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Acute toxicity of insecticide, Diazinon and fungicide, Tilt (Propiconazole) on Pacific white Shrimp, *Litopenaeus vannamei* postlarvae and *Palaemon adspersus* juveniles

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ABSTRACT:

Acute toxicity of Diazinon and Tilt (Propiconazole) agricultural toxins was studied on *Litopenaeus vannamei* postlarvae and *Palaemon adspersus* juveniles, aiming to determine the 50% lethal concentration (LC_{50}) in 96 h time duration. The experiments were conducted at static condition and standard method in four days. Physical and chemical factors were controlled through the experiment so that the amount of dissolved oxygen was fixed on 8 mg/L, temperature: 25 ± 1 ^{0}C, pH: 7.5 to 8 and salinity: 31 ppt. The results showed that the mean LC50 values of Diazinon at 24, 48, 72 and 96 h were 0.298, 0.255, 0.237, 0.226 mg/L, and the mean LC50 values of Tilt at 24, 48, 72 and 96 h were 9.021, 4.227, 4.032, 3.635 mg/L, to the *Litopenaeus vannamei* postlarvae. Also, the results showed that the mean LC50 values of Diazinon at 24, 48, 72 and 96 h were 0.391, 0.330, 0.294, 0.277 mg/L, and the mean LC50 values of Tilt at 24, 48, 72 and 96 h were 0.789, 0.763, 0.674, 0.611 mg/L, to the *Palaemon adspersus* juveniles.

Keywords:
Acute toxicity, Diazinon, Tilt, *Litopenaeus vannamei, Palaemon adspersus.*

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INTRODUCTION

White shrimp *Litopenaeus vannamei* (Boone) is distributed throughout the Pacific coast from the Gulf of California to northern Peru. It is the major species of penaeid shrimp in the east hemisphere and contributes 30% of farmed production of penaeid shrimp in the world (Pe´rez Farfante and Kensley, 1997). This species inhabits wide ranges of salinity, including brackish water of 1–2 ppt and saline water of 40 ppt (Menz and Blake, 1980). Gomishan region in Golestan province is one of the main areas for culturing the *Litopenaeus vannamei* in Iran. Distribution areas of *Palaemon adspersus*, Rathke include the North Sea, Baltic Sea, Eastern Atlantic, Mediterranean, and Black Sea (Udekem d'Acoz, 1999; Janas et al., 2004). *Palaemon adspersus* inhabits mainly Zostera covered bottoms only (Berglund, 1980; Baden and Pihl, 1984). Also, this prawn exist in Caspian sea and Gomishan lagoon, Iran. Pesticide use causes serious environmental problems, especially in the dry season when the dilution capacity of the water systems is low, increasing the risk of high concentrations of toxic chemicals. Moreover, the dry season is often the critical period for many animals, especially aquatic animals such as: fish and shrimp (Adedeji et al., 2009). Pesticide usage all over the world has increased dramatically during the past few decades, coinciding with changes in farming and intensive agriculture practices. Hence the environmental pollution caused by pesticides, especially in aquatic ecosystems, has become a grave problem (Chilke, 2012). Direct or indirect contamination of water by pesticides can lead to fish and shrimp deaths, reduced productivity or elevated concentrations of undesirable chemicals in edible fish tissue, which can affect the health of humans eating these aquatic animals (Adedeji et al., 2000). The organophosphate insecticide Diazinon (*O,O*-diethyl *O*-[6-methyl-2-(1-methylethyl)- 4-pyrimidinyl] phosphorothioate) has agricultural and commercial uses and it is used to control a variety of insects, primarily aphids, beetles, scales and pill bugs, in the household environment (Cox, 1992). Trade names for Diazinon include Knox-out, Dianon and Basudin (EPA**,** 2004). Tilt, with the chemical name of Propiconazol, is a systemic fungicide, which in high usage is against various kinds of rice diseases, such as stem rottenness and etc (Mohammad Nejad Shamoushaki, 2005). Golestan province is a great pole of agriculture in the north of Iran and above 1.5 millions hectares of agricultural fields in areas are specified to cultivate various kinds of farming products and dry farming. Thus different kinds of chemical fertilizers and vegetable pesticides are highly in use in Golestan. From a total degree of around 35000 metric tons of vegetable pesticides, which are distributed in the Iran, around 25000 metric tons of agricultural toxins are used by farmers in agricultural fields of Golestan province (Mohammad Nejad Shamoushaki, 2005). Toxicology of environmental parameters on *Litopenaeus vannamei* mainly focused on ammonia, nitrite, and some heavy metal ions (Li et al, 2008), while there is no report on the toxicology of Diazinon and Tilt to this species and *Palaemon adspersus*. As Gomishan area in Golestan province is only reproducing western white shrimp in the northern part of Iran, the acute toxicity pesticides Diazinon and Tilt, which is used a lot in Golestan district, on the *Litopenaeus vannamei* poslarvae and *Palaemon adspersus* juvenile were studied.

MATERIALS AND METHODS

Shrimp and chemical supply

To determine the acute toxicity of Diazinon and Tilt from *Litopenaeus vannamei* postlarvae and *Palaemon adsperus* from *Litopenaeus vannamei* postlarvae and *Palaemon adspersus* of Gomishan shrimp brooding and culture center in Golestan province (north of Iran). In several processes some of these postlarvae (PL=20) and *palaemon adspersus* (1-2 gr) have been transferred to tanks to adapt to the new condition for 5-7 days. The experiments were carried out in 20 lit

aquariums (10 shrimps for each aquarium) with static condition based on O.E.C.D method (TRC, 1984) with five treatments and one blank with three repetitions. Physical and chemical factors were controlled through the experiment. Dissolved oxygen was fixed on 8 mg/L, temperature: 25 ± 1 ^{0}C, pH: 7.5 to 8 and salinity: 31 ppt.

Acute toxicity tests

The first experiment was conducted to determine the effects of acute toxicity (LC50 in 96 h) of Diazinon (60 EM) and Tilt in two groups (*Litopenaeus vannamei* postlarvae and *Palaemon adspersus* juvenile). For this purpose, five treatments and one blank were used to test toxicity; each treatment had three replications and 10 shrimps per tank with 20 litres water capacity. Mortality records were taken every 24 h (24, 48, 72, 96 h). Movements and behaviors of the shrimps were investigated at the time of experiments. Finally, after early experiments, Diazinon concentration on *Litopenaeus vannamei* postlarvae was determined to be 0.15 - 0.3 mg/L and 2 - 8 mg/L, concentration of Tilt can affect on *Litopenaeus vannamei* postlarvae. Then, based on this experiments LC_{10}, LC_{50} and LC_{90} in 24, 48, 72 and 96 h were measured. Also, early experiments showed 0.2 - 0.4 mg/L concentration of Diazinon and 0.4 - 1 mg/L concentration of Tilt can affect *Palaemon adspersus* juvenile. Then LC_{10}, LC_{50} and LC_{90} in 24, 48, 72 and 96 h of Diazinon and Tilt were measured for *Palaemon adspersus* juvenile.

Statistical Analysis

After obtaining the final results, the information was analysed statistically by probit program version 1.5 (USEPA, 1985) and mortality was assessed at 24, 48, 72, and 96 h after the early and dead shrimps removal. Finally, LC_{10}, LC_{50} and LC_{90} values at 24 48, 72 and 96 h, the maximum allowable concentration (MAC) value (LC50 in 96 h divided by 10) (TRC, 1984), the degree of toxicity, mean of ineffective concentration (LOEC (Lowest Observed Effect Concentration)) (Finney, 1971), of Diazinon and Tilt to *Litopenaeus vannamei* poslarvae and *Palaemon adspersus* juvenile were determined.

RESULTS AND DISCUSSION

The results showed that the mean LC50 values of Diazinon at 24, 48, 72 and 96 h were 0.298, 0.255, 0.237, 0.226 mg/L, and the mean LC50 values of Tilt at 24, 48, 72 and 96 h were 9.021, 4.227, 4.032, 3.635 mg/L, to the *Litopenaeus vannamei* postlarvae **(Table.1 and table. 2).** Also, MAC value of Diazinon and Tilt were determined to be 0.0226 and 0.3635 mg/L to *Litopenaeus vannamei* postlarvae. The results showed that the mean LC50 values of Diazinon at 24, 48, 72 and 96 h were 0.391, 0.330, 0.294, 0.277 mg/L, and the mean LC50 values of Tilt at 24, 48, 72 and 96 h were 0.789, 0.763, 0.674, 0.611 mg/L, to the *Palaemon adspersus* juveniles **(Table.3 and table. 4).** Also, MAC value of Diazinon and Tilt were determined to be 0.0277 and 0.0611 mg/L to *Palaemon adspersus* juveniles, respectively. Also, LOEC (Lowest Observed Effect Concentration) which is called LC10 in 96 h for Diazinon and Tilt were determined 0.149 and 0.244 mg/L, respectively to *Litopenaeus vannamei* and to *Palaemon adspersus* were determined 0.212 and 0.377 mg/L, respectively. The results showed that how pesticides tested concentration increases, shrimps died in less time. In fact, for 24 hours the mortality of shrimp in the amount of toxin is needed

Table1. Acute toxicity of Diazinon in 96 h on *Litopenaeus vannamei* postlarvae

Concentration	24 h	48 h	72 h	96 h
LC10	0.2	0.176	0.158	0.149
LC50	0.298	0.255	0.237	0.226
LC90	0.443	0.370	0.360	0.342

Table2. Acute toxicity of Tilt in 96 h on *Litopenaeus nnamei* postlarvae

Concentration (mg/L)	24 h	48 h	72 h	96 h
LC10	3.162	1.448	1.375	1.244
LC50	9.021	4.227	4.032	3.635
LC90	25.730	12.340	11.825	10.621

Table3. Acute toxicity of Diazinon in 96 h on *Palaemon adspersus* juvenile

Concentration (mg/L)	24 h	48 h	72 h	96 h
LC10	0.275	0.243	0.214	0.212
LC50	0.391	0.330	0.294	0.277
LC90	0.557	0.447	0.404	0.362

Table4. Acute toxicity of Tilt in 96 h on *Palaemon adspersus* juvenile

Concentration (mg/L)	24h	48h	72h	96h
LC10	0.539	0.525	0.447	0.377
LC50	0.789	0.763	0.674	0.611
LC90	1.157	1.066	1.018	0.989

more than 96 hours. Also, according to **Table 5** (determination of toxicity in different pesticides) Diazinon has high toxicity and Tilt has medium toxicity to *Litopenaeus vannamei*. Also, Diazinon and Tilt have high toxicity to *Palaemon adspersus*. The result of Shrimp behaviors in different concentrations of toxin showed that both shrimps, in high concentration of Diazinon and Tilt, had fast swimming, permanently then got tired and died. While in low concentrations, during the first hours, there were no obvious reactions, then gradually faint. The main effect of concentrations of toxins was nervous system disorder, other external signs such as: imbalance, spiral swimming, and skin darkening were recorded to *Litopenaeus vannamei* postlarvae and *Palaemon adspersus* juvenile. So far no study has been reported on the effects of Diazinon and Tilt feature and other pollutants on *Litopenaeus vannamei* and *Palaemon adspersus* in Iran. This could be due to the recent entry of *Litopenaeus vannamei* into Iran and there are still many studies on this shrimp that can be made by researchers. But since there is little information on the toxic effects of agricultural toxins to crustaceans, further studies should be conducted to understand the toxic effect and mechanisms of agricultural toxins on crustaceans. As far as we know, agricaultural toxin such as Diazinon and Tilt can rapidly be accumulated in the water and reach toxic concentrations via several processes, such as agricultural chemicals, laundry products, irrigation drain water, mining and processing, and coal burning, so it presents a danger to aquatic organisms (Li et al, 2008). Toxicology of environmental parameters on *Litopenaeus vannamei* mainly focused on ammonia, nitrite, and some heavy metal ions were studied by Li et al, (2008), while there is no report on the toxicology of Diazinon and Tilt to *Palaemon adspersus*. The 96 h LC50 values of ammonia-N on *L. Vannamei* juveniles were 24.39 mg/L at 15ppt; 35.4 mg/L at 25ppt; 39.54 mg/L at 35ppt, respectively. The 96 h LC50 values of NH3-N (un-ionized ammonia as nitrogen) were 1.20 mg/L at 15ppt; 1.57 mg/L at 25ppt; 1.60 mg/L at 35ppt, respectively (Lin and Chen, 2001). The 96 h LC50 value of nitrite-N on *L. vannamei* juveniles was 76.5 mg/L at 15ppt, 178.3 mg/L at 25ppt, 321.7 mg/L at 35ppt (Lin and Chen, 2003). The 96 h LC50 values of boron were 25.05 mg/L at 3.0 ppt and 80.06 mg/L at 20.0ppt for *Litopenaeus vannamei* (Li et al, 2008). Acute toxicity of ozone-produced oxidants (OPO) to juvenile Pacific white shrimp, *Litopenaeus vannamei*, was assessed and found to be 0.50 mg/L (Schroeder et al, 2010). Also, the results of this study and its comparison to the literature finally showed that the range of sensitivity to agricultural toxins (e.g: Diazinon and Tilt), is more than that to the ammonia and nitrite toxicity. But as to the pesticide Diazinon there has been many studies on different species of fish. In other studies, 96 h LC50 value of Diazinon were determined for the following species: *Anguilla Anguilla*: 0.08 mg/L, Blue gill: 0.46 mg/L, Fathead minnows (*Pimephales promelas)*: 7.8 mg/L and zebra fish (*Brachydanio rerio*): 2.12 mg/L (Ansari et al.,

Table5. Determination of toxicity in different pesticides (Piri Zirkoohi and Orfog, 1997)

LC50 (mg/L)	Degree of toxicity
Up to 100	Nearly no poison
10-100	toxicity Low
1-10	toxicity Medium
0.1-1	toxicity High
Less to 0.1	toxicity Very high

1987). Also, 96 h LC50 value of Diazinon were determined for silver carp (*Hypophthalmichthys molitrix)* and *Abramis brama,* 1.9 mg/L and 8.1 mg/L, respectively (Nasri Tajan, 1996); *Acipenser persicus:* 4.38 mg/L (Pajand, 1999); *Acipenser nudiventris:* 4.6 mg/L (Khoshbavar-Rostami and Soltani, 2002); *Acipenser gueldenstadtii:* 6.09 mg/L (Soltani and Khoshbavar-Rostami, 2002); *Huso huso:* 4.99 mg/L (Khoshbavar-Rostami et al., 2004); *Acipenser stellatus:* 4.98 mg/L (Khoshbavar-Rostami et al., 2005); grass carp (*Ctenopharyngodon idell*a): 15.13 mg/L (Pourgholam et al., 2006); *Silurus glanis:* 4.142 mg/L (Kprücü et al., 2006); African catfish (*Clarias gariepinus*): 6.6 mg/L (Adedeji et al., 2009), *Bufo regularis:* 0.44 mg/L (Lawrence and Isioma, 2010). The results of this study and its comparison to the literature finally show that range of sensitivity to diazinon toxicant is, as shown below: *Anguilla anguilla* > *Litopenaeus vannamei* > *Palaemon adspersus*> *Bufo regularis* > blue gill> *Hypophthalmichthys molitrix* > *Brachydanio rerio* > *Silurus glanis*> *A. persicus* > *A nudiventris* >*A. stellatus* > *Huso huso*> *A. gueldenstaedtii* > *Clarias gariepinus* > *Pimephales promelas* > *Abramis brama* > *Ctenopharyngodon idell*a

Also, 96 h LC50 values of Tilt was determined as 3.9 mg/L at *A nudiventris* (Mohammad Nejad Shamoushaki, 2005).

CONCLUSION

The survey results indicated that the Diazinon and Tilt are very toxic to *Litopenaeus vannamei* and *Palaemon adspersus* and endangers their health. Therefore, the shrimp farms in the vicinity of the pesticide use are in danger. In fact, *L. vannamei* shrimp farms in the vicinity of the use of pesticides can cause harm to their health and affect the shrimp industry.

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