

# Field efficacy of expanded polystyrene and shredded waste polystyrene beads for mosquito control in artificial pools and field trials, Islamic Republic of Iran

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## النَّجَاحَةُ المِيدَانِيَّةُ لمُكَافَحَةِ البَعُوضِ بِخَرَزَاتِ البُولِي سَتِيرِينِ الموسعة وشرائط فضلات خَرَزَاتِ البُولِي سَتِيرِينِ فِي بَرَكِ صَنَاعِيَّةٍ وَتِجَارِبِ مِيدَانِيَّةٍ فِي جُمهُورِيَّةِ إِيرَانَ الْإِسْلَامِيَّةِ

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الخلاصة: أدت مشاعر القلق من مبيدات الهوام الكيميائية التقليدية إلى زيادة البحوث من أجل الوصول إلى طرق مكافحة مبتكرة للبعوض. وتقارن هذه الدراسة فعالية نوعين مختلفين من خرزات البولي ستيرين في مكافحة يرقات البعوض في جنوب شرق جمهورية إيران الإسلامية. وقد أجريت تجارب محاكاة ميدانية في برك صناعية وتجارب ميدانية في قريتين في منطقة مأهولة بالمalaria، باستخدام الطرق الموصى بها من منظمة الصحة العالمية. وقد أدى تطبيق الخرزات الموسعة للبولي ستيرين أو شرائط فضلات رقائق البولي ستيرين على أسطح البرك إلى اختلاف يعتد به في كثافة البعوض قبل المعالجة وبعدها (حدث خفض بمقدار 86% و 78% بعد أسبوعين من المعالجة). ولم يوجد فرق يعتد به في النجاعة بين النوعين. وعلى هذا فإن استخدام خرزات البولي ستيرين كمكون ضمن التدبير المتكامل للنواقل مع سائر التدابير الأخرى، يمكن أن يساعد في مكافحة الأمراض المنقولة بالبعوض في جمهورية إيران الإسلامية والبلدان المجاورة لها.

**ABSTRACT** Concerns about traditional chemical pesticides has led to increasing research into novel mosquito control methods. This study compared the effectiveness of 2 different types of polystyrene beads for control of mosquito larvae in south-east Islamic Republic of Iran. Simulated field trials were done in artificial pools and field trials were carried out in 2 villages in an indigenous malaria area using WHO-recommended methods. Application of expanded polystyrene beads or shredded, waste polystyrene chips to pool surfaces produced a significant difference between pre-treatment and post-treatment density of mosquitoes (86% and 78% reduction respectively 2 weeks after treatment). There was no significant difference between the efficacy of the 2 types of material. The use of polystyrene beads as a component of integrated vector management with other supportive measures could assist in the control of mosquito-borne diseases in the Islamic Republic of Iran and neighbouring countries.

## Effacité sur le terrain des billes de polystyrène expansé et de copeaux de polystyrène recyclé pour la lutte contre les moustiques dans des bassins artificiels et des essais sur le terrain en République islamique d'Iran

**RÉSUMÉ** Les préoccupations concernant les pesticides chimiques traditionnels ont conduit à intensifier la recherche sur de nouvelles méthodes de lutte contre les moustiques. La présente étude a comparé l'efficacité de deux types de billes de polystyrène dans la lutte antilarvaire dans le sud-est de la République islamique d'Iran. Les simulations d'essais sur le terrain ont été réalisées dans des bassins artificiels alors que les essais sur le terrain ont été menés dans deux villages d'une zone où le paludisme est autochtone, avec des méthodes recommandées par l'Organisation mondiale de la Santé. L'application de billes de polystyrène expansé ou de copeaux de polystyrène recyclé sur la surface des bassins a entraîné une différence significative entre la densité des moustiques avant et après le traitement (86% et 78% de réduction respectivement après deux semaines de traitement). Il n'y avait pas de différence significative entre l'efficacité des deux types de matériaux. L'utilisation de billes de polystyrène en tant que composante de la gestion intégrée des vecteurs avec d'autres mesures d'appui pourrait contribuer à la lutte contre les maladies transmises par des moustiques en République islamique d'Iran et dans les pays voisins.

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Received: 28/02/10; accepted: 26/05/10

## Introduction

Growing concerns about the potential health and environmental risks surrounding traditional chemical pesticides [1,2] have led environmental protection agencies to ban or place severe restrictions on the use of many pesticides that were formerly used in mosquito control programmes. There are now fewer adulticides available than there have been for the last 20 years [3]. It is likely, therefore, that mosquitoes will quickly develop high levels of resistance to the remaining available adulticides. This has led to concern among operational mosquito control personnel that effective insecticides may not be available in the near future [4]. Hence, it is imperative that novel mosquito control methods are developed and put into general use as soon as possible.

One potential alternative bio-environmental control measure in these habitats is the use of expanded polystyrene (EPS) beads. Besides hindering the respiration of larvae, thick layers of polystyrene beads prevent the emergence of adult mosquitoes and the layers present a dry surface unsuitable for egg laying [5,6]. These beads are nonpolluting and socially acceptable and their long-lasting, non-hazardous nature and one-time application renders it a cost-effective and feasible solution to mosquito control [1,6,7]. Shredded, waste polystyrene (SWAP) chips are another cheap and readily available product with similar properties.

According to previous studies in the Islamic Republic of Iran there are several genera of mosquitoes, including *Anopheles* spp., *Aedes* spp., *Culex* spp. and others, which normally share the same breeding places [8]. In some southern regions of the country, due to the lack of a piped water supply most villagers use stored water for drinking and washing. These habitats provide ideal conditions for mosquito breeding. Various re-emerged malaria epidemics have occurred in Iranian cities in the

recent years. A number of methods for mosquito control in the country have been reported, including source reduction, biocontrol (*Bacillus thuringiensis* and larvivorous fishes), larviciding and adulticiding [9–12].

The objective of the present study was to evaluate the efficacy of locally made industrial polystyrene products for mosquito control under simulated field and actual field trial conditions in Sistan and Baluchestan province, Islamic Republic of Iran. The expected outcome was to determine a safe, cheap, feasible, applicable and environmental friendly measure for mosquito larvae control.

## Methods

### Simulated field trials

Application of polystyrene beads was tested for their efficacy in controlling mosquito larvae and pupae of *Anopheles* spp. and *Culex* spp. under simulated field conditions in 2 phases during different seasons: December 2006 and June 2007.

The study was carried out in artificial pools at Bandar Abbas health research centre. Two months prior to the tests 6 artificial pools (5 m<sup>3</sup>: dimensions 1 × 1 × 0.5 m) were excavated and the base of the pools was covered with 2 thick layers of waterproof plastic. The artificial pools were filled with well-water in order to prepare breeding places for mosquitoes under natural conditions. Water levels in the artificial pools were monitored and maintained at 0.4 m depth by carefully adding fresh water periodically during the trials. Three artificial pools were selected for the larval density tests and remained uncovered. Three artificial pools were selected for the adult emergence test and covered with emergence traps (1 m<sup>3</sup> made of net with 156 holes/inch<sup>2</sup>) to record the daily number of adults emerging. For each set of tests, 1 pool was treated with EPS beads, 1 pool treated with

SWAP chips and 1 control pool was left untreated.

Before adding the polystyrene beads to the water surface, we calculated the daily density of larvae (density per 10 dippers) and adult emergence (by mouth aspirator) over 3 days. Then on the 3rd day polystyrene beads were added to the pools, and the densities of larvae and adults emerging were calculated and recorded daily for up to 2 weeks. Supplies of EPS and SWAP were purchased from a local Iranian factory. EPS beads (2 mm diameter) were applied in a 1 cm thick floating layer and SWAP was applied in a 1–2 cm floating layer. Air temperature and relative humidity were recorded daily. At the end of the experiments, water samples from the pools were analysed by gas chromatography for the levels of dissolved monostyrenes.

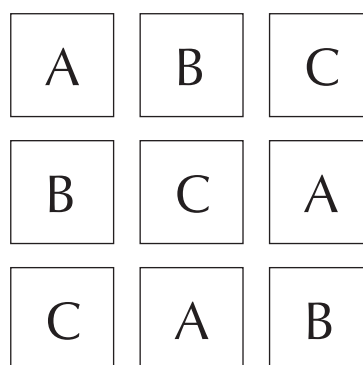
### Field trials under natural conditions

Field evaluations of polystyrene beads were carried out in 2 villages near Chabahar (Comb and Tis) where malaria is indigenous. The villages are located in Sistan and Baluchestan province in the south-east of the Islamic Republic of Iran (25°17'N, 60°38'E). The trials were carried out from September to November 2007.

Breeding sites were selected for the field trials based on high larval populations and evidence of continued oviposition. Among the various types of mosquito breeding habitats that were available in the study area, 9 cement water reservoirs, of over or underground construction, were selected for the present study. Before the polystyrene application, information on the mosquito species in the breeding sites was obtained by collecting samples of immature adults after emergence from all pools. These were kept separately in the laboratory according to habitat for species identification. Information about the general topography of the area,

weather conditions, water temperature, and pH of water also was recorded.

The pre-treatment density of mosquito larvae was calculated in all pools for 3 days. On the 3rd day EPS beads and SWAP chips were added to the water surface of treated pools, based on a Latin square design (Figure 1). The efficacy of the 2 types of polystyrene against larval and pupal density was evaluated and compared with control pools for 2 weeks after treatment and then after 1 month. Larval density was measured using a standard dipper consisting of a white aluminium bowl (diameter 9 cm, 300 mL capacity) [13]. A fixed number of 10 dippers were taken in the periphery and the centre of the pools. Larvae and pupae collected by dipping were pooled by site in white trays, counted and released back into the respective habitats. Samples of late-stage larvae and pupae were collected and brought to the laboratory for species identification after mounting. Pre-treatment and post-treatment densities of immature mosquitoes (I + II instars, III + IV instars, and pupae) per 10 dippers were recorded separately for both treatment and control sites.



**Figure 1 Schema of the field trials: A = expanded polystyrene beads; B = shredded, waste polystyrene chips; C = controls**

The efficacy and durability of effectiveness of the 2 type of polystyrene were determined from the post-treatment counts of larvae and pupae in treated and control sites as compared to the pre-treatment populations.

### Statistical analysis

Percentage reduction was calculated using the formula [13]: % reduction =  $100 - (C1 \times T2 / T1 \times C2) \times 100$ .

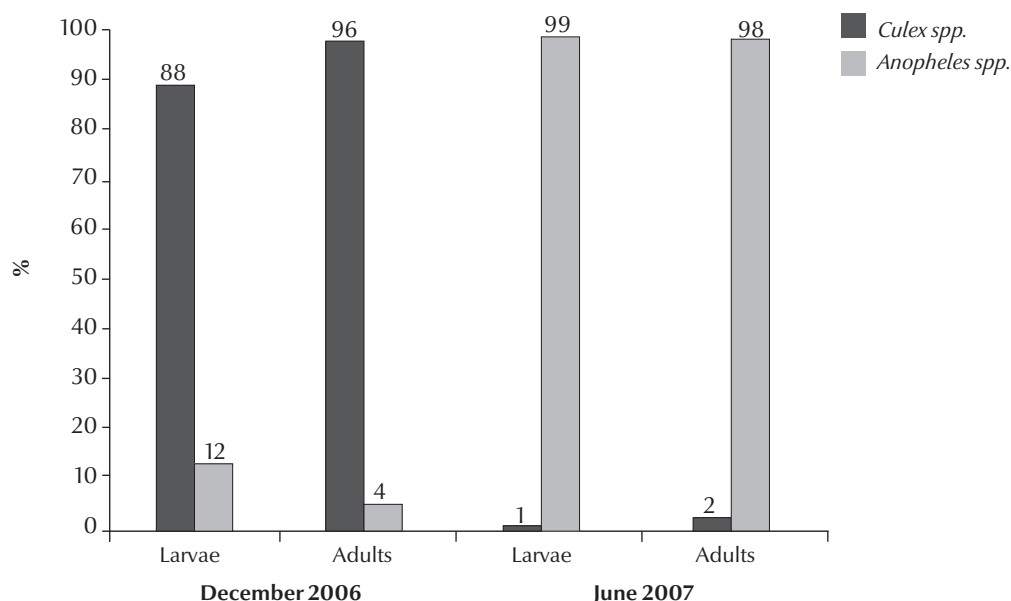
Where C1 and T1 are the pre-treatment immature densities in the control and treated habitat and C2 and T2 are the post-treatment

immature densities in control and treated habitat. Means were compared using statistical tests of non-parametric (Mann–Whitney U-test) using SPSS, version 11.5 software.  $P < 0.05$  indicated significant difference between treatments.

## Results

### Simulated field trials

The results of species identification of larvae and adult mosquitoes collected in artificial pools in 2 different seasons



**Figure 2 Comparison of the percentage of *Anopheles* spp. and *Culex* spp. mosquitoes (larvae and adults) collected in artificial pools at 2 different seasons, Bandar Abbas, 2006–07**

of 2007 are showed in Figure 2. This shows that in December *Culex* spp. mosquitoes were the dominant species, whereas in June, *Anopheles* spp. were dominant.

Trends of larval densities and adults emerging in EPS- and SWAP-treated and untreated artificial pools in the 2 different seasons are shown in Figure 3. Analysing the data for the 2 seasons combined showed no significant difference in the pre- and post-treatment periods in the number of mosquitoes in control artificial pools, either larvae ( $P = 0.410$ ) or adults ( $P = 0.376$ ). However, significant differences were clearly observed in the numbers of mosquitoes (adults and larvae) in the 2 treated artificial pools pre- and post-treatment, both for larva ( $P = 0.041$  and  $P = 0.007$  for EPS and SWAP respectively) and for adult stages ( $P$

$= 0.001$  and  $P = 0.012$  for EPS and SWAP respectively).

The levels of dissolved monostyrene are shown in Figure 4. This shows that the rate of detected monostyrene at all pools was considerably less than the Environmental Protection Agency's standard of polystyrene level for potable water (0.32 ppm) [14].

The prevalence of identified mosquitoes showed that *An. stephensi* and *Cx. pipiens* were the dominant species in Bandar Abbas (data not shown).

### Field trials

The larval densities pre- and post-treatment in treated and control pools are showed in Figure 5. The results showed no significant difference comparing the pre-treatment and post-treatment density in control pools ( $P = 0.621$ ), whereas a significant difference

was observed between pre-treatment and post-treatment density in both kinds of treatment pools ( $P = 0.001$  and  $P = 0.002$  for EPS and SWAP respectively). There was no significant difference in larval densities between EPS- and SWAP-treated pools ( $P = 0.15$ ).

In EPS-treated pools, a more than 86% reduction was observed in immature mosquito densities comparing the pre-treatment period with the 2 weeks post-treatment ( $P < 0.001$ ). The figure for SWAP-treated pools was a 78% reduction over the same period ( $P < 0.001$ ) (Figure 6).

The results of identified mosquito based on species composition showed the dominance of the vector species *An. stephensi* in this area (82%); considerably higher than *An. culicifacies* (9%), *An. dthali* (4%), *An.*

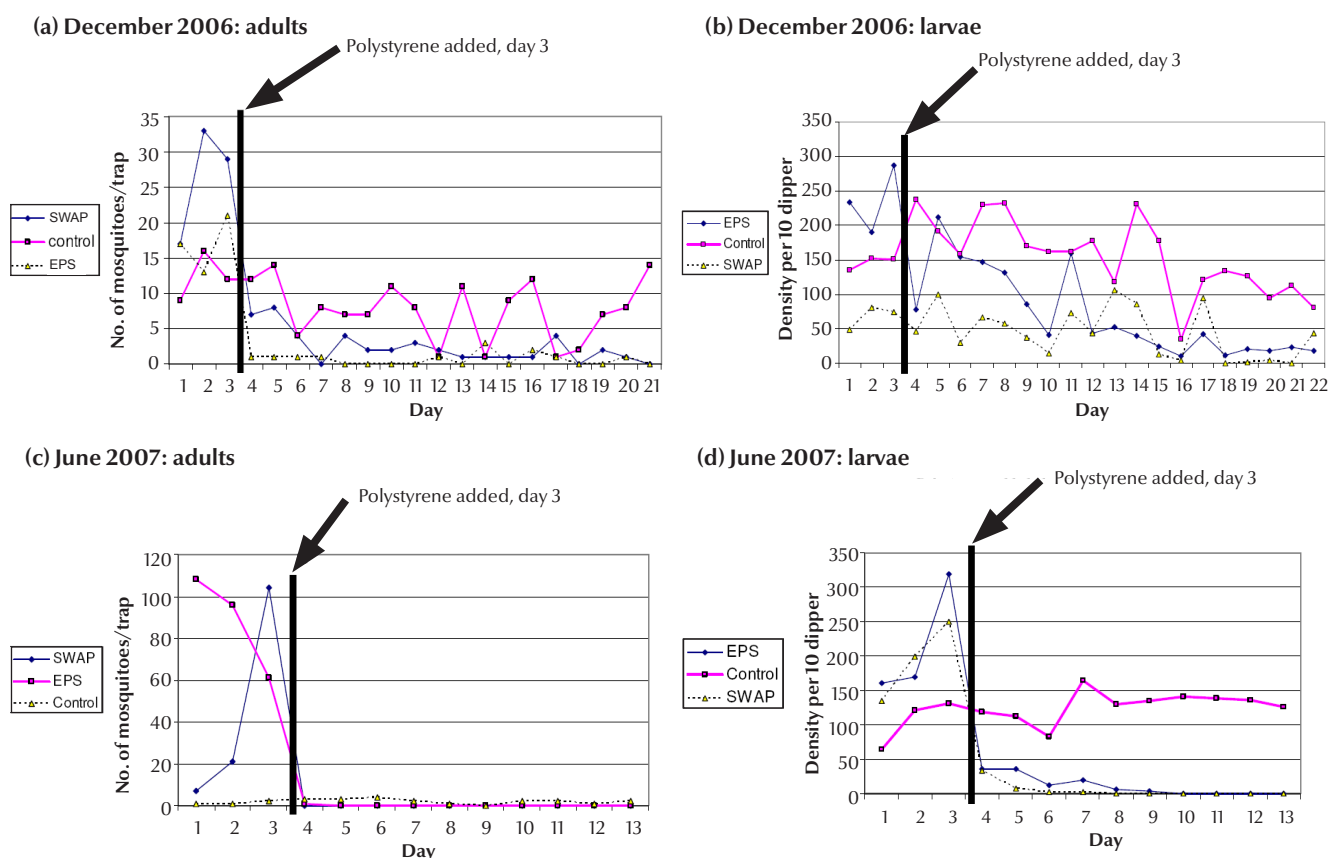


Figure 3 Trend of adult and immature populations of mosquitoes in treated and untreated artificial pools in 2 different seasons, Bandar Abbas, 2006-07, comparing treatment with expanded polystyrene (EPS) beads and shredded, waste polystyrene (SWAP) chips

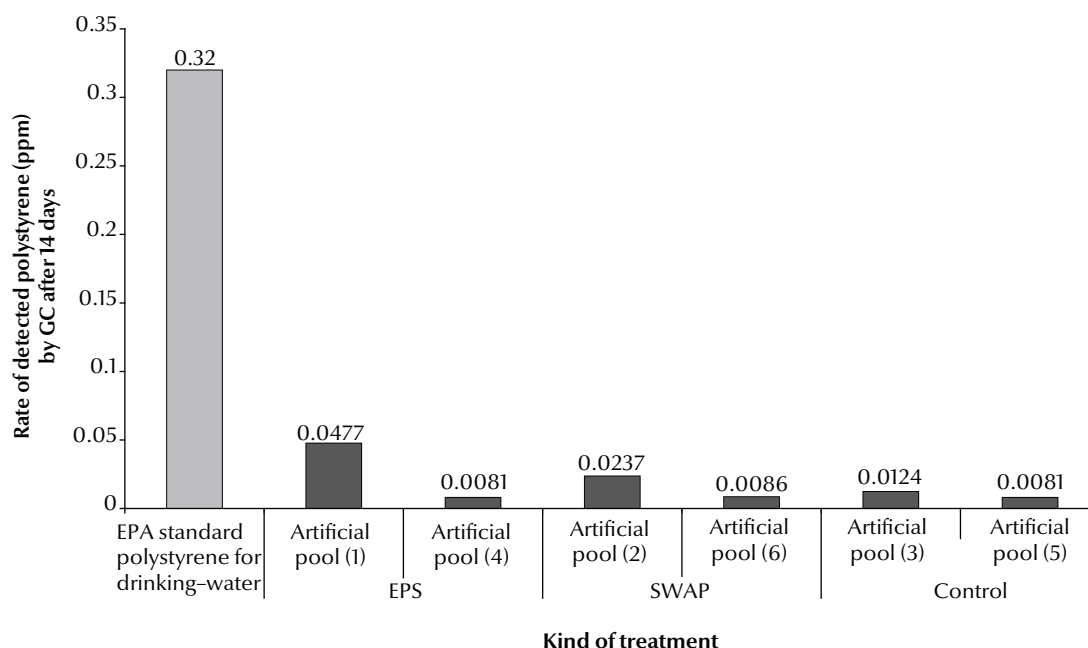


Figure 4 Monostyrene concentrations in water of artificial pools, untreated and treated with expanded polystyrene (EPS) beads and shredded, waste polystyrene (SWAP) chips, compared with the Environmental Protection Agency (EPA) standard

*turkhudi* (2%), *An. superpictus* (2%) and *An. sergentii* (1%).

## Discussion

There are several reports from other parts of the world concerning the use of polystyrene beads for elimination of

mosquito emergence in cesspits, flooded cellars, soakage pits, water tanks, gem pits and industrial complexes [5,15–26]. We have previously evaluated the efficacy of locally made industrial beads under laboratory conditions [27]. The polystyrene layer reduces the density of mosquitoes by suffocating larvae and pupae and inhibiting

mosquito egg-laying in water bodies. Our results also showed that EPS beads and SWAP chips are effective and practical methods for reduction of *An. stephensi*, *Cx. pipiens* and other mosquitoes, under simulated field and actual field trial conditions. Larval mortality increased significantly using both types of polystyrene.

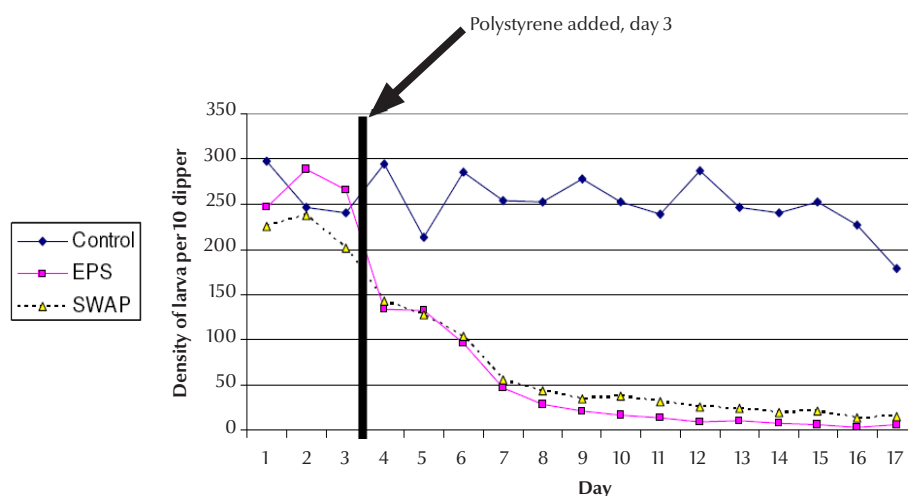
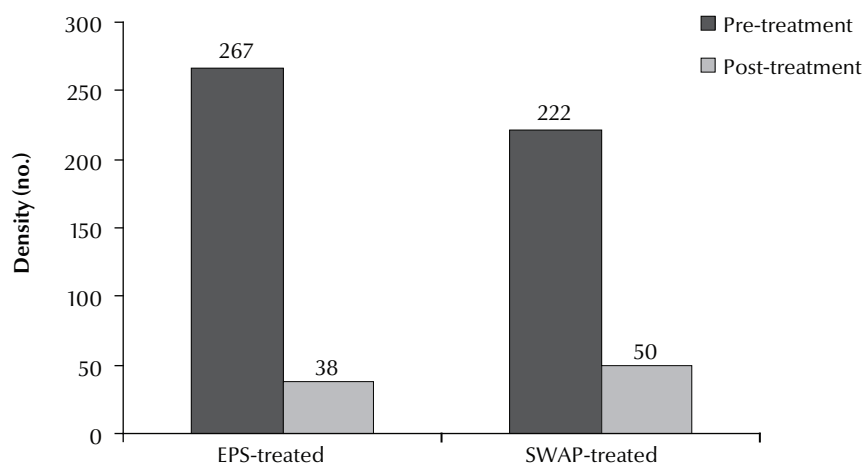


Figure 5 Trend of larvae population in field trials, Chabahar 2007, comparing treatment with expanded polystyrene (EPS) beads and shredded, waste polystyrene (SWAP) chips





**Figure 6** Density of immature mosquitoes (I + II instars, III + IV instars, and pupae) pre- and post-treatment in field trials, Chabahar 2007, comparing treatment with expanded polystyrene (EPS) beads and shredded, waste polystyrene (SWAP) chips

We observed an 86% and 78% reduction rate of immature mosquito densities for EPS- and SWAP-treated pools respectively in actual field trial situations. A range of surface treatments for mosquito control including polystyrene beads have been tested in other studies. Similar studies with EPS in Zimbabwe, India and Zanzibar showed a mortality rate of 72.3%–100% [6,7]. Reduction of emergence of *Ae. triseriatus* was found using scrap EPS beads in the laboratory and in field trials in tree holes [28]. The superiority of polystyrene beads over oil application to water surfaces on the control of mosquito breeding was found in cesspits in Egypt [29]. Reduction of *Cx. quinquefasciatus* and *Ae. Albopictus* densities using EPS beads in septic tanks was demonstrated in Tanzania [30]. A laboratory and field trial in the Caribbean using SWAP was not able to gain 100% efficacy against emergence of adult mosquitoes in the laboratory. The authors concluded that the irregular shapes of the SWAP chips reduced their capacity to spread over the water surface and the interstitial air spaces also permitted respiration and development of mosquito larvae. However, in field trials in pit latrines with careful application of SWAP the mean number of *Cx. quinquefasciatus* mosquitoes declined from 487 on day 1 to only 1 on day 63 and the control was sustained for

2 months [31]. The efficacy of Agnique® MMF, a monomolecular film formulation, was tested against immature forms 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 a 75% reduction of late instars and 100% reduction of pupae on day 1 [32].

In the present study the figures for mortality and inhibition of emergence with the SWAP material were lower than that of EPS beads, which may be due to variability in bead diameter and irregular shapes which are not able to cover the water surface completely. Nevertheless, both materials had high larval control efficacy and there was in fact no significant difference between their efficacies in field trials, which suggest that both of them could be used in the control of mosquitoes. Analysis of our data for mosquito densities showed significant differences between pre-treatment and post-treatment density for both kinds of treatment. Levels of dissolved monostyrenes were well within the Environmental Protection Agency standard. EPS beads are therefore a promising vector control strategy to minimize the mosquito breeding sources in integrated vector management programmes, while SWAP appears to offer good prospects as an alternative to EPS for inexpensive,

long-term mosquito control in selected habitats.

To be cost-effective appropriate vector control tools need to be directed in the right place and at the right time. Such methods should not be simply prescribed; instead site-specific control methods should be developed and implemented with a partnership approach. Application of polystyrene beads requires effective coverage. Polystyrene beads should be applied according to the nature of the habitats and can be applied in regions where water sources are restricted and water supply systems are non-piped, such as in the endemic malarious areas of Islamic Republic of Iran.

We conclude that the use of EPS beads and SWAP chips as a component of integrated vector management, together with other supportive measures, could assist in the elimination of mosquito-borne diseases in the Islamic Republic of Iran and neighbouring countries.

## Acknowledgements

The authors are grateful to the staff of Bandar Abbas health research centre for their close collaboration during the study. This project was financially supported by the Centre for Environmental Research, Tehran University of Medical Sciences.

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