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Original Article

Toxicity of fipronil and atrazine on *Metapenaeus affinis* (Milne-Edwards, 1837) and their effects on oxygen consumption

Rajaa N. Al-Yassein*

Department of Fisheries and Marine Resources, College of Agriculture, University of Basrah, Iraq.

Abstract: Increasing the use of pesticides has led to declines in crustaceans in aquatic systems. In this study, the effects of acute doses of fipronil insecticides and atrazine herbicides on the toxicity in sublethal concentrations and their effect on oxygen consumption of adult *Metapenaeus affinis* at 20±1°C and 4 psu salinity were investigated. Series of fipronil (0.05-2 $\mu g.l^{-1}$) and atrazine (625-2000 $\mu g.l^{-1}$) lethal concentrations were used. Fipronil showed high toxicity to adult shrimp *M. affinis* compared to the atrazine. The median lethal concentration (LC_{50}) for 96h of fipronil and atrazine were 0.47 and 8280.02 $\mu g.l^{-1}$, respectively. A decrease in the rate of oxygen consumption with increasing sublethal concentrations after 24h was observed in fipronil and atrazine exposed shrimps. A significant difference in oxygen consumption was found between the control and the experimental treatments. The oxygen consumption of high concentration of fipronil 0.2 $\mu g.l^{-1}$and high concentration of atrazine 6000 $\mu g.l^{-1}$were 0.190 and 0.373 $\mu g.l^{-1}$/O2/gm/h, respectively, compared to control one (0.540 $\mu g.l^{-1}$/O2/gm/h).

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Introduction

Human activities such as agricultural operations, industrial effluents, and increased urbanization posed a serious threat to the freshwater ecosystem (Meijide et al., 2018). Pesticides are extensively used to control and decrease unwanted plants and animals (Choung et al., 2013). They can enter aquatic environments and cause negative effects on non-target species (Bradley et al., 2017). Fipronil is a phenylpyrazole insecticide used to control insect pests in agricultural and residential environments (Gunasekara et al., 2007).

The mode of action in fipronil is different from other insecticides like organophosphates, carbamates, and several pyrethroids, all traditional insecticides, to which many insects have evolved resistance. (Cole et al., 1993). Fipronil has adverse effects on aquatic organisms with high acute and chronic toxicity and the ability to accumulate (Ngim and Crosby, 2001). Its environmental fate is unusual due to desulfinyl derivatives, which are two times more toxic to aquatic invertebrates (Schlenk et al., 2001). Fipronil has a low to moderate water solubility, favors lipophilic (organic) matrices, and is stable at ambient temperatures (Aajoud et al., 2003).

Atrazine is a common triazine herbicide used worldwide, mostly in developing countries (Roustan et al., 2014). Although atrazine has been banned for many years, it remains one of the most frequent herbicides with extreme persistence in water bodies (Jablonowski et al., 2011). Therefore, it has become one of the main concerns of aquatic life (Solomon et al., 2008). Due to high levels in soil and widespread application, atrazine has been found in various environmental samples, like ground and surface water, at levels substantially over the legal limits (Tappe et al., 2002). Therefore, it became one of the main concerns for aquatic life (Solomon al., 2008). In most environments, atrazine concentrations have been recorded as one $\mu g.l^{-1}$ or lower (de Albuquerque et al., 2020).

Crustaceans serve an important role in the coastal ecosystem, having high economic importance

*Correspondence: Rajaa N. Al-Yassein
E-mail: rajaa.alyassein@uobasrah.edu.iq
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(Mehanna et al., 2012). *Metapenaeus affinis* inhabits the northern part of the Persian Gulf and migrates up the estuary of Shatt Al-Arab to the Iraqi inland waters (Salman et al., 1990). It is the most abundant commercial shrimp on the Iraqi coast (Saoud et al., 1993). Shrimp are commonly used in toxicity experiments to assess the potential hazards of poisonous chemicals on different aquatic organisms (Key et al., 2003). Therefore, this work aimed to study the toxicity of two pesticides of, fipronil and atrazine, as sublethal concentrations and their effect on oxygen consumption in *M. affinis.*

Material and Methods

Collection and Maintenance: Adult *M. affinis* was collected from aquariums in the marine science station, University of Basrah, Iraq. Under laboratory settings of 20±1°C and 4 PSU salinity, the shrimps were acclimated in 10-gallon aquaria using a 14h light: 12h dark photoperiod. The weight of the specimens was 7.8-10.5 g, with a mean length of 5.4-8.2 cm. The shrimps were acclimated for ten days before experiments. Throughout the acclimation period, water quality parameters such as dissolved oxygen (Oxygen Meter DO-5509), salinity (American Optics Refractometer), temperature, and pH) were monitored daily. Shrimp were fed daily during the acclimation period, and their feeding was stopped during acute toxicity tests (Buikema et al., 1980).

Stock solution preparation: One gram of highly purified fipronil (98% purity) was dissolved in 1000 ml of pesticide grade acetone 98.0%. A similar procedure was used for atrazine. A diluted daily stock in deionized water was prepared for toxicity tests.

Acute toxicity tests: The dilute fipronil daily stock solution was used to prepare fipronil exposure concentrations of 2, 1, 0.40, 0.20, 0.10 and 0.05 $\mu g.l^{-1}$and atrazine exposure concentrations of 20000, 10000, 5000, 2500, 1250 and 625 $\mu g.l^{-1}$. Each beaker (1L) was wrapped in acetone-rinsed tin foil to eliminate any contamination and reduce evaporation before use (Konwick et al., 2005). Each beaker was stocked with five shrimps and each treatment with three replicates of fipronil and atrazine concentration and controls without any pesticide. Water changes were done every 24 hours. Toxicity studies were performed at regular conditions of 20±1°C, 4 PSU salinity, pH=7.5-7.8, and DO >7.2 $mg.l^{-1}$. Cumulative mortality of the animals was recorded for each dose/replicate.

Oxygen consumption measurement: Adult *M. affinis* were exposed to fipronil or atrazine and their oxygen consumption in treatments and control groups was measured after 24h. Shrimps were placed in an oxygen consumption detection flask filled with the test concentrations of pesticide. At intervals of 30, 60, 90, 120, and 180 minutes, the oxygen consumption rate was determined as mgO2/g/h based on Chinni et al. (2000).

Statistical analysis: The probit method was used to determine the LC_{50} values with 95% confidence limits (CI) and the used concentrations to estimate the 24-96h LC_{50} concentrations (Razzaghi, 2013). Also, data were analyzed using SPSS (22.0 version) for mean and standard deviation and evaluating the regression coefficient at a 5% level of significance.

Results

In acute toxicity tests, increasing concentrations of fipronil and atrazine showed rising mortality in adult *M. affinis*. At 96h of exposure to fipronil, the mortality of 0.05, 0.10, 0.20, 0.40, 1 and 2 $\mu g.l^{-1}$ treatments were 10, 20, 40, 70, 100, and 100%, respectively (Fig. 1). In the atrazine treatments of 625, 1250, 2500, 500, 10000 and 20000 $\mu g.l^{-1}$, the mortality were 5, 10, 20, 45, 70, and 100%, respectively (Fig. 2). Based on the results, fipronil was more toxic than atrazine. The LC_{50} values of fipronil at 24, 48, 72, and 96 h were calculated as 0.96, 0.68, 0.47 and 0.47 $\mu g.l^{-1}$, respectively (Table 1). Atrazine LC_{50} were 20432.31, 12913.22, 8280.02 and 8280.02 $\mu g.l^{-1}$, in the 0.05, 0.10, 0.20, 0.40, 1 and 2 $\mu g.l^{-1}$ treatments, respectively (Table 2).

A significant reduction in oxygen ratio consumption was observed in exposure shrimps to sublethal concentrations of fipronil and atrazine. There was a significant difference in oxygen consumption (P<0.01) in *M. affinis* at all treatments.

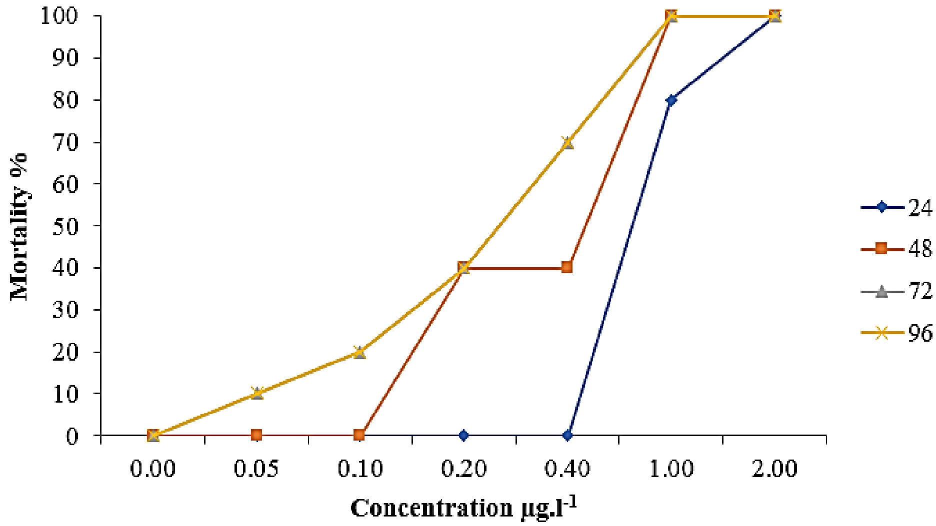

Figure 1. Adult *Metapenaeus affinis* mortality in each fipronil concentration (µg.l^{-1}) after 24- 96h of exposure.

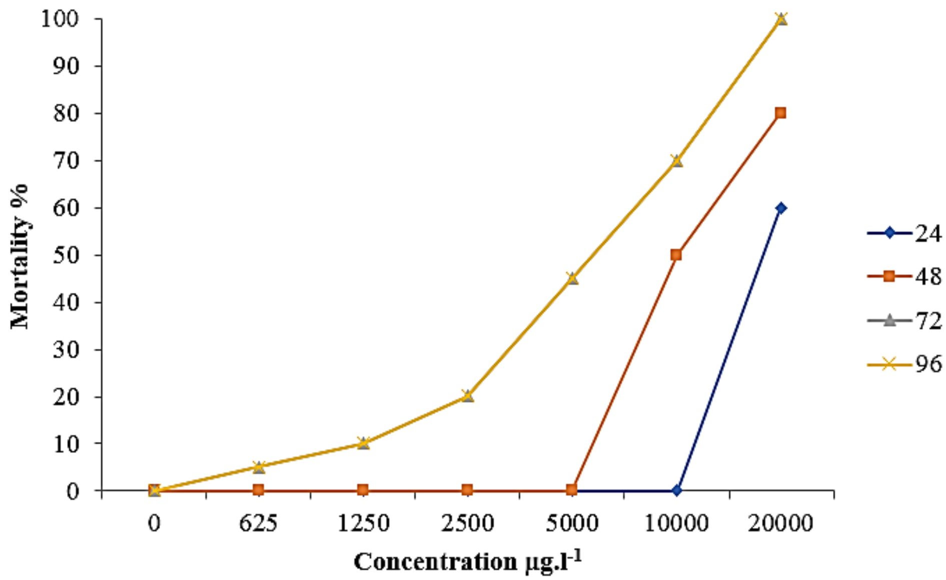

Figure 2. Adult *Metapenaeus affinis* mortality in each atrazine concentration (µg.l^{-1}) after 24- 96h of exposure.

Oxygen consumption rates in the shrimps with sublethal treatments of fipronil were 0.330, 0.250 and 0.190 µg.l^{-1} /O2/gm/h, respectively, and in the sublethal atrazine treatments were 0.445, 0.441 and 0.373 µg.l^{-1}/O2/gm/h (Figs. 3, 4). That of the control group was 0.540 µg.l^{-1}/O2/gm/h.

Discussion

The mortality ratio of *M. affinis* exposed to various concentrations of fipronil and atrazine raised as concentration increased. Based on the results, fipronil was more toxic to the *M. affinis* than atrazine in 96h LC_{50}. Each toxic chemical has a different effect related to specific mechanisms of its action (Barbieri et al., 2013). The toxicity of fipronil to the adult grass shrimp, *Palaemonetes pugio* was 96h LC_{50} (Key et al., 2003). After 72 hours of exposure, fipronil was found highly toxic to *Daphnia magna* at concentrations of 0.1, 1, 10, and 100 µg.l^{-1} (Bownik and Szabelak, 2021). Chandler et al. (2004) found that fipronil is highly toxic to the adult copepod *Amphiascus tenuiremis* with LC_{50} of 6.8 mg.l^{-1} after 96 h. A decrease in survival of shrimp, *Penaeus monodon* by 24h mortality exposed to fipronil has also been reported (Hook et al., 2018). Montagna and Collins (2008) found that mortality increased with increasing

Table 1. Logarithm of fipronil LC_{50} (µg.l^{-1}) values, Standard error and 95%Confidence limits (Cl) to the adult *Metapenaeus affinis* at 24, 48, 72 and 96h of exposure.

Time (h)	LC_{50} (µg.l^{-1})	Std Error	95% Confidence limits
24	0.96	1.09	6.042-10.315
48	0.68	0.272	3.016-4.038
72	0.47	1.76	2.199-2.88
96	0.47	1.76	2.199-2.88

Table 2. Logarithm of atrazine LC_{50} (µg.l^{-1}) values, Standard error and 95%Confidence limits (Cl) to the adult *Metapenaeus affinis* at 24, 48, 72 and 96h of exposure.

Time (h)	LC_{50} (µg.l^{-1})	Std Error	95% Confidence limits
24	20432.31	0.581	6.085-5.361
48	12913.22	0.425	3.772-5.437
72	8280.02	0.157	2.008-2.662
96	8280.02	0.157	2.008-2.162

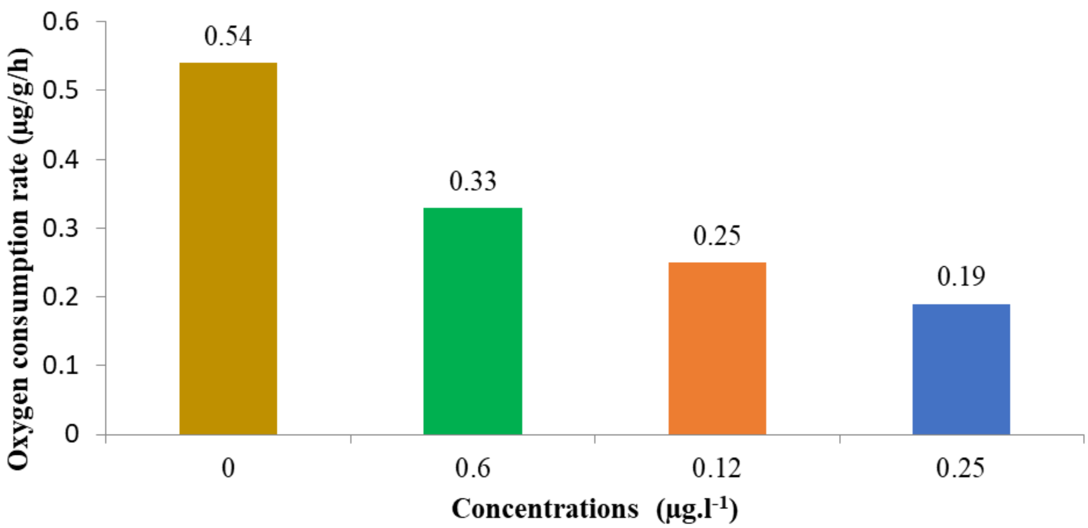

Figure 3. Oxygen consumption rate in *Metapenaeus affinis* at different concentrations of fipronil.

concentration of organophosphate chlorpyrifos and endosulfan insecticides in freshwater crab, *Trichodactylus borellianus* in 24h of exposure.

Atrazine was less toxic to the *M. affinis* as reported by Key et al. (2007) when exposed to *P. pugio*, with an LC_{50} 96h of 9000 µg.l^{-1}. Atrazine has been recorded to be moderate to severely toxic to aquatic species e.g. its toxicity values for atrazine in freshwater shrimp, *Paratya australiensis* with 96h LC_{50} value was 6500 to 9900 µg.l^{-1} (Phyu et al., 2005). However, values of LC_{50} have been reported at 6100 and 4900 µg.l^{-1}, respectively for mussels *Perna viridis* and *Paphia malabarica* (Iqbal and Navalgund, 2021). Atrazine was reported to be less toxic compared to three other insecticides (carbofuran, dichlorvos, and malathion) in copepodids, *Tigriopus brevicornis* with LC_{50} value of 153.2 µg/ l^{-1} (Forget et al., 1998). There was no mortality in the marine copepod *T. japonicus* in response to 20000 µg. l^{-1} of atrazine (Yoon et al., 2019). Measurement of oxygen consumption is an important indicator of sublethal stress levels for predicting chemical substance risks (Ansari et al., 2010). According to Barbieri et al. (2013), the presence of pollutants in the ecosystem is the main cause of a decline in the regular amount of oxygen and impairment of the crustacean respiratory system. In many crustaceans, oxygen consumption is reduced because of a greater number of pesticides being

Table 3. Measured concentrations (mean±standard error), Confidence limits (Cl) with F- value of fipronil and atrazine in sublethal test concentrations solutions of *M. affinis*.

Pesticides Cons ($\mu g.l^{-1}$)	Mean±Std	95% confidence limits (CL)	F-value
	Fipronil		
Control	0.5400±0.001	(0.5375-0.5425)	70075.000
0.6	0.3300±0.001	(0.3275-0.3325)	
0.12	0.2500±0.001	(0.2475-0.2525)	
0.25	0.1900±0.001	(0.1875-0.1925)	
	Atrazine		
Control	0.5400±0.001	(0.5375-0.5425)	15446.750
1500	0.4500 ±0.001	(0.4475-0.4525)	
3000	0.4100±0.001	(0.4075-0.4125)	
6000	0.3730±0.001	(0.3705-0.3755)	

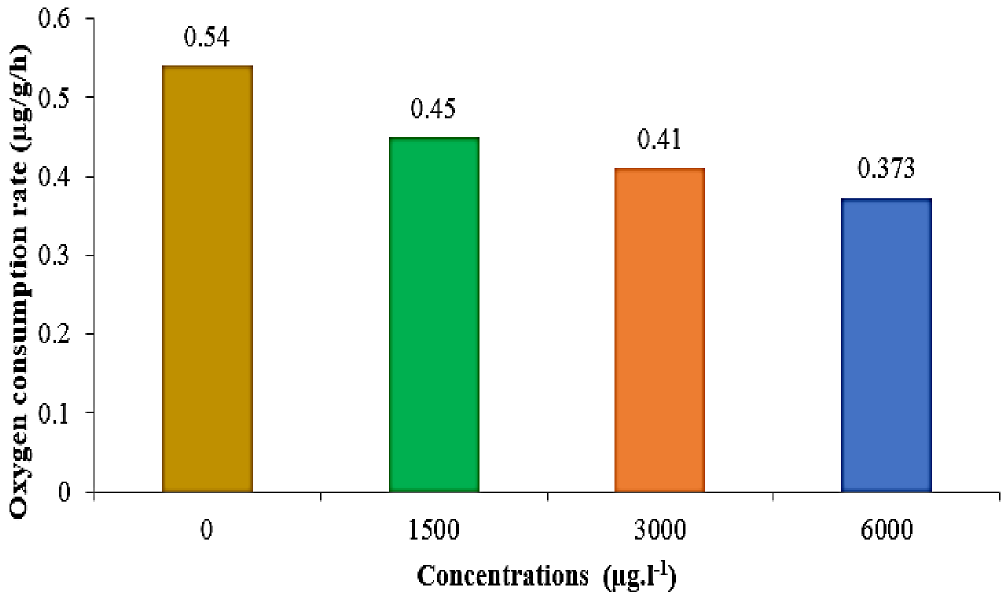

Figure 4. Oxygen consumption rate in *Metapenaeus affinis* at different concentrations of atrazine.

absorbed through the gills, which are vulnerable and more susceptible to damage by pollutants (Montagna and Collins, 2008).

The current study revealed that increasing the concentration and exposure time of fipronil and atrazine decreases the amount of oxygen consumption. Oxygen consumption in the highest concentrations of fipronil and atrazine (2 $\mu g.l^{-1}$; 95% CI, 0.1875-0.1925 $\mu g.l^{-1}$ and 6000 $\mu g.l^{-1}$; 95% CI, 0.3705-0.3755) were 0.190 and 0.373 $\mu g.l^{-1}$/O2/gm/h, respectively. Dillon (1983) recorded a decline in the rate of oxygen consumption in *P. pugio* exposed to dimethyl-naphthalene concentrations of 0.24 $\mu g.l^{-1}$ at 18-22°C. A decrease in oxygen consumption has also been reported in *T. borellianus* exposed to chlorpyrifos and endosulfan (Montagna and Collins, 2008). In contrast, there was an increase in the respiratory rate of *M. affinis*, up to 44.4% when exposed to different concentrations of naphthalene (Ansari et al., 2010). The reduction in the oxygen consumption rate of post-larvae *Penaeus indicus* after 24h at 29±1°C during exposure to different concentrations of lead has been reported by Chinni et al. (2000). In another study, inhibition in the oxygen consumption rate of 52.63% was proved in Penaeid shrimps, *Xiphopenaeus kroyeri* at 20°C when exposed to Cadmium and Zinc (Barbieri et al., 2013).

Conclusion

The results indicated that mortality increases with increasing fipronil and atrazine concentrations. Fipronil was more toxic to adult shrimp *M. affinis* compared to atrazine, with LC_{50} 96h of fipronil and atrazine were 0.47 and 8280.02 μg. l^{-1}, respectively. Oxygen consumption rate decrease with increasing sublethal concentrations in shrimp exposed to fipronil or atrazine.

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