifers as a model receptor (Marcial *et al*., 2005; Snell and Hicks, 2011; Cooper *et al*., 2014; Wang *et al*., 2014; Zhang *et al*., 2013, 2014, 2015a, 2015b). Population dynamics of rotifers are well-known in laboratory and field conditions (Edmondson, 1965). Rotifers are so important for population dynamic studies, that are considered and presented as pollution indicators, and bioassay organisms (Snell, 1991) as well as models in experiments of population dynamics (Snell, 1978). They work as grazers on phytoplanktons and involve in nutrient recycle procedures in aquatic ecosystems (Ejsmont-Karabin, 1983).

Early studies, in ecotoxicological experiments, were only emphasized on asexual reproduction phase of rotifer life cycle. Snell and Carmona (1995) compared sexual and asexual sensitivity of freshwater rotifer *B. calyciflorus* to toxins. The influence of endocrine disrupters on the sexual reproduction of freshwater rotifers was studied by Preston *et al.* (2000). Nowadays, investigations on full life cycle of rotifers are required to evaluate the ecological effect of pollutants. A lot of tests exist in bibliography about the acute and chronic toxicity of different pesticides on freshwater rotifer but due to differences in genotype and environment, these results are very different. Therefore, it is recommended that these experiment, to be performed at various locations (Sarma, 2000).

Pyrethroid pesticides such as deltamethrin are widely used in the world. Application of these pesticides are considered to be a potential danger for various environments including aquatic ecosystems (Richterová and Svobodová, 2012; Qadir et al., 2015). Utilization of imidacloprid has been greatly increased in the last two decades (Jemec *et al.,* 2007). This neoticotinoid insecticide is considered as a potential pollutant for surface and ground water in the world (Tippe, 1987).

Chemical pesticides with stable molecules (long half-life) will pose a threat on aquatic organisms and human populations that consume them (McKim, 1985). Therefore, the present study attempts to evaluate the effect of deltamethrin and imidacloprid insecticides on the population growth and reproduction dynamics of freshwater rotifer, *Brachionus calyciflorus*.

Materials and methods

The rotifer *B. calyciflorus* used in this experiment was isolated from Chahnime water reservoirs (30'45''N 61'38''E) in northern part of Sistan and Blouchestan Province-Iran. Rotifers were cultured for three months in hard synthetic freshwater (EPA, 2002) and fed on *Chlorella vulgaris* (1-2×10^6 cells m L^{-1}) during adaptation period and attempts to obtain the required amounts of resting eggs. Resting eggs were produced under highly controlled conditions and stored at 4 ºC in darkness. Hatching of resting eggs was started by transferring them to EPA medium (Peltier and Weber, 1985) at 25 ºC and 3000 lux of light intensity. Pure algae (*Chlorella vulgaris*) stock was obtained from the Hamoun International Wetland Research Institute, Iran. They were cultured in semi-intensive system in Zinder (Z-8) medium at 25±1ºC temperature and 16:8h light-dark regime (3000 lux) (Lucía-Pavón *et al.*, 2001).

In order to gain the same chronological age of rotifers, after 16-18 hours, the homogeneous group (same age) of neonates were isolated and transferred to test tubes using pipette (0.4 mm in diameter). Each test tube was containing 10 mL of culture medium and 100 newly hatched neonates (with average age of less than 2 hours). Advantage of using hatched resting eggs is that it is working with the homogeneous groups with similar physiological and genotype conditions and this will greatly reduce the experiment errors (Fernandez-Casalderrey *et al.*, 1991).

Acute toxicity experiments were carried out statically based on standard methods (Opschoor and Vos, 1989) in order to determine the LC_{50} 24h. According to range-finding test and logarithmic calculations that were carried out based on the method described by Snell and Persoone

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(1989), we defined eight concentrations of deltamethrin (0.7, 0.8, 0.9, 1, 1.2, 1.3, 1.4 and 1.5 mg L^{-1}), nine concentration of imidacloprid (17.5, 35, 70, 105, 140, 175, 210, 245 and 280 mg L^{-1}) and a control treatment with three replicates to determine the lethal concentrations of these pesticides. Then, the LC_{10}, LC_{50} and LC_{90} of deltamethrin and imidacloprid were determined using Probit Analysis (Kuçukgul Gulec et al., 2015).

For evaluation of pesticides effects, the rotifers were exposed to five concentrations of deltamethrin (0.00, 0.05, 0.10, 0.21 and 0.53 mg L^{-1}) and imidacloprid (0.00 (control), 6.22, 12.45, 24.91 and 62.27 mg L^{-1}) based on results of LC tests. To begin the test, 27 tubes [((2×4 level of toxin concentration)+1 control)× 3 replicates] containing 10 ml of EPA medium, chlorella (2×10^6 cells mL^{-1}) and toxin were prepared and 100 newly hatched neonates were added to each tube. The number of mictic females, amictic females, ovigerous females and nonovigerous females were determined according to their color, shape and body size and monitored (Snell, 1978) and then the rotifers were transferred to new tubes which was supplied with fresh food and toxin solution daily (Fernandez-Casalderrey *et al.,* 1991). During the experiment, test tubes were kept under 3000 lux of light intensity and 16:8h of light/dark regime without aeration, and the algae were suspended by micropipette every 12 hours (Xi and Feng, 2004). The rate of population growth in different treatments were calculated by the following exponential equation 1 (Fernandez-Casalderrey *et al.*, 1992):

$$r = (LnN_t - LnN_0)\ t^{-1} \quad (1)$$

Where N_0 is the initial population density, N_t is the final population density and t is time.

Data analysis performed using SPSS Ver.16 and normality of data distribution was tested using the Shapiro–Wilk test. Analysis of variance (One-way ANOVA) and Duncan test used for the general comparison between the treatments and for the segregation of different groups (p<0.05). The LC50 values for pesticides were measured using probit analysis test.

Results

According to lethal concentration tests, the LC_{50} 24h of deltamethrin and imidacloprid for freshwater rotifer (*B. calyciflorus*) were obtained as 1.06 and 124.54 mg L^{-1}, respectively (Table 1).

Table 1: Acute 24 h toxicity of technical deltamethrin and imidacloprid insecticides in *Brachionus calyciflorus.*

	NOEC	LOEC	LC_{50}	LC_{50} 95% Confidence limits	LC_{10}	LC_{90}
Deltamethrin (mg L^{-1})	0.70	0.80	1.06	1.02-1.11	0.81	1.40
Imidaclopride (mg L^{-1})	17.5	35	124.54	93.98-155.11	55.51	279.43

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Principally, pesticide concentrations selected for the chronic toxicity test were based on the result of lethal concentration test, in a manner that the highest concentration of LC_{50} was half a fold and the lowest concentration was 0.05 times of LC50 for each insecticide. In this study, the result showed that population density of rotifer exposed to 6.22 mg L^{-1} of imidacloprid had the same trend as the control group until the sixth day and the same trend was observed for deltamethrin (0.05 mg L^{-1}) until the fifth day. Moreover, deltamethrin at 0.53 mg L^{-1} (half of LC_{50}) and imidacloprid at 24.91 mg L^{-1} and 62.27 mg L^{-1} (half of LC_{50}) indicated a decreasing trend of population density during the trial period (Figs. 1, 2). The growth rate of rotifer population significantly decreased in concentrations of 0.01, 0.21 and 0.53 mg L^{-1} deltamethrin, and 12.45, 24.91 and 62.27 mg L^{-1} of imidacloprid in 10 days (Figs. 3,4).

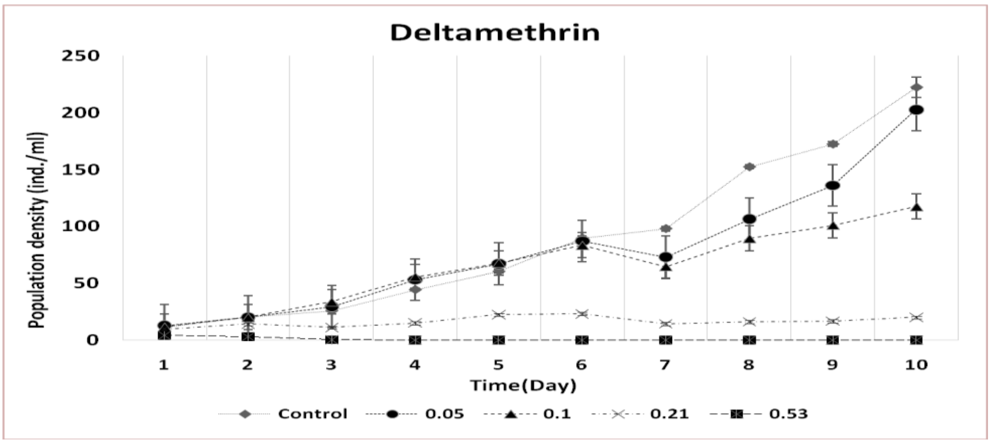

Figure 1: Population density of *Brachionus calyciflorus* exposed to five concentrations of deltamethrin (0.00 (control), 0.05, 0.1, 0.21 and 0.53 mg L^{-1}) at 10 days. The values are mean±standard error and shown based on three replicate recordings.

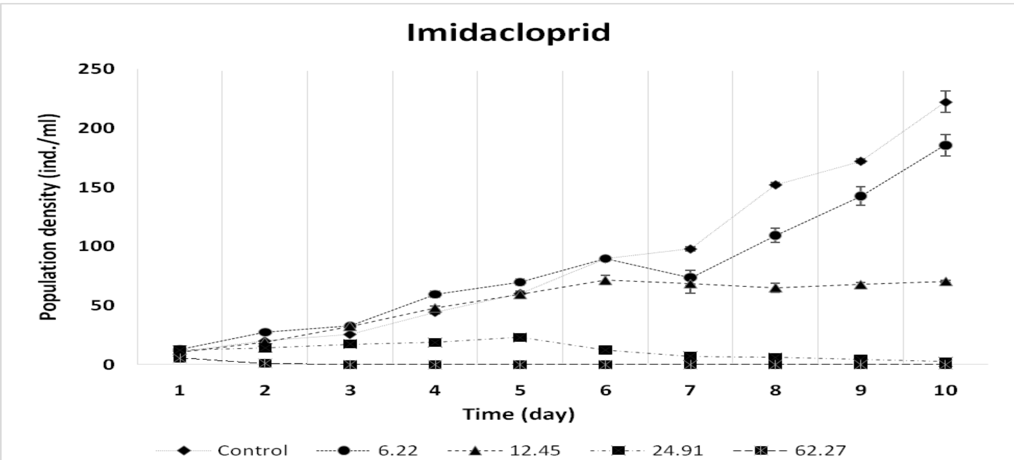

Figure 2: Population density of *Brachionus calyciflorus* exposed to five concentrations of imidacloprid (0.00 (control), 6.22, 12.45, 24.91 and 62.27 mg L^{-1}) at 10 days. The values are mean±standard error and shown based on three replicate recordings.

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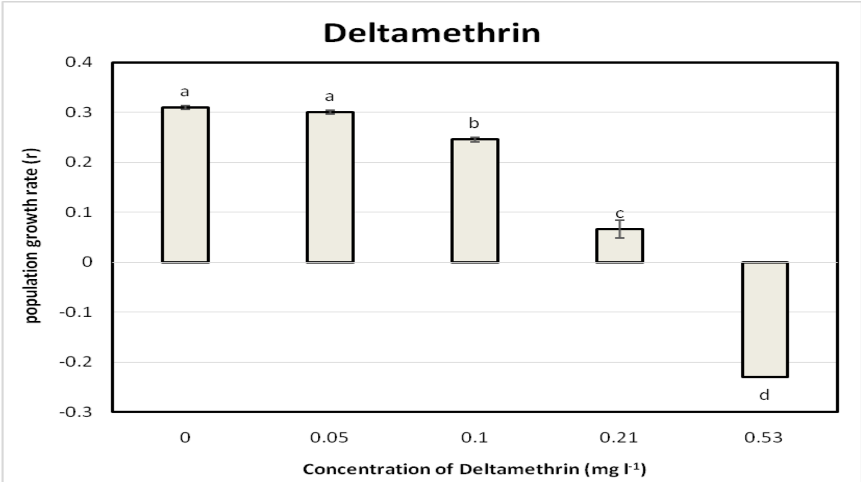

Figure 3: Population growth rate of *Brachionus calyciflorus* exposed to five concentrations of deltamethrin (0.00 (control), 0.05, 0.1, 0.21 and 0.53 mg L^{-1}) at 10 days. The values are mean±standard error and shown based on three replicate recordings.

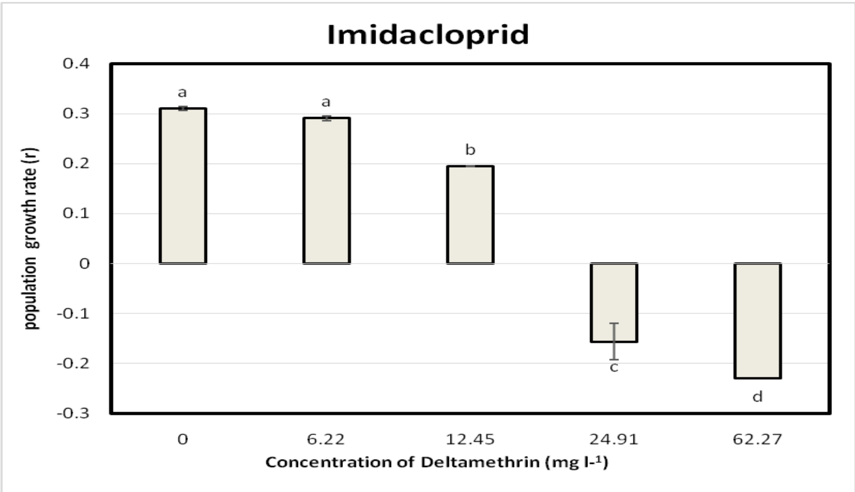

Figure 4: Population growth rate of *Brachionus calyciflorus* exposed to five concentrations of imidacloprid (0.00 (control), 6.22, 12.45, 24.91 and 62.27 mg L^{-1}) at 10 days. The values are mean±standard error and shown based on three replicate recordings.

Discussion

Results of our research clearly indicated that *B. calyciflorus* has more sensivity to deltamethrin in comparison to imidacloprid. This result might be related to the rapid effect of deltamethrin on the nervous system of target organisms. Deltamethrin at sublethal concentrations had no effect on duration of embryonic stage of rotifer but it prolongs the juvenile stage at 0.06 and 1.2 mg L^{-1} (Xi and Hu, 2003). The effect of acute toxicity of deltamethrin studied on *Daphnia magna*, showed that the 24 and 48 hours LC_{50} for this toxicant were 0.11 and 0.037 mg L^{-1}, respectively (Toumi *et al.*, 2015). In another study, deltamethrin caused 50% mortality in Mysid shrimp, *Mysidopsis bahia*, population after 96h at 0.0017 ppb (EPA, 2002). Based on the report of Oros and Werner (Oros and Werner, 2005), it seems that fish embryos are more resistant than larvae against pyrethroids. The 48-hour lethal concentration of deltamethrin for Common carp embryos and larvae has been reported as 0.21 and 0.74 ppb, respectively (Köprücü and Aydin, 2004). The imidacloprid toxicity to aquatic invertebrates is very diverse and Daphnia is one of most resistant invertebrates to this insecticide (Jemec *et al.,* 2007). It means that toxicity of imidacloprid is largely depended on the species and the results could not be generalized.

Roex *et al*. (2000) reported that in high toxin stresses, the density may increase at first, but then the growth rate could decline significantly. In our research, when the population growth rates in all treatment groups were compared with the control group, it showed that this index decreased significantly (p<0.05) in deltamethrin at concentrations higher than 0.05 mg L^{-1} and imidacloprid at concentrations higher than 6.22 mg L^{-1}.

Rao and Sarma (1986) and Janssen *et al.* (1994) found that DDT at 30.0 µg L^{-1} and lindane at concentrations from 15.0 to 20.0 mg L^{-1} decreased significantly the population growth rate of *B. patulus* and *B. calyciflorus*, respectively. Xi *et al.* (2007) reported that DDT at 0.64 mg L^{-1} concentration significantly decreased the population growth rate of *B. calyciflorus*. Toxicants at low concentrations could not cause the death of phytoplanktons because in general phytoplanktons are more resistant than zooplanktons (Kerrison *et al.*, 1988), but at high concentrations they may kill algal cells. Due to the fact that dead Chlorella has much less effect than live Chlorella on the rotifer population growth, at least *Brachionus* sp. (Sarma *et al.*, 2001), the reduction in the population growth rate of *B. calyciflorus* under toxic stress could be the result of a reduced swimming speed, egg production and food uptake because filtration and ingestion rates of *B. calyciflorus* after exposure to sub-lethal levels of imidacloprid and deltamethrin (Halbach, 1984; Fernandez-Casalderry *et al.*, 1992).

Based on the reproductive strategy of rotifer in favorable environmental conditions is asexual reproduction that makes resting egg production and sexual reproduction can be physiologically more complex, energy consuming and difficult. But with deterioration of the conditions, rotifer tends to undergo sexual reproduction in order to be able to maintain its genetic resources. By comparing the results of population growth and mic/amic ratio, we found that in concentrations of 0.05 and 0.10 mg L^{-1} of deltamethrin and concentrations of 6.22 and 12.45 mg L^{-1} of imidacloprid, the resting egg production and mic/Amic proportion increased significantly in the middle of the trial period (Figs. 5, 6), while we had seen that the growth rate was increasing before that. But at concentrations of 0.21 mg L^{-1} of deltamethrin and 24.91 mg L^{-1} of imidacloprid that are $\frac{1}{5}$ of LC50 concentrations, the sexual reproduction and resting egg production occurred from the beginning of experiment. Xi *et al.* (2007) found DDT at 0.0025 and 0.01 mg L^{-1} increased significantly the mic/amic ratios in *B. calyciflorus*. On

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ninth day, mic/amic ratio in control treatment was significantly over the other treatments ($p<0.05$), that indicating the dominance of sexual reproduction in rotifer life cycle because of the increase in population density of *B. calyciflorus* and the results stated here supported the general hypothesis that repression of sexual reproduction was a general response to environmental stresses of many types (Snell and Boyer, 1988).

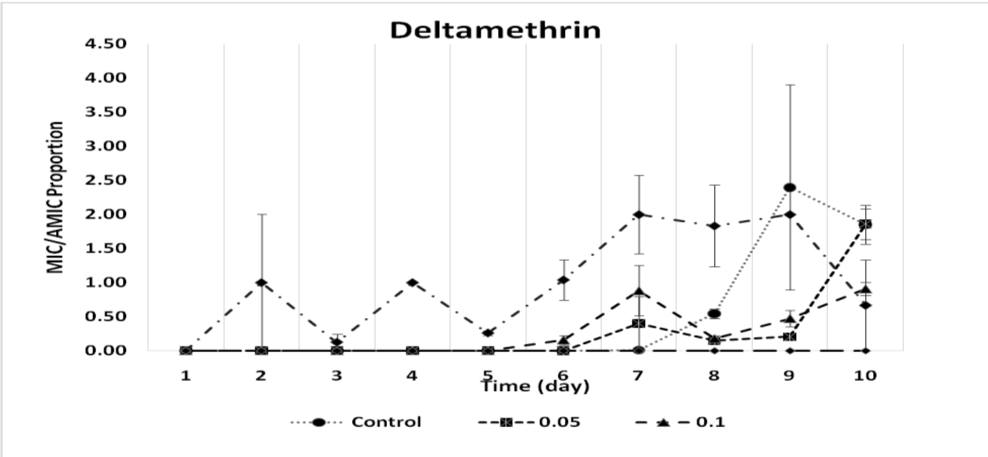

Figure 5: Effect of five concentrations of deltamethrin (0.00 (control), 0.05, 0.1, 0.21 and 0.53 mg L^{-1}) on mic/amic proportion of *Brachionus calyciflorus* during 10 days. The values are mean±standard error and shown based on three replicate recordings.

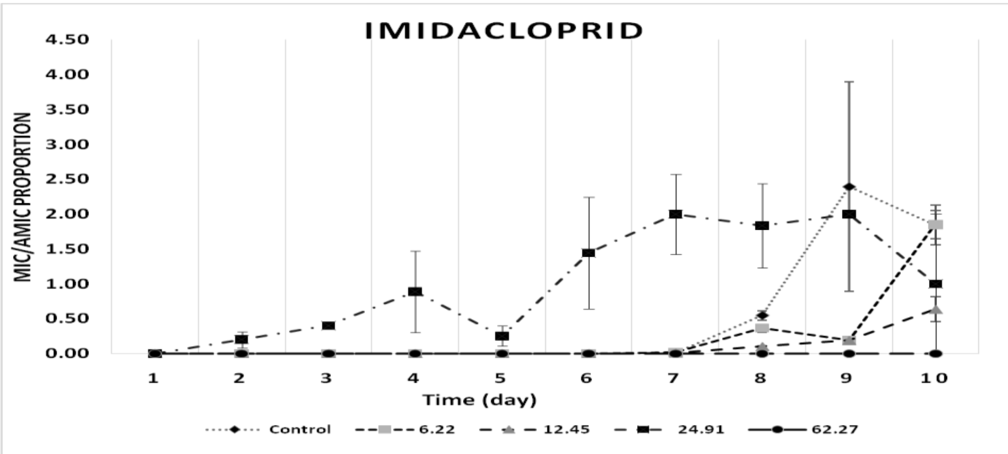

Figure 6: Effect of five concentrations of imidacloprid (0.00 (control), 6.22, 12.45, 24.91 and 62.27 mg L^{-1}) on mic/amic proportion of *Brachionus calyciflorus* during 10 days. The values are mean±standard error and shown based on three replicate recordings.

The results showed that deltamethrin at 0.21 mg L^{-1} concentration on the eight day and imidacloprid at 24.91 mg L^{-1} concentration on the third and eight days increased OF/NOF ratio (Figs. 7, 8). According to the results, it can be explained that the effect of pollutants on OF/NOF extremely depends on pesticide type. In 0.21 mg L^{-1} treatment of deltamethrin from second to ninth day of the test, the OF/NOF ratio was significantly higher than the other treatments and control group ($p<0.05$) (Figs. 7, 8). Xi *et al.* (2007) reported that lindan at 7 mg L^{-1} concentration increased the OF/NOF proportion on the third day but DDT at concentrations higher than 0.24 mg L^{-1}, dicophol at 1.2 mg L^{-1} and Andosulfan at 7 mg L^{-1} in third day decreased this proportion. It might explain the effects of pollutants on the OF/NOF ratio in a rotifer population.

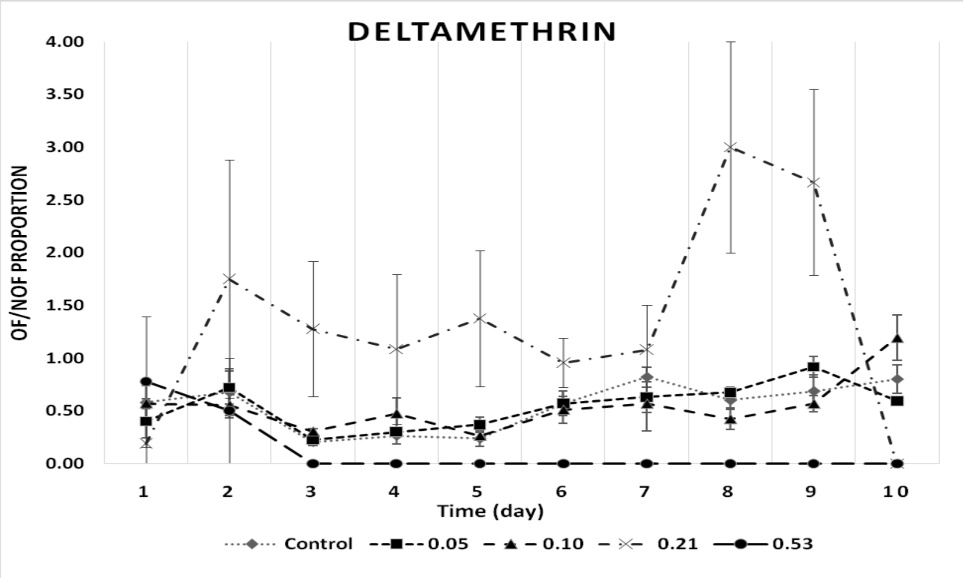

Figure 7: Effect of five concentrations of deltamethrin (0.00 (control), 0.05, 0.1, 0.21 and 0.53 mg L^{-1}) on OF/NOF proportion of *Brachionus calyciflorus* during 10 days. The values are mean±standard error and shown based on three replicate recordings.

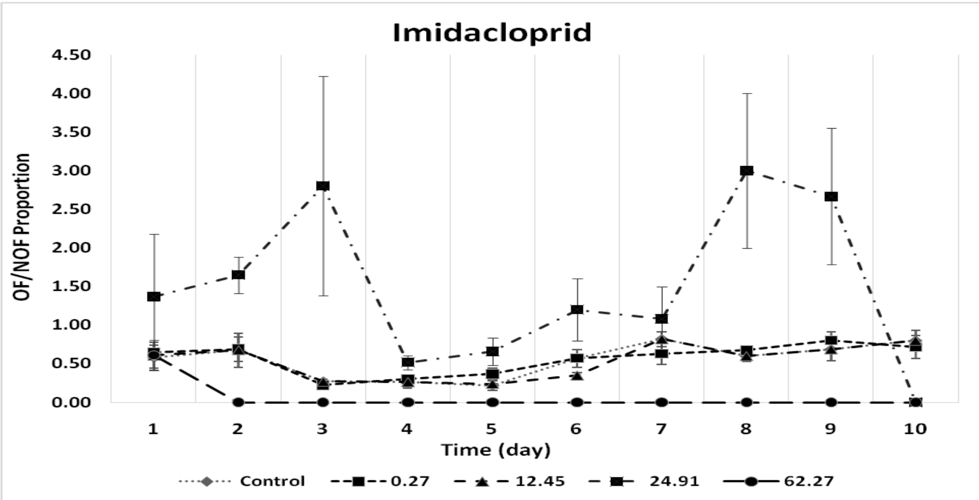

Figure 8: Effect of five concentrations of imidacloprid (0.00 (control), 6.22, 12.45, 24.91 and 62.27 mg L^{-1}) on OF/NOF proportion of *Brachionus calyciflorus* during 10 days. The values are mean ± standard error and shown based on three replicate recordings.

Our results demonstrated that *B. calyciflorus* is a more resistant species to imidacloprid than to deltamethrin pesticide. Since, rotifers are very important in freshwater and coastal marine environments as the main food of many invertebrates and larval period of fish, they can play the role of toxicant carrier in chain food. As these pollutants accumulate in the environment, a higher amount of them will accumulate into the fish body.

Therefore, we hope that the findings of the current study can be helpful for further studies in aquatic environments s, aquaculture and fisheries sciences. Moreover, these studies could contribute to create a network for the production of new supplements.

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