Imposex Study on *Thais tuberosa* from Port and Non-Port Areas along the West Coast of Peninsular Malaysia

(Kajian Imposeks dalam *Thais tuberosa* dari Kawasan Pelabuhan dan Bukan-Pelabuhan Sepanjang Pantai Barat Semenanjung Malaysia)

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ABSTRACT

Imposex was found to occur among Thais tuberosa snails collected from eight locations, consisting of port and non-port areas, along the west coast of Peninsular Malaysia. All (100%) female individuals of this species were found to have male sexual characteristics (imposex) at various stages. The highest discovered imposex level was Stage 5 with the Relative Penis Length Index (RPLI) of 56.35%; this was found in samples collected from Lumut Port (ALP; an area with high shipping and boating activities). On the other hand, samples collected from areas with lower maritime activities were observed to have moderate imposex stages (Stage 3 and Stage 2). Samples from an isolated area of NTP were recorded to have Stage 1 imposex. These results demonstrate the potential influence of maritime activities (a higher leachate of tributyltin (TBT)) on the degree of imposex in T. tuberose individuals.

Keyword: imposex, Peninsular Malaysia, port, snail, *Thais tuberosa*, tributyltin

ABSTRAK

Imposeks telah ditemui berlaku di kalangan siput Thais tuberosa yang diambil dari lapan lokasi, merangkumi kawasan pelabuhan dan bukan-pelabuhan, di sepanjang pantai barat Semenanjung Malaysia. Semua (100%) individu betina spesies ini telah mempunyai sifat-sifat seksual jantan (imposeks) pada pelbagai peringkat. Peringkat tertinggi yang ditemui adalah Peringkat 5 dengan Indeks Panjang Penis Relatif (RPLI) sebanyak 56.35% dan ditemui pada sampel yang diambil dari Pelabuhan Lumut (ALP; kawasan dengan aktiviti perkapalan dan bot yang tinggi). Sebaliknya, sampel-sampel yang diambil dari kawasan yang kurang aktiviti-aktiviti maritime mempunyai peringkat-peringkat imposeks sederhana (Peringkat 3 dan Peringkat 2). Sampel-sampel dari kawasan terpencil Tanjung Pelepas (NTP) telah direkodkan mempunyai Peringkat 1 imposeks. Kesemua keputusan ini telah menunjukkan potensi pengaruh aktiviti-aktiviti maritim (seterusnya larut resap tributiltimah (TBT) lebih tinggi) terhadap tahap imposeks di kalangan individu T. tuberosa.

Katakunci: imposeks, Semenanjung Malaysia, pelabuhan, siput, *Thais tuberosa, tributyltin*

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INTRODUCTION

Tributyltin (TBT) has been used for almost three decades as an effective antifouling agent added in marine paint formulations used on pleasure boats, ships, vessels, docks, fish nets and also as lumber preservatives and slimicides in cooling systems (Fent, 1996). It can be found in many coastal waters, in both industrial and developing countries, with the highest levels in harbours and shipping lanes (Tanabe et al., 2000). In isolated areas, for example in the Arctic and Antarctic environments, TBT has also been detected close to harbours and shipping lanes (Negri et al., 2004; Strand and Asmund, 2003). According to King et al. (1989), TBT has been listed among the most toxic compounds ever deliberately introduced by societies into natural waters. Numerous biological effects as a result of exposure to TBT have been well documented. In particular, lower tropical organisms were found to be acutely sensitive to TBT (Bushong et al., 1988; Hall Jr., 1988). TBT adversely affects molluscs in various ways such as shell malformation in oysters (Alzieu, 1996; Waldock et al., 1996), mortality of the larvae of mussels (Beumont and Budd, 1984) and endocrine disruptions including imposex in gastropods (Bryan and Gibbs, 1991; Matthiessen and Gibbs, 1998). TBT also exhibited toxic effects in fishes particularly during early life stages (Fent, 1992), which may result in sex hormone imbalances (Fent et al., 1998).

Imposex in gastropods has been used worldwide as a bioindicator of TBT contamination as its development in snails is a more sensitive biomarker of TBT than detection limits from analytical chemistry. The use of long-living neogastropods as bioindicators in specific biological effect monitoring of TBT has previously been questioned, as some studies were unable to find correlations between imposex levels and TBT (Ide et al., 1997; Nicholson et al., 1998). However, other studies have found correlations between imposex and organotin body burdens or intensities of ship traffic (Strand and Jacobsen, 2002; ten Hallers-Tjabbes et al., 2003). It has also been put forward that a weak relationship is believed to be largely due to the fact that imposex development is an irreversible phenomenon and may not necessarily reflect present TBT concentrations.

Peninsular Malaysia is surrounded by busy waterways such as the Straits of Malacca and the South China Sea. These waterways are known to have the highest maritime activity in Southeast Asia. The Straits of Malacca is reportedly being used by more than 140,000 vessels annually, including 50,000 supertankers carrying about one-quarter of the world's traded goods (EIA, 2008; Freeman, 2003; Ismail et al., 2004). It is clear that this scenario could lead to organotin contamination in marine and coastal environments. However, there are only a few reports on organotin contamination, particularly TBT, in Malaysian coastal ecosystems. Most studies discuss the occurrence of TBT in sediments and mussels from selected areas in Peninsular Malaysia (Tong et al., 1996; Sudaryanto et al., 2002). Concentrations of TBT in the Malaysian coastal environment have been reported to be high enough to lead to imposex in gastropods. Reports on the occurrence of imposex in *Thais* spp. collected along the west coast of Peninsular Malaysia include studies by Ismail et al., 2004 and Mohamat-Yusuff et al., 2010. Ismail et al. (2010) also reported imposex cases among *Thais* spp. collected from a number of locations along the east coast of Peninsular Malaysia. The aim of this study was to observe the influence of maritime activities on imposex occurrence in *Thais tuberosa* by assessing samples collected from both port and non-port areas along the west coast of Peninsular Malaysia.

MATERIAL AND METHODS

Samples of *T. tuberosa* were collected from eight locations along the Straits of Malacca (Table 1, Figure 1). Descriptions of respective location are listed in Table 1. The sites are mainly distinguished as port or non-port areas. The selection of each sampling location was primarily based on the presence of *T. tuberosa* in the area.

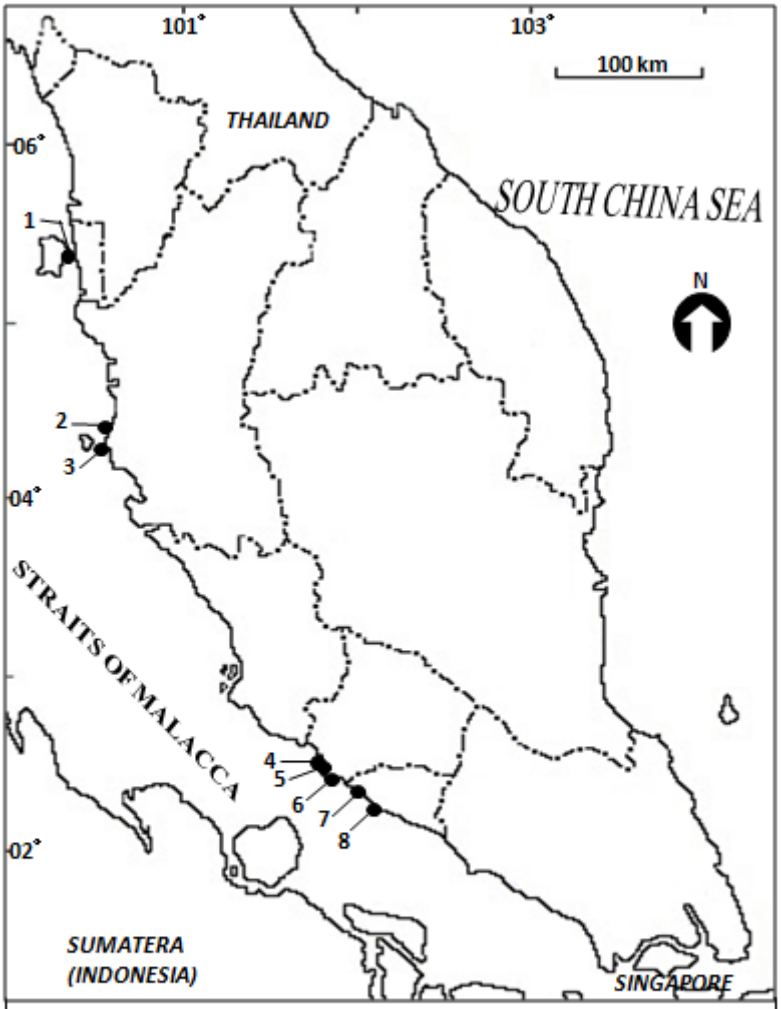

Figure1: Sampling locations in port and non-port areas along Straits of Malacca.

Live adult *T. tuberosa* snails were randomly collected from the sampling sites and brought back to the laboratory in Universiti Putra Malaysia (UPM) for further analysis. The shell length of each snail was measured using a vernier caliper (±0.01 mm). The shell was then removed by cracking it using a bench vice. The sex (female, male or imposex) of the snails were determined under a dissecting microscope (±64 magnification). Female snails were determined based on the presence of a pallial oviduct and a straight oviduct connected to the gonad. Male snails were determined through their small prostate gland and a seminal vesicle in addition to a large penis.

The level of imposex was characterized by the frequency of females expressing imposex and the imposex severity according to the vas deferens sequence (VDS) protocol as introduced by Gibbs et al. (1987). There are six imposex stages, categorized by the development of the vas deferens in the female (i.e. examination of the penis, vas deferens, capsule gland and genital papilla). The Relative Penis Length Index (RPLI) was also used by comparing female penis length as a percentage of male penis length as described by Gibbs et al. (1987).

Table 1: Location of sampling sites along the west coast of Peninsular Malaysia

Location	Code	Coordinate	Area Descriptions
Penang Royal Custom	PRC	05°22.99'N 100°20.05'E	Port area; reclaimed, mainly rocky beach with concrete structures; close distance to a major coal terminal and Penang Port; urban area
Teluk Senangin	ATS	04°17.90'N 100°34.75,E	Non-port area; mainly sandy beach but rocky at both ends; used by a few small traditional fishing boats; about 10 km away from busy Lumut port; remote area
Lumut Port	ALP	04°13.95'N 100°37.578'E	Port area; mainly sandy beach with some parts reclaimed for port structures; close proximity to a naval base, Lumut commercial and trading ports, and dockyard; urban area
Negeri Sembilan Royal Custom	NRC	02°31.25'N, 101°47.841'E	Port area; reclaimed rocky beach; close proximity to an unloading terminal for oil tanker and coal vessels; fishing and government boats terminal; urban area
Teluk Kemang	NTK	02°27.69'N 101°51.002'E	Non-port area; mainly sandy beach but rocky at both ends; tourism and recreational beach; used by a few small traditional fishing boats; urban area
Teluk Pelanduk	NTP	02°25.05'N 101°53.536'E	Non-port area; sandy-muddy beach with some parts reclaimed; used by a few small traditional fishing boats for fish landing; traditional village area
Tanjung Meriam Patah	MTM	02°22.22'N 101°58.826'E	Non-port area; sandy-muddy beach; used by a few small traditional fishing boats for fish landing; traditional village area
Tanjung Keling	MTK	02°12.98'N 102°9.575'E	Port area; sandy beach but rocky at nearby cape; close proximity to main petroleum refinery area and Malacca Port; recreational and tourism beach; urban area.

RESULTS AND DISCUSSION

In this study, 100% of female *T. tuberosa* collected along the west coast of Peninsular Malaysia showed the development of imposex characteristics at various stages. Table 2 shows the respective percentages for frequency of imposex and RPLI of female *T. tuberosa* from port and non-port areas along the west coast of Peninsular Malaysia. Stage 5 imposex was the highest examined imposex stage among all samples. It occurred in 47.62% of the total female snails collected from Lumut Port (ALP) area as shown in Table 3. The RPLI value for samples collected from ALP was 56.35%.

The second highest imposex stage was Stage 4. 100% of female *T. tuberosa* snails collected from the Penang Royal Custom (PRC) and Teluk Senangin (ATS) were observed to have Stage 4 imposex characteristics. The RPLI values of imposex snails from both PRC and ATS are 13.33% and 50% respectively. Similar imposex stages were also found in more than 52.38% of female snails collected from ALP.

Stage 3 levels of imposex was discovered in 58.33% of female snails collected from Negeri Sembilan Royal Custom (NRC) and 35% of the female snails from Teluk Kemang (NTK). The RPLI values recorded for NRC and NTK were 8.33% and 5.86% respectively. On the other hand, Stage 2 imposex was found to occur in 30% of female snails from NTK, 40% of total female snails collected from Tanjung Meriam Patah (MTM) and 43.75% of the female snails collected from Tanjung Keling (MTK). Finally, the initial imposex stage (Stage 1) was found in 100% of total female snails collected from Teluk Pelanduk (NTP) and more than 40% of the total female snails collected from MTK and MTM. However, the RPLI value cannot be calculated since there is no penis development at Stage 1 imposex.

Table 2: Frequency of imposex and RPLI values in female *T. tuberosa* from port and non-port areas along the west coast of Peninsular Malaysia.

Site Code	Total Sample (*n*)	Number of Females (n_f)	Imposex Frequency (%)	RPLI (%)
PRC	8	3	100	13.33
ATS	25	10	100	50.00
ALP	30	21	100	56.35
NRC	30	12	100	8.33
NTK	30	20	100	5.86
NTP	30	22	100	0.00
MTM	11	5	100	1.57
MTK	30	16	100	3.40

Table 3: RPLI values and respective percentages of imposex cases in *T. tuberosa* along the west coast of Peninsular Malaysia.

Location	RPLI	% Stage 1	% Stage 2	% Stage 3	% Stage 4	% Stage 5
PRC	13.33	-	-	-	100.00	-
ATS	50.00	-	-	-	100.00	-
ALP	56.35	-	-	-	52.38	47.62
NRC	8.33	41.67	-	58.33	-	-
NTK	5.86	35.00	30.00	35.00	-	-
NTP	0.00	100.00	-	-	-	-
MTM	1.57	40.00	40.00	20.00	-	-
MTK	3.40	56.25	43.75	-	-	-

In general, the occurrence of imposex among *T. tuberosa* indicates the spread of TBT contamination along the Straits of Malacca, particularly along the western coastline of Peninsular Malaysia. Imposex occurred in snails collected in areas both exposed and unexposed to maritime activities. The frequency of imposex stages in female *T. tuberosa* is summarized in Figure 2. All female snails of this species were observed to show imposex characteristicss in various stages up to Stage 5. Based on the variability of imposex stages recorded, it can be said that imposex cases in *T. tuberosa* showed a relationship with the level of maritime activities in the sampling areas. Snails collected from locations within ports, marinas and dockyards, or in close proximity with these areas showed higher stages of imposex occurrance. However, lower imposex stages were still found in snails collected from areas with low maritime activities or far from port areas.

In the case of NTP (a remote area), only Stage 1 imposex was found in all female *T. tuberosa* samples. Since no maritime activities occur nearby, it is possible that TBT leachate originated from the busy shipping route of the Straits of Malacca. This is consistent with a report by Swennen et al. (1997); TBT originating from large vessels affects snails far removed from shipping lanes. According to Tong et al. (1996), the water from remote or unexposed coastal areas were found to have <3.4 - 20.0 ng/L TBT concentration. Horiguchi et al. (1994) estimated that concentrations of TBT more than 10.0 ng/g wet tissue could initiate imposex in *T. clavigera* and *T. bronni*.

The increment of imposex stages in this study correlated with the increment of maritime activities in a particular area. This result is supported by Bryan et al. (1988); a strong correlation was discovered between imposex in neogastropods and organotin (specifically TBT) concentrations in the environment. The present study also showed a similar result, especially in samples collected from MTM and MTK which showed Stage 2 imposex characteristics. Although these areas are located near a few traditional villages and far from industrial and shipping areas, additional TBT leachate from sources other than the shipping lane could include traditional fishing boats anchored along the

beach. The local communities, most of whom are traditional near-shore fishermen, probably use TBT-based antifouling paints for their boats.

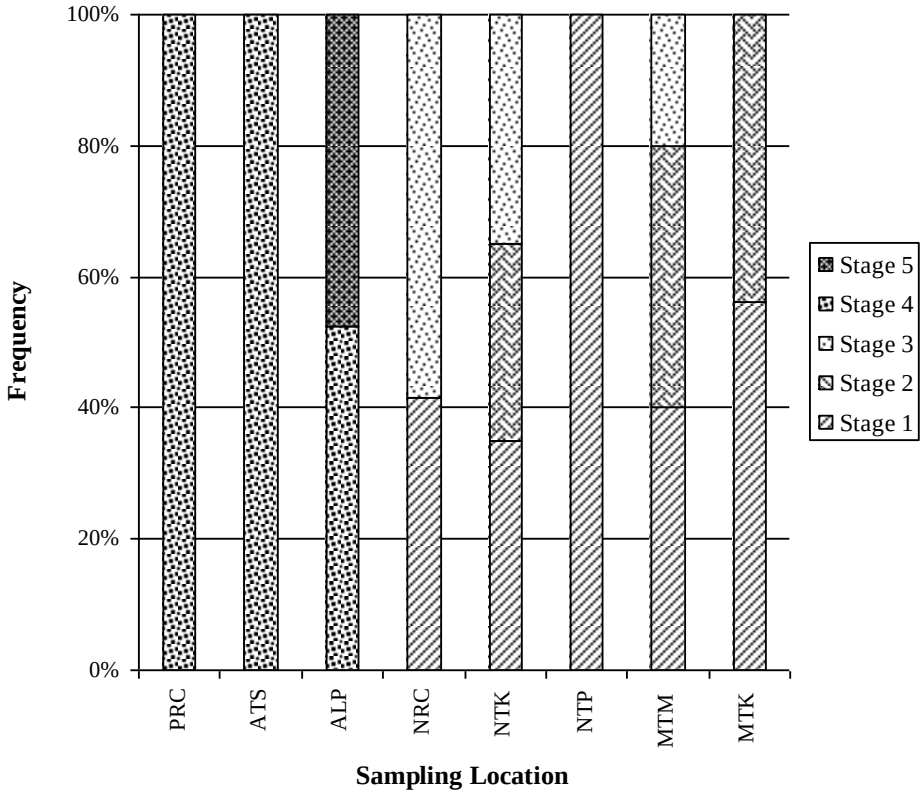

Figure 2: Stages of imposex in ports and non-port areas along the west coast of Peninsular Malaysia.

In contrast, NRC and NTK have moderate marina, recreational and fishing boat activities occurring in their vicinity. These conditions might contribute relatively moderate loads of TBT leachate into the surrounding environment. Thus *T. tuberosa* samples collected from these areas showed Stage 3 imposex. This is almost similar to a report by Bech (2002) who observed that *Chicoreus capucinus* samples collected from active marinas had Stage 4 imposex.

In addition, Stage 4 imposex characteristics occurred in 100% of female snails collected from ATS even though the station is situated about 10 km away from the major port area of ALP. Although ATS is isolated from domestic and industrial areas, there is a small number of recreational sea crafts and traditional near-shore fishing boats anchored along the beach. Furthermore, there is a possibility that TBT contamination spread from the nearby busy ALP port area which has the highest maritime activity (e.g. navy and trading ships, passenger and fishing boats) of all the sampling sites. According to Ismail et al. (2003), imposex can be detected up to 20 to 30 miles from a source area. Imposex in the snails collected from ALP occurred at Stage 4 and Stage 5 (the highest observed stages in this study). The area could possibly be contaminated by organotin of more than 30.0 ng/l, as demonstrated by Tong et al. (1996).

These findings suggest that imposex in neogastropod snails and the intensity of maritime activities are closely related. Other studies have found correlations between imposex and the intensity of ship traffic (Ten Hallers-Tjabbes et al., 2003; Strand and Jacobsen, 2002). Since imposex development is an irreversible phenomenon, we also predicted that imposex developed during the juvenile life stages and increased with maturity. Experiments with *Buccinum undatum* have shown that TBT induced imposex in juveniles and not in adults (Mensink et al., 2002). Imposex was found in the Arctic whelk *Buccinum finmarkianum* at several marine stations off Thule Air Base, a US military

facility in Northwest Greenland which indicates widespread contamination by the antifouling agents TBT and TPT in the area (Jacob et al., 2006).

This short survey showed that the west coast of Peninsular Malaysia and the Straits of Malacca have been contaminated with organotins, particularly TBT. Previous reports on imposex cases in the Straits of Malacca (Tan, 1997; Ismail et al., 2004) and high levels of TBT in the Straits of Malacca (Tong et al., 1996; Hashimoto et al., 1998; Sudaryanto et al., 2002) showed that the Straits of Malacca is already exposed to a certain level of TBT contamination and that it has an effect on marine organisms. Although TBT is not persistent in most natural waters (i.e. it is susceptible to natural degradation processes and it is absorbed by particulate matter), it is considerably more persistent in sediments, having an estimated half-life of 2.5 years (Hashimoto et al., 1998) and in heavily contaminated sediments it becomes even more persistent (Stewart and de Mora, 1990). Levels in sediments are commonly 1000 times greater than levels in overlying waters (Stewart and de Mora, 1990). Any disturbance by sea currents or human activities will cause a resuspension of sediment-bind-trace elements (including TBT) in the environment and probably in soluble forms readily absorbed by aquatic organisms (Zulkifli et al., 2010). Hence, further monitoring programs are needed to substantiate previous studies.

The contamination of TBT along the west coast of Peninsular Malaysia is no longer localized or restricted to port or non-port areas. TBT pollution is now widely spread along the Straits of Malacca and obviously affects marine life in the coastal areas. With increasing shipping activities, the expansion of ports and marinas, increasing industrial and fishery activities, continuous elevation of TBT in the coastal environment is expected.

CONCLUSION

The various stages of imposex occurrence in *T. tuberosa* indicate a widespread contamination of TBT on the west coast of Peninsular Malaysia; even though these sites are non-port areas situated far from TBT source areas. Serious concern and further comprehensive research are needed to ensure healthier coastal marine life in the Straits of Malacca.

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