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A novel animal model for accumulated palytoxin bioassay in associated communities with zoanthids using Dara Index

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Abstract

Palytoxin (PTX) is a potent marine toxin that produced by zoanthids in coral islands of the Persian Gulf. The purpose of this study was to collect more information on *Zoanthus sansibaricus* toxicity as the dominant species of Hormuz Island and the consequences of exposure to PTX on associated communities with it. Hence, zoanthid colonies were collected during reef walk at low tide in April 2016. Also, among associated communities, snails were collected from the rock for experimental purpose as they are very abundant on and around Hormuz Island reef. In this model for each transect, 36 snails were divided into 4 set, each set had 3 replicates and working mucus solution, as PTX exist in the mucus of zoanthids, was injected in 2 different doses. Results showed that no snails were dead during the study period. However, calculation of Dara Index (DI) was indicated PTX accumulation in high toxin concentrations in snail's foot and thus snail can be introduced as an indicator of ecotoxicity conditions.

Keywords: Palytoxin, Zoanthus sansibaricus, Dara Index, Snail, Hormuz Island.

Introduction

All species under the order Zoantharia can produce a deadly chemical call palytoxin (PTX). PTX generally exist in mucus and gonads (Borneman, 2004). It was first isolated and purified from corals belonging to the family Zoanthidae, order Zoantharia and phylum Coelenterata in Hawaii (Moore and Scheuer, 1971). This zoanthid was subsequently identified as *Palythoa toxica* (Walsh and Bowers, 1971). Indigenous people used this neuromuscular agent to tip spear for hunting (Borneman, 2004). The toxin has a very complex molecule structure, and it is known as the second most poisonous non-protein based toxic (Riobo and Franco, 2011). As PTX is a neurotoxin, they can paralyze prey animal and enemies by attacking the sodium-potassium channel, which is responsible for controlling the ionic balance that is important to cellular function (Na+/K+ -ATPase), crucially change these specialized channel into non-selective pores (Deeds and Schwartz, 2010).

PTX is widespread in the marine environment, importantly it can be found in the food chain including marine organism which preys on zoanthids or predators prey on these creatures such as fish, crabs, molluscs (Hashimoto et al., 1969). Human can be exposure to PTX in many way like eating seafood that contain PTX, inhalation of marine aerosols and dermal interaction with zoanthids. PTX can affect the heart, muscles, and nerves leaving its victim in paralysis, which can be deadly to human and may be responsible for some of the human sickness (Tubaro et al., 2011).

Many studies on the effect of PTX have been done on different animals (monkeys, dogs, rabbits, guinea pig, rats and mice). However these studies are mainly base on PTX found in *P. tuberculosa*, *P. caribeaorum*, *P. mammilosa*, *P. toxica*, *P. vestitus* (Munday, 2011). Evaluation of PTX toxicity using various animal models revealed that PTX is an extremely potent neurotoxin. As previous study mentioned all species of *Palythoa* contain PTX, however the toxicity of PTX found in *Zoanthus sansibaricus* are not well study.

Organisms like corals, sponges, tube worms and nematodes are found occupying same niche of zoanthids and therefore space competition is observed (Mirzabagheri et al., 2008). Snails, although motile, are found within the zoanthid colony for food and shelter (Khushali et al., 2014). They live among *Zoanthus* polyps and under the edges of *Palythoa* incrustations, on which they feed and to which they and their egg masses are attached with sturdy but elastic mucus threads (Robertson, 1967). The ability of snails to accumulate elements (Madejon et al., 2013) and the identification of snails as quantitative indicators of environmental conditions in terrestrial habitats (Shimek, 1930; Berger and Dallinger, 1993) and aquatic habitats (Elder and Collins, 1991; Choubisa and Sheikh, 2013) are well known. Hence, they can be used as a suitable animal model for toxicity detection (Tallarico et al., 2014).

In littoral of Hormuz Island in the Persian Gulf of Iran, among identified zoanthids (*Z. sansibaricus*, *P.* cf. *mutuki* and *P. tuberculosa*), the highest number of toxic colonies per unit area is related to *Z. sansibaricus* which can have a significant impact on associated communities with it (Mirzabagheri et al., 2008).

This study aims to investigate and provide more information on the toxicity of PTX in *Z. sansibaricus* and the consequences of exposure to PTX on associated communities with it using aqatic snails, *Planaxis sulcatus*, and the calculation of Dara Index (DI) using the innovative formula and novel model given by Mirzabagheri during the PhD thesis.

The advantage of using this new method is easy access to samples of animal models in the same study area to assess the ecotoxicity and low cost and high speed process than other animal models. Bioassays tested for some marine invertebrates and evidence from environmental populations exposed to the toxins also give indications of the high impact that these toxins may have on natural food webs. The recognition of their wide distribution coupled with the poisoning effects that these toxins can have

on animals, and especially on humans, have concerned the scientific community (Gleibs and Mebs, 1999).

Materials and Methods

Study area

At least 6 individual colonies of *Z. sansibaricus* were collected from the reef flat and outer slope of reef crest on the Eastside and Westside of Hormuz Island that is located at 27°03'51"N and 56°27'20"E in north of the Strait of Hormuz of the Persian Gulf (Fig 1). Colonies were collected during reef walk at low tide in the morning and evening on the 21th April 2016. Coral rubble or "live rock" that had *Z. sansibaricus* colonies was collected using hammer and chisel.

Snails were collected from the rock for experimental purpose as they are very abundant on and around Hormuz Island reef. All animals were released back to its natural habitat as no animal die or injured during this study.

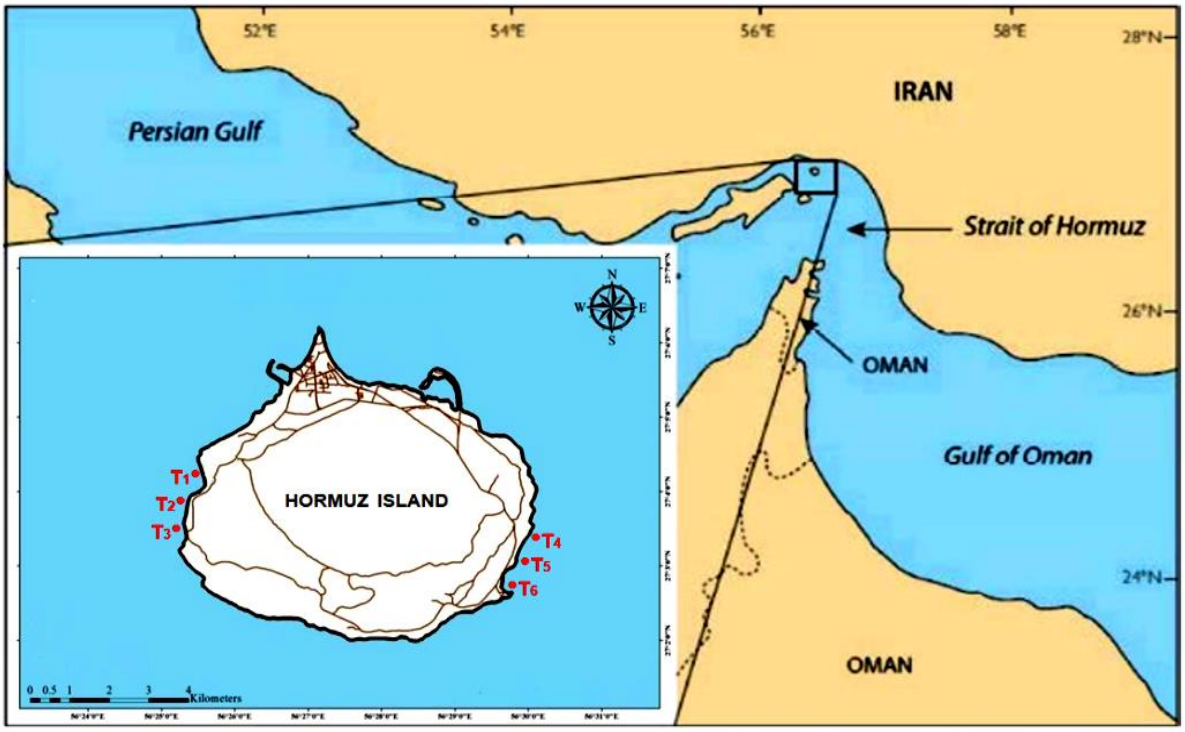

Figure 1: Map of Hormuz Island in the Persian Gulf, showing the sampling transects

Solution preparation

As PTX exist in the mucus of *Z. sansibaricus*, mucus were scribe using forceps or squeezed by hand with protective gloves on. A total of 0.5ml of mucus were collected and put in a 10ml tube. Mucus was then stored in the fridge for later use.

Dilute stock 0.5ml mucus solution with 4.5ml of seawater to make working mucus solution (5ml).

Snail sample and setting

36 snails were used for each transect and each of them weighed around 4-4.5g and placed separately in different plastic container. 36 snails were divided into 4 set, each set will have 3

replicates. Set 1: Control group (Sae water), Set 2: Working mucus solution (Original solution), Set 3: Treatment 1 (+2ml of sea water), Set 4: Treatment 2 (+4ml of sea water).

It should be noted that in each transect, sea water samples were taken from the surrounding environment of the zoanthid colonies.

Injection

• Prepare 2 new 10ml tubes and 1ml of working mucus solution was added into each of the tube: For Treatment 1, add 2ml of seawater and for Treatment 2, add 4ml of seawater.
• Mix well, and place in the tube rack for later use.
• Each snail was double dosed, a single dose is injected on the 22/04/2016 and the next single dose is injected 24 hours later.
• Hold the snail upside down to allow access to the foot.
• 1cc/ml syringe fitted with a 30G needle were used to withdraw about 0.03cc/ml of solution. New set of 1cc/ml syringe and 30G needle should be used for each solution.
• 0.03cc/ml of solution in the syringe was then injected into the foot of the snail.
• Snails were then placed back into its own plastic container.
• 24 hours later, 2nd dose were injected in the same snail, followed the same method as the 1st dose.
• Reaction of the snails was observed 24hrs after the 1st dose and 24 hours after the 2nd dose.

Calculation of DI

The formula calculation of DI is as follows:

$$DI = (CT_{24} + CT_{48}) - (SB_{24} + SB_{48}) \quad (1)$$

CT_{24}: Number of the snails crawl up to the top 24hrs after the 1st dose
CT_{48}: Number of the snails crawl up to the top 24hrs after the 2nd dose
SB_{24}: Number of the snails stay on the bottom 24hrs after the 1st dose
SB_{48}: Number of the snails stay on the bottom 24hrs after the 2nd dose

It should be noted that after injection, if snails die must be calculated as *SB* in formula 1.
DI generally varies between $-2n$ and $2n$ in each set that is as follows:

$$-2n \le DI \le 2n \quad (2)$$

n: Total number of snails into each set
Maximum individual poisoning: $DI = -2n$
Minimum individual poisoning: $DI = 2n$
Higher individual poisoning: $DI < n$
Lower individual poisoning: $DI > n$

Statistical analyses

Scores of DI were calculated using the Microsoft Excel software. For analysis of DI, a dendrogram was prepared to graphically visualize the differences among the 4 set in each transect.

Results

Results showed that no snails were dead during the study period. However, the changes of snail's fitness were observed. Snails injected with the working mucus solution (highest concentration of mucus) and with treatment 1 became less active and the foot of the snails was less sticky compare to the control snails (injected with seawater).

Table 1 shows that more snails injected with seawater and treatment 2 crawled up to the top over the night, whereas more snails injected with working mucus solution and snails injected with treatment 1 tend to stay on the bottom.

The result from the fitness change with calculation of DI was enough to draw a significant outcome. As a result, there were sufficient evidences to conclude the toxicity of PTX exist in *Z. sansibaricus* and accumulate in snails.

The dendrogram in Fig 2 shows two different groups. In all transects, Set 1 and Set 4 are grouped together with a low poisoning; Set 2 and Set 3 are grouped together with a high poisoning.

Table 1: The calculation of DI after injection

Transect No.	Number of transect individual (*N*)	Set No.	Number of set individual (*n*)	Number of exposured individual				DI	Coverage range of *Z. sansibaricus*
				24 hr		48 hr			
				*CT**	*SB***	*CT**	*SB***		
T_1	36	S_1	9	9	0	8	1	16	Wide
		S_2	9	0	9	0	9	–18	
		S_3	9	4	5	2	7	–6	
		S_4	9	7	2	7	2	10	
T_2	36	S_1	9	9	0	9	0	18	Middle
		S_2	9	1	8	0	9	–16	
		S_3	9	5	4	3	6	–2	
		S_4	9	7	2	6	3	8	
T_3	36	S_1	9	9	0	9	0	18	Narrow
		S_2	9	1	8	1	8	–14	
		S_3	9	5	4	3	6	–2	
		S_4	9	8	1	7	2	12	
T_4	36	S_1	9	8	1	8	1	14	Wide
		S_2	9	1	8	0	9	–16	
		S_3	9	4	5	2	7	–6	
		S_4	9	7	2	6	3	8	
T_5	36	S_1	9	9	0	8	1	16	Middle
		S_2	9	1	8	0	9	–16	
		S_3	9	4	5	2	7	–6	
		S_4	9	8	1	7	2	12	
T_6	36	S_1	9	9	0	9	0	18	Narrow
		S_2	9	0	9	0	9	–18	
		S_3	9	5	4	4	5	0	
		S_4	9	8	1	8	1	14	

**CT*: Crawl Top **SB*: Stay Bottom

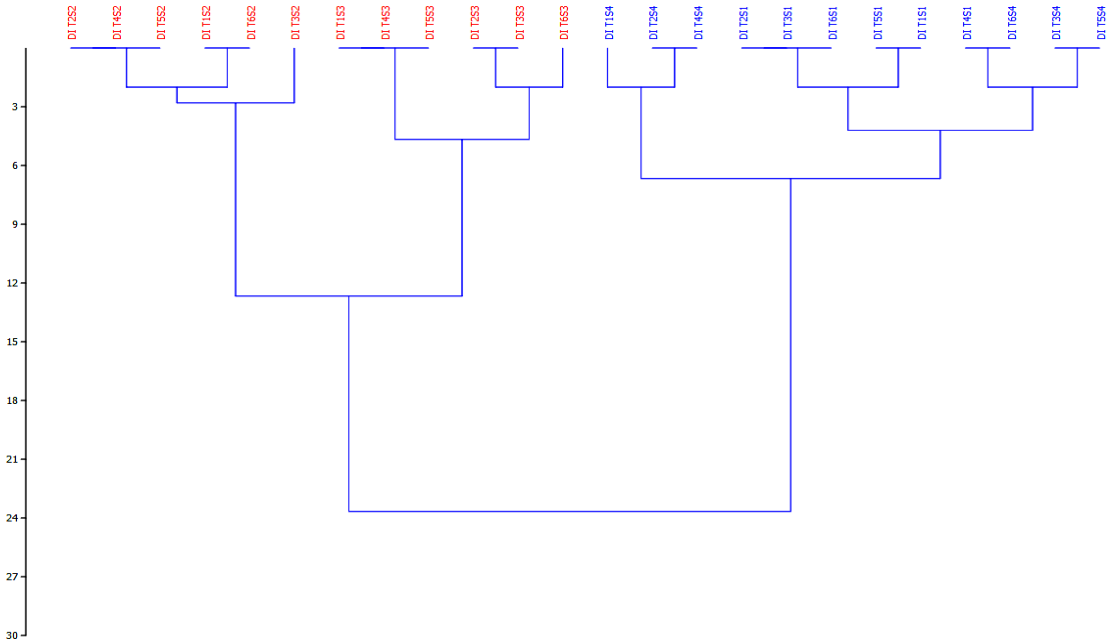

Figure 2: Dendrogram based on DI with data from table 1; Transect (T) and Set (S)

Discussion

PTX is a marine toxin found in all species of the order Zoantharia. PTX found in all species of *Palythoa* were well studied. However, only a little study on the PTX existed in species of *Zoanthus* such as *Z. solanderi* and *Z. sociatus* (Gleibs et al., 1995).

According to the result of this study on the PTX in *Z. sansibaricus*, the fitness of the snails had slightly decreased, however as there are no significant result of death, without using DI it is hard to determine the toxicity of PTX in the mucus of *Z. sansibaricus*.

Most of the previous studies indicated that PTX is very toxic to animal when injected. In mammal, the rabbit, monkey and rat are most affected by PTX. For example LD_{50} in rats was 0.03-0.45g/kg, and dose above the LD_{50} deaths can occur around 1-5 hours after the injection (Munday, 2011). PTX can cause illnesses varying from maculopapular dermatitis to myalgia, respiratory and cardiac problem (Deeds and Schwartz, 2010). Gastropods have been identified as the dominant symbiont with Zoanthids (Robertson, 1967; Yamamoto, 1973). But is there no report based on the testing of PTX injected into snails and the effects of exposure to it.

The result of this study using DI indicated that the fitness of snail tend to have slightly decreased as they become less active and its foot become less sticky, that is probably due to the effect of PTX on the mucus gland located on snail's foot as the gland produces sticky for locomotion (Achaval, 2005).

Results revealed by the dendrogram analyses in Fig 2 showed significant differences in DI in the different transects which implies that coverage range of *Z. sansibaricus* plays an important role on the community association with zoanthids (Trivedi et al, 2014). In transects with wide coverage range of *Z. sansibaricus* (Table 1), PTX is high in sea water of zoanthid surrounding environment that accordingly, in associated communities with it, the amount of poisoning is high.

The sufficient evidence of this study results clearly revealed that PTX exist in *Z. sansibaricus* not only for associated communities with it was acute, even there is feeding possibility of *Z. sansibaricus* by them without any deaths that eventually lead to accumulate of PTX in their organs and to carry in the food chains (Gleibs and Mebs, 1999), so that the transport and accumulation of toxins in the food chain, especially in marine organisms, is a normal phenomenon (Yasumoto and Murata, 1993).

There are reliable evidences from this study to conclude the toxicity of the PTX exists in the mucus of *Z. sansibaricus*, but PTX concentration in the mucus is unknown. In order to get a more reliable result, future study on the toxicity and the consequences of PTX found in *Z. sansibaricus* will need to extraction and purify the PTX found in *Z. sansibaricus*. For the entire Persian Gulf, further experimental studies with more replicates, higher level and more detailed experimental design are also required to provide valuable information about the toxicity of zoanthids.

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