LARVAL HABITATS OF MAIN MALARIA VECTORS IN HORMOZGAN PROVINCE AND THEIR SUSCEPTIBILITY TO DIFFERENT LARVICIDES

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Abstract. Hormozgan Province is located in the south of Iran bordering the Persian Gulf. In this area, malaria is still considered a major health problem. In the province, eleven species of *Anopheles* were identified: *An.pulcherrimus, An.sergenti, An.apoci, An.multicolor, An.subpictus, An.turkhudi, An.fluviatilis, An.stephensi, An.d'thali, An.superpictus,* and *An.culicifacies*, among which only the last five species play an important role in malaria transmission. Larval habitats of malaria vectors varied from clean to brackish water, as well as sewage water, under palm trees, marshland, pools, pounds, drainage, irrigation canals, wells, and water containers. Malathion, fenitrothion, chlorpyrifos, and temephos were tested against *An.stephensi, An.fluviatilis* and *An.d' thali* larvae at diagnostic doses recommended by WHO. The results showed susceptibility to malathion, chlorpyrifos and temephos, but fenitrothion was not able to yield 100% mortality.

INTRODUCTION

Despite the considerable research and control efforts devoted to malaria since the turn of the century, it is still the most prevalent and most devastating parasitic disease in the tropics. An estimated 400-500 million new cases occur each year, 1.2 to 2.7 million people die each year from malaria and complications (Najera and Zaim, 2002). At present, in Iran, malaria can be considered a health problem only in three provinces of Hormozgan, Kerman and Sistan, and Baluchistan (Eshghi *et al*, 1976; Manouchehri *et al*, 1992; Ministry of Health and Medical Education, 2003). Recently, according to the Ministry of Health and Medical Education of Iran, a total of 9,000 malaria cases was reported mainly imported, and some indigenous, cases. The duration of malaria transmission is up to 10 months in coastal and 8 months in mountainous areas of Hormozgan. Two distinct peaks of malaria transmission occur in this area, in April-May and in October-November. About 1.5 million people live in the area of Hormozgan Province. Malaria can be transmitted, so that several indigenous malaria cases have been reported annually. The mosquito control program in the area has a high cost every year (Ministry of Health and Medical Education, 2003, unpublished data). The main control activities in the area are use of lambdacyhalothrin as a residual spray, and chlorpyrifos methyl and *Bacillus thuringiensis* as larvicides. *Gambusia affinis* and *Aphanius dispar*, larvivorous fishes have been introduced to breeding places.This investigation was made to determine the breeding places and susceptibility levels of *An.stephensi* (laboratory and field strains) as well as, *An.fluviatilis* and *An.d'thali* larvae to the WHO recommended diagnostic doses of organophosphate insecticides, using the standard method (WHO, 1981).

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MATERIALS AND METHODS

Study area

Hormozgan Province (Fig 1) on the southern border of Iran, adjacent to the Persian Gulf and Oman Sea, has two different areas; a coastal band in the south and a mountainous area in the north. The study area is located in a mountainous part of Bandar Abbas County, with surface area of 65,379 km^2, located between 25˚, 24´-28˚, 57´ N latitude and 52˚, 41´-59˚, 15´ E longitude. The area is subtropical and is a suitable place for maintaining and transmitting malaria. The total annual rainfall in 2002 was 100-150 mm, and the mean annual relative humidity was 59.5%. The maximum and minimum mean annual temperatures were 52˚ and 6˚C, respectively. The larvae were collected in different parts of the province by a dipper and identified according to Shahgudian (1969).

Larval susceptibility test

The following larvicides were used: malathion (3.125 mg/l), temephos (0.25 mg/l), fenitrothion (0.125 mg/l), and chlorpyrifos (0.025 mg/l) at diagnostic doses. Butanone 2% in absolute ethanol was used as control. All concentrations were provided by the WHO.

Fig 1- Iran map showing Hormozgan Province.

In each test, there were two control and 4 replicate beakers for testing a single diagnostic dose of larvicide. Mosquito larvae were tested at the late 3rd and early 4th instar according to the method described by WHO (1981). The larvae were exposed to a diagnostic dose of larvicide. At each concentration, at least 100 larvae, representing four replicates of 25, were tested. The larvae were fed with Bemax and mortality counts were made after 24 hours' exposure.

RESULTS

Anopheles stephensi breeds in a wide range of both urban and rural habitats throughout its distribution in Hormozgan Province. In urban areas of Hormozgan, this species breeds in all sorts of water bodies, such as wells, cisterns, fountains, ornamental ponds, and in water used for building construction. Larvae can be collected from ponds, pools, stream margins, catch basins, and seepage canals. It is found in water with high salinity, sometimes reaching or even exceeding that of seawater. In the city of Bandar Abbas, larvae were found in sewage, wells and ponds, and even in small amounts of water leaking from air conditioners. In rural areas, the breeding places are pools, streambeds, palm irrigation canals, at the margin of streams and rivers, seepages, and marshy areas with a gentle water flow.

Anopheles d'thali breeds in pebbly margins of rivers, springs, pits around springs with or without vegetation, pools in dried-up river beds, and palm irrigation canals in Hormozgan. In Bandar Abbas County, larvae were also found in mineral water. It is found in water with high salinity. The water temperature of breeding places ranging between 13ºC and 28ºC, with a pH of 6.9-8.0.

The results of larvicide tests showed that the larvae of *An.stephensi* (laboratory and field) strains, *An.fluviatilis* and *An.d'thali* had 100% mortality after 24 hours' exposure to diagnostic doses of temephos, malathion, chlorpyrifos, and fenitrothion. The mortality for a diagnostic dose of fenitrothion for field strain of *An.stephensi* was 73±3. The figures for *An.fluviatilis* and *An.d'thali* were 80 ± 4 and 96 ± 2, respectively (Table 1).

DISCUSSION

The results of our study exhibited a wide variety of mosquito habitats. *An.stephensi* and *An.d'thali* had almost the same breeding places, and were often caught altogether in larval checks. A study of mosquito breeding in brackish water showed that larvae of *An.stephensi* survived well in freshwater but some were able to tolerate up to 50% seawater (Roberts, 1996). A study of mosquito breeding in wells revealed the dominance of *An.stephensi* among the malaria vectors.

Table 1
Mortality rates of larvae of malaria vectors to diagnostic doses of different larvicides in Bandar Abbas County, Hormozgan Province, Iran, 2002.

Species / Larvicides	*An.stephensi* laboratory strain		*An.stephensi* field strain		*An.fluviatilis*		*An.d'thali*	
	Mortality	SE	Mortality	SE	Mortality	SE	Mortality	SE
Malathion	100	0.00	100	0.00	100	0.00	100	0.00
Temephos	100	0.00	100	0.00	100	0.00	100	0.00
Fenitrothion	100	0.00	77	3.0	80	4.0	96.0	2.0
Chlorpyrifos	100	0.00	100	0.00	100	0.00	100	0.00
Control	4	2	3	2	6	2	10	3

However, no anopheline species was encountered when the well water level exceeded 12 m (Rajnikant *et al*, 1993). A study of *Anopheles* mosquito breeding in irrigated areas showed habitat characteristics most correlating with the occurrence of anophelines were the physical water conditions and the absence of fauna, particularly predators. The existence and abundance of *Anopheles* immatures were not significantly correlated with water temperature, dissolved oxygen, electroconductivity and pH (Herrel *et al*, 2001). A study of breeding habitats of *An.fluviatilis* in India showed it breeding in all types of habitats. Streams were most productive for this species and breeding was intense during winter and early summer. In the rainy season, breeding occurred in terraced paddy fields (Sahu *et al*, 1990).

In a parallel study carried out in the region, it was shown that, in the mountainous area of Hormozgan Province, *An.fluviatilis* larvae were found in stream and river margins, slow-flowing water, pits in stony river beds, and pits around spring and irrigation drains with vegetation. Oviposition sites and breeding places of *An.fluviatilis* are fresh, slow-flowing or stagnant waters, vast marshes, river banks, pits in the beds of stony or sandy rivers and rainfall pits (Eshghi, 1976). Furthermore, larvae of this species were collected from the coastal plain of Minab (Tomgohar). The favorite pH for this species in about 7 and usually Graminae plants exist in their breeding places. Larvae were more abundant during September and October, which is the end of the hot season in southern Iran (Manouchehri *et al*, 1992). Results of the susceptibility tests against the insectary strain larvae of *An. stephensi* showed no indication of resistance to the tested larvicides, confirming preceding studies (Limui, 1989). The results for the field strain of this species showed 77±3% mortality in a diagnostic dose of fenitrothion, but 100% in other larvicides. Resistance of this species to fenitrothion has been reported from India, Iraq, Iran, and Pakistan (WHO, 1992). Based on previous studies, two genetic factors have a role in resistance of *An.stephensi* to insecticides (Hearth and Davidson, 1981; WHO, 1982). Susceptibility tests on *An.fluviatilis* larvae showed 80±4% mortality in diagnostic doses of fenitrothion. Reports of susceptibility tests on this species to insecticides in countries neighboring Iran showed resistance to DDT and dieldrin in Afghanistan and Pakistan, and to dieldrin in Saudi Arabia (WHO, 1992; Thavaselvam *et al*, 1993). There was no report of resistance to insecticides in Iran for this species. A study in India reported that *An.fluviatilis* had developed resistance to HCH (Sahu, 1995). In this investigation, larvae of *An.d'thali* had a mortality of 96±2% in a diagnostic dose of fenitrothion. This species is resistant to chlorpyrifos, fenitrothion, bromofos and some carbamate insecticides in Egypt, and temephos in Jordan (WHO, 1992). Also there is resistance to DDT in adult *An.d'thali* in Iran (WHO, 1992).

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