Original Article

Use of Expanded Polystyrene (EPS) and Shredded Waste Polystyrene (SWAP) Beads for Control of Mosquitoes

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Abstract

Background: Mosquitoes transmit several diseases to human. There are several measures for control of larvae. As part of Integrated Vector Management (IVM) program, the utility of floating layers of polystyrene beads (EPS) is a potential alternative in habitats of mosquito larva. EPS beads prevent oviposition of mosquito as well as killing the immature stages by forming a tick layer on the water surface. They are cheap, environmentally safe and do not need frequent application and remain on the surface of water for long time. The objective of the current study was to asses the effectiveness of two types of polystyrene beads of (EPS) and (SWAP) for control of mosquito larvae under laboratory conditions.

Methods: *Anopheles stephensi* and *Culex quinquefasciatus* were used for experimental purposes. In each tray 250 larvae of late 3rd and early 4th instars were introduced. The experiment was conducted on 4 replicates for *An. stephensi*, *Cu. quinquefasciatus* and combination of both. Emerging of adult mosquitoes were calculated every day until the end of experiments.

Results: Mortality rate and Inhibition of Emerge (IE) for *Cu. quinquefasciatus, An. stephensi* and combination of both species was 97.8%, 100% and 99.07%, respectively using EPS. In average, EPS was able to kill 98.9% of larvae. The figures with SWAP were 63%, 91.05% and 72.65%, respectively. The average mortality for mosquitoes was 75.57%

Conclusion: EPS and SWAP beads can be very effective and practical for elimination of *An. stephensi* and *Cx. quinquefasciatus* under the laboratory conditions.

Keywords: Polystyrene beads, Mosquitoes, *Anopheles stephensi*, *Culex quinquefasciatus*, Iran

Introduction

Mosquitoes belonging to the family of Culicidae transmit some of the worldwide important diseases such as malaria, filariasis, yellow fever and dengue hemorrhagic fever. These diseases are rising and some of them re-emerged in many tropical and subtropical areas (WHO 1999). Therefore, approaches to reduce the incidence of vector-borne diseases by controlling mosquito populations are highly warranted (Subbiah and Brij Kishore 2006).

Urbanization with poor sanitation increases the number of breeding places for mosquitoes, especially *Culex* spp, such as pit latrines, cess pits, cesspools, disused wells, blocked open drains, open septic tanks, polluted puddles, uncovered overhead tanks (Das and Rajagopalan 1989, Nathan et al. 1996). In some parts of Iran due to the lack of water pipe system, villagers use the stored water for drinking and washing. These kind of breeding places have ideal conditions for *Anopheles*, *Aedes*

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and *Culex* mosquito habitats. Re-emerge of malaria epidemic have occurred in the cities of Iran in the recent years (Edrissian 2006). Mosquito control with the use of insecticides in some areas is not very effective because of vector resistance, exophilic vector behavior, environmental concern and low community acceptance (Malaviya et al. 2006).

Additionally only few number of insecticides have been recommended for control of mosquitoes in potable water. Many populations of mosquito vectors of diseases have developed resistance to synthetic organic insecticides (organochlorines, organophosphates, carbamates and pyrethroids) that have been used mostly during the last half of the 20^{th} century. Thus interest in alternate strategies as well as Integrated Vector Management (IVM) have been increased in recent years (WHO 1982a, b, WHO 1986). Therefore, methods of control are effective to produce the desired results, including the use of bio-control agents and environmental management.

One of the effective non-chemical methods for control of mosquito's larvae is usage of expanded polystyrene (EPS). These agents eliminate/minimize the mosquito breeding places leading to decrease of adult population. An alternative and plentiful source of expanded polystyrene is the waste material destined for landfill sites, packaging, from perishable foodstuffs and shipping electrical and other appliances.

Unexpanded polystyrene beads are produced by the petrochemical industry with pentane in solid solution in each bead. Industrially, the beads are heated with steam to make EPS beads, which are then heated and pressed to form blocks or slabs for packaging, insulation (Curtis et al. 1989, Sivagnaname et al. 2005). Also shredded waste polystyrene (SWAP) is produced from this products and a suitable alternative to use of commercially available, SWAP appears to offer an effective, cheep and readily available alternative to EPS for mosquito controlling purposes.

The transport of large volumes of EPS beads over long distances is cost prohibitive and thus expansion using locally available resources is suggested (Curtis and Feachem 1981). A 2 cm thick layer of 2 mm beads is sufficient to eliminate mosquito breeding. Floating layers of 2 mm expanded polystyrene were found to be most effective for suffocating *Anopheles* larvae and pupae. They must be placed in sacks or dumps to take them to the pits where they are needed. If piled loose in an open trunk, they would blow away before reaching their destination.

Storage of beads for quite a long time would result in failure of the beads to expand to the maximum when boiled due to loss of pentane; hence it has been suggested that beads should be boiled soon after procurement from the production firm (Madurai and Nadu 1995).

In Iran there are several methods for control of mosquito larvae in breeding places. Use of some organophosphate insecticides, patchy application of *Bacillus thuringiensis*, and introducing of larvivorus fishes are the main control measures. The bionomics of malaria vectors have been studied in the region (Vatandoost and Moinvaziri 2004, Vatandoost et al. 2004, Vatandoost et al. 2006, Hanafi-Bojd et al. 2006).

The objective of the present study was to evaluate the efficacy of local made industrial beads for mosquito control under laboratory conditions.

Material and Methods

Mosquito strains

An. stephensi is the main malaria vector in Hormozgan Province. The filed strain of Bandar Abbas was collected and reared in the insectray of Bandar Abbas Health Research Center, southern Iran related to Tehran University of Medical Sciences, School of Public Health. *Cu. quinquefasciatus* also was collected from the field and maintained in the insectary.

EPS and WASP

EPS and SWAP were purchased from The National Petrochemical Company (NPC), in Iran.

Experimental design

Sample size was designed according to WHO methods (WHO 2005) with some minor modifications. A total of 36 trays of 35×25×10 cm were used. A total of 250 larvae of mosquito (250 *Anopheles* or 250 *Culex* or mixed 125 *Culex*+125 *Anopheles*, based on designed pattern) at the late 3rd and early 4th instars were released into each tray. Based on kind of treatment, four replicates were used for each species (Fig. 1). Temperature in the insectaria was 27±3^{o} C, with a period of 14-10 h light-dark. Relative humidity was maintained at 70-80%. Larvae were fed by fish flakes. Feeding and water volume were prepared for all trays as required. Subsequently the EPS and SWAP were added to the water surface of treated trays to make a layer of 0.5-1 cm. One group of 1000 larvae was used as control for each species. Fig. 1 shows the design of the experiment.

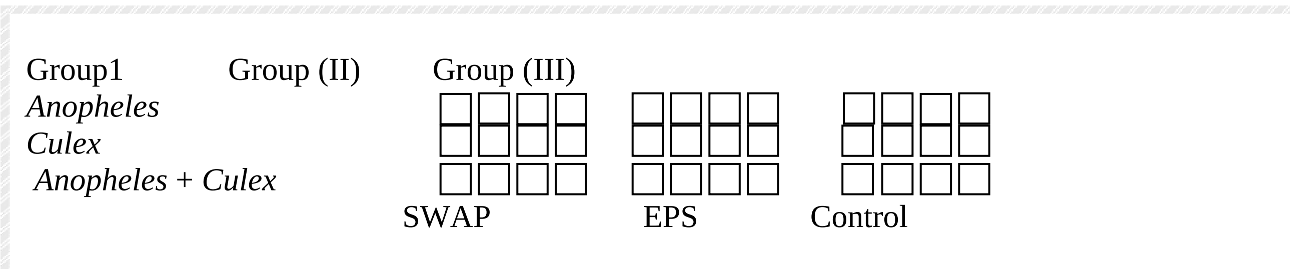


Fig. 1. Schematic diagram of the tests performance

The fish food was added daily to the rearing trays, and then the surface of treated water was covered with the EPS or SWAP. The rearing trays were covered with net to prevent the adult mosquito escaping during the test period. Mosquitoes were emerged daily and trapped under the net cover. This allowed us to catch them using a mouth aspirator and to identify the genus. Adult emergence was calculated daily up to 20 d. At the end of experiments all the dead and alive stages of mosquitoes were counted and recorded. After emerging all mosquitoes, the dead adults, pupae and larvae from the polystyrene layers were counted and subjected to statistical analysis.

Statistical analysis

The larval mortality data were corrected according control mortality by the Abbott's formula (Abbot 1925). Inhibition of emerge (IE%) or mortality of mosquitoes is calculated using the following formula:

$$IE(\%) = 100 - \left(\frac{T \times 100}{C}\right) ,$$

Where *T*= percentage emergence in treated trays and *C*= percentage emergence in the control. If adult emergence in the control is less than 80%, the test should be discarded and repeated (Mulla and Darwazeh 1975). Where the percentage is between 80% and 95%, the data are corrected using Abbott's formula.

ANOVA was carried out with SPSS 11.5. Means were compared with Post Hoc Tests (LSD), (P< 0.05).

Results

Results of mosquitoes mortality based on life stages are given in Table 1. From a total of 5750 out of 9000 larvae in all rearing trays, the highest number of mortality (2973) was recorded in EPS, followed by SWAP (2362), and control (415) rearing trays.

If the control mortality was between 5% and 20%, the mortalities of treated samples were corrected according to Abbott's formula. Results of adult mosquitoes collected live using mouth aspirator based on used materials, are showed in Fig. 2 during 20 d of the study period. We can observe differences between three methods clearly.

In another step of the experiment, we assessed and compared the survival means by Post Hoc Tests (LSD) based on untreated control and treated samples. Details of daily emergence based on mosquito groups and the tested materials are presented in Fig. 2. As shown in the Table1, the adult emergence in the control is 86.17% (mortality 13.83%), therefore our treatment results were corrected using the Abbott's formula. Fig. 3 has compared percentage emergence of mosquitoes with 95% confidence intervals for each types of polystyrene, species of mosquitoes and control group.

Table 1. Mortality of mosquitoes in treated samples and untreated control

			EPS group			
Mosquito species	Larva	Pupa	Adult	Total	%Mr	Corrected Mr(IE%) *
Culex	981	0	0	981/1000	98.1	97.8
Anopheles	998	2	0	1000/1000	100	100
Anopheles+Culex	984	8	0	992/1000	99.2	99.07
Total	2963	10	0	2973/3000	99.1	98.96
			SWAP group			
Mosquito species	Larva	Pupa	Adult	Total	%Mr	Corrected Mr *
Culex	379	25	269	673/1000	67.3	63.0
Anopheles	549	83	293	925/1000	92.5	91.05
Anopheles+Culex	402	26	336	764/1000	76.4	72.65
Total	1330	134	898	2362/3000	78.73	75.57
Control group						
Mosquito species	Larva	Pupa	Adult	Total	%Mr	
Culex	109	7	0	116/1000	11.6	
Anopheles	146	16	0	162/1000	16.2	
Anopheles+Culex	137	0	0	137/1000	13.7	
Total	392	23	0	415/3000	13.83	

* Percentage of mortality (IE%) is corrected by Abbott's formula
Mr: Mortality

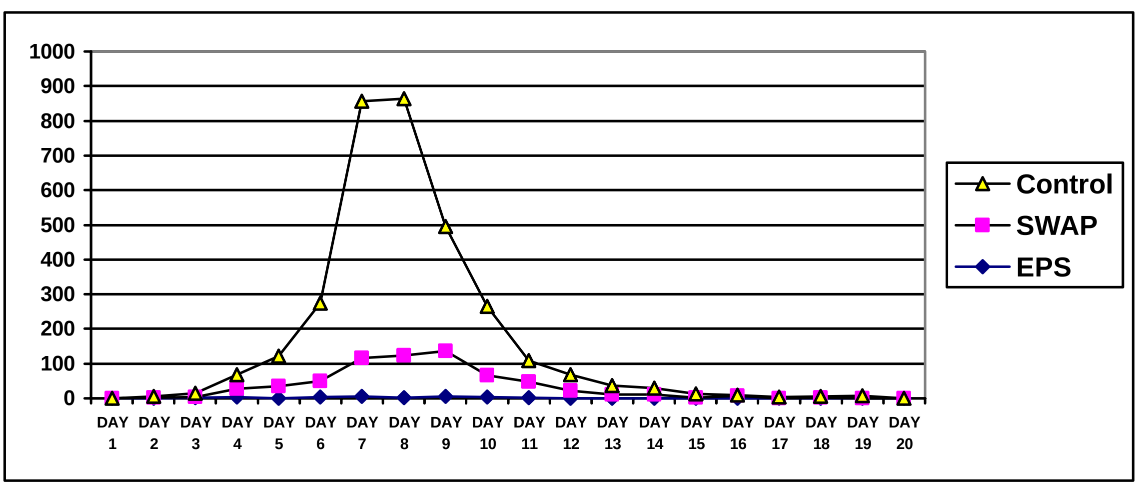
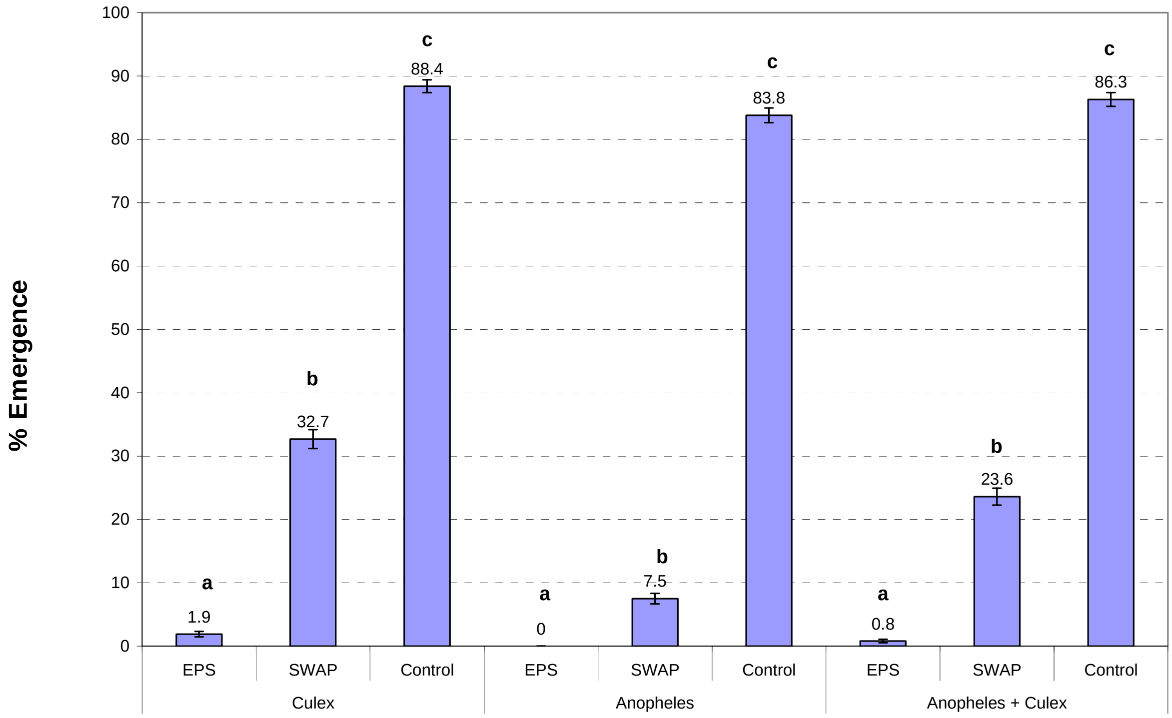


Fig. 2. Number of mosquitoes collected by aspirator from different rearing trays in treated and control experiments



The mean difference is significant at the 0.05 level

Fig. 3. Comparing the emergence of mosquitoes in two types of polystyrene

Discussion

There are several reports concerning use of polystyrene beads for elimination of mosquito emergence in the world in the cess pits, flooded cellars, soakage pits, water tanks, gem pits and industrial complex (Rietre 1978, Chavasse et al. 1995, Curtis and Minjas 1985, Curtis 1994, Maxwell et al. 1990, Reuben et al. 2001, Chandrahas and Sharma 1987, Yapabandara and Curtis 2002, Dua et al. 1989, Rieter 1985, Sharma 1985, Chandrahas et al. 1993). The layer of this product reduces the density of mosquito by suffocating larvae and pupae and inhibiting mosquito egg-laying in water surface.

Our results showed that utility of EPS and SWAP beads can be completely effective and practical for elimination of *An. stephensi* and *Cx. quinquefasciatus* under the laboratory conditions. Larval mortalities increased significantly ($P< 0.05$) with using both polystyrene forms and the mortality was greater in those larvae treated by EPS than SWAP ($P> 0.05$). Mortality rate for *Cx. quinquefasciatus, An.stephensi* and combination of both species was 97.8%, 100% and 99.07%, respectively using EPS. In average EPS was able to kill 98.9% of mosquitoes. Observations of the present study show that EPS beads induced 98.9% mortality in the tested mosquitoes, representing 98.61% and 0.34% in the larval and pupal stages, respectively. The figures for SWAP were 63%, 91.05% and 72.65%. The average mortality for mosquitoes was 75.57%. The SWAP beads caused 75.57% mortality: 42.55% larvae, 4.29% pupae and 28.73% in newly emerged adults that were trapped in SWAP beads.

Inhibition of Emerge (IE) for EPS obtained 100%, 97.8 and 99.07 in the case of *An. stephensi*, *Cx. quinquefasciatus* and *An. ste-*

phensi+Cx. quinquefasciatus, respectively. The IE for SWAP was calculated 91.05, 63.0 and 72.65 in *An.stephensi, Cx. quinquefasciatus* and *An.stephensi+Cx. quinquefasciatus*, respectively. Similar studies showed a mortality rate of 72.3-100% (Curtis et al. 1989, Sivagnaname 2005).

The observed mortality using EPS imply that this material provides an appropriate and unique floating layer on the water surface, however the SWAP beads due to its variability in beads' diameter and irregular shapes are not able to cover water surface completely, mortality and inhibition of emerge is less than that of EPS. This factor leads to adult emergence in SWAP treated trays. Although a relatively high number of mosquito larvae could converted to adults, but most of them were trapped among layers of SWAP beads consequence of trapping and dead. Some of emerging adults showed morphological abnormalities such as wing, leg, head, thorax and abdomen malformations at rearing trays treated by SWAP.

Difference in survival rate of *Anopheles* and *Culex* mosquitoes is another important point. The emergence rate for *An. stephensi* was 46.31%, among which 22.49% of them were trapped among SWAP beads and resulted to dead, so the survival rate for this species was evaluated as 23.82%. Concerning *Cx. quinquefasciatus,* 75.91% of larvae completed their development cycle and changed to adult stage, while 39.27% of them were dead and therefore the survival rate was calculated as 36.27%. The comparison between the survival rates of these two mosquito species showed that *Culex* has more relative ability to survive than *Anopheles* (1.5-fold). This ability may be is attributed to the morphological and physiological characteristics and respiratory system of the *Culex* larvae that allow them to breathe easier than *Anopheles* under the covering layers on water surface. Additionally *Culex* mosquitoes are able to survive in organic polluted water bodies. Parallel to our study, Nathan et al. (1996) conducted laboratory and filed trail on the use of commercially available, expanded-polystyrene beads for the control of *Cx. quinquefasciatus*. They were not able to gain 100% mortality in the population of mosquitoes in the laboratory. They concluded that the irregular shape of the SWAP particles greatly reduced their capacity to spread over the water surface and the interstitial air spaces also permitted respiration and development of mosquito larvae. Under field conditions and in wet pit latrines the control sustained for two months.

Efficacy of Agnique MMF, a monomolecular film formulation, was tested against immature of *An. stephensi* in India. Simulated field trials carried out in cement tanks showed 100% inhibition of adult emergence for up to 3 weeks. In tanks and wells it produced 75% reduction of late instars and 100% reduction of pupae on day 1 (Batra et al. 2006).

Beehler and DeFoliart (1991) used EPS for control of *Aedes triseriatus* in the laboratory and the field. EPS reduced the emergence of adults by preventing normal eclosion from the pupae. In the field, tree holes containing EPS had significantly fewer larvae than controls.

Bekheit et al. (1991) compared the oils and expanded polystyrene beads for the control of mosquito breeding in Egypt. Results revealed the superiority in effectiveness of beads over oil for the control of mosquito breeding. Mosquito catches from cesspits treated with polystyrene beads dropped to zero soon after application of the beads and remained so throughout five months observation period. A field trial of the use of EPS to control the breeding of mosquito larvae in household septic tanks was conducted by Chang et al. (1995). One week after treatment, the breeding of *Cx. quinquefasciatus* and *Ae. albopictus* was reduced by 100% and 68.7%, respectively. For both species combined, a 57.25% reduction in the adult emergence rate was achieved. No adult was caught in the emergence trap one month after treatment.

Data analysis of our results for mosquito's survival showed that there are significant differences between two polystyrene forms (*P*< 0.05). Using EPS, a significant difference observed between all mosquitoes (*Anopheles*, *Culex*, and their combination) as compared to the control group (*P*< 0.05), additionally there was significant difference between two species (*P*= 0.012). Similarly, in the SWAP tests a significant difference observed between all species and control (*P*< 0.05). Therefore, the present study clearly indicates that EPS beads could provide excellent larval control for mosquitoes. In addition, it was found that both materials had high larval control and both of them could be used in the control of mosquitoes. EPS beads could be used as one of the promising vector control methods to eliminate/ minimize the mosquito breeding sources in IVM programs, while SWAP appears to offer good prospects as an alternative to expanded-polystyrene beads for inexpensive, long-term mosquito control in selected habitats.

In total, regardless of efficacy of the method in the control of mosquito larval stage under laboratory conditions, the extensive field studies in the area (Iran) are recommended to evaluate and may be introduce this method as an easy and inexpensive way in mosquito control, especially in non-drinking water sources.

Trials of using polystyrene beads for control of mosquito have been conducted in several parts of the world (Curtis et al. 2002). The method greatly and substantially reduced the vector population. Utility of EPS and SWAP as a component of Integrated Vector Management with other supportive measures could assist considerably the process of elimination of mosquito-borne disease. Further study is required to the practicality of using these products in community-based, mosquito-control programs.

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