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Efficacy of Expanded Polystyrene Beads (EPB) and Diesel oil for mosquitoes (Diptera: Culicidae) control in integrated vector control management (IVM) methods

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Abstract
The aim of this 1st time conducted study in Pakistan was to assess the efficacy of expanded polystyrene beads and diesel oil to control mosquitos' density. Expanded polystyrene beads and diesel oil was applied in mosquito larvae breeding sites and their effect on adult emergence reduction was evaluated over time. The % reduction in emergence of adult mosquitoes in expanded polystyrene beads treated sites ranged from 88.3% to 100%, whereas in Diesel oil treated sites were varied from 58.4% to 90.2%. So Expanded Polystyrene beads were found more effective and also durable than the diesel oil, whereas diesel oil remains effective for maximum 1 week and have to repeat application afterwards. Breeding sites treated with Expanded Polystyrene beads showed statistically significant ($p =< 0.04$) reduction in emergence of mosquitoes, whereas no significant reduction calculated in diesel oil applied sites. This analysis also reveals the superiority in effectiveness of expanded polystyrene beads over diesel oil. The use of this intervention as a component of integrated vector management (IVM) coupled with other supportive measures could help to control the menace of vector borne diseases.

Keywords: Expanded polystyrene beads, Diesel oil, Mosquito, Larvae

1. Introduction
Vector-borne diseases are illnesses caused by pathogens and parasites in human populations [1]. Every year there are more than 1 billion cases and over 1 million deaths from vector-borne diseases such as malaria, dengue, schistosomiasis, human African trypanosomiasis, leishmaniasis, Chagas disease, yellow fever, Japanese encephalitis and onchocerciasis, globally [1]. Vector-borne diseases account for over 17% of all infectious diseases coupled with globalization, unplanned urbanization and environmental challenges such as climate change are having a significant impact on disease transmission in recent years [2]. Some diseases, such as dengue, chikungunya and West Nile virus, are also emerging in countries where they were previously unknown [2].

Dengue is a mosquito-borne viral disease that has rapidly spread in all regions of WHO in recent years [3]. Dengue virus is transmitted by female mosquitoes mainly of the species *Aedes aegypti* and, to a lesser extent, *Aedes albopictus*. This mosquito also transmits chikungunya, yellow fever and Zika infection [3]. In 2010, 2013 and 2015, nearly 2.4 million cases were reported annually. Although the full global burden of the disease is uncertain [3]. Several mosquito species have been found to be naturally infected with Zika virus, including *Aedes africanus*, *Aedes luteocephalus*, *Aedes hensilli*, *Aedes polynesiensis*, *Aedes dalzielii*, *Aedes albopictus*, *Aedes apicoargenteus*, and *Aedes aegypti* among others, but little is known about their vector competence [4, 5]. In Pakistan, major vector borne diseases (VBDs) in the country include malaria, dengue/dengue haemorrhagic fever (DHF), leishmaniasis and Crimean Congo Haemmorhagic Fever (CCHF) [6]. Since 2005, Pakistan facing regular outbresks of dengue/DHF across the country, claimimg more than 155 human lives during last five years[6]. This shows that there is rising trend of VBDs in country [6]. Malaria is 2nd most prevalent and devastating disease and accounts 16% disease load of the country [7, 8].

Culex pipiens and *Culex quinquefasciatus* are important vectors of many diseases, such as West Nile virus, St. Louis encephalitis virus, Eastern Equine Encephalitis virus, Japanese Encephalitis virus, Chikungunja virus,

Wucheria bancroftii and the pathogens that cause lymphatic filariasis [9]. These mosquitoes are widespread globally and especially common in tropical and temperate regions [10]. They are often resistant to insecticides, a feature that may be related to their habit of breeding in habitats that are frequently exposed to insecticide runoff, such as sewers and drainage ditches [11]. The larvae prefer eutrophic waters with a high organic content. Exceptionally high densities of larvae may be found in the septic water associated with oxidation ponds, sewage drains, cesspools, and septic tanks [12]. The larviciding oils are probably the least studied of the mosquito larvicides, despite their long period of use for mosquito control. Early mosquito control measures were often drastic, with broad and dramatic consequences. Campaigns in this crusade included spreading crude oil, kerosine, or diesel oil across the surface of water bodies to suffocate mosquito larvae [13]. Unexpanded polystyrene beads contain an expanding agent that when heated rapidly expands the beads up to 30 times in volume [14]. EPS beads do not need frequent application since they remain on the surface for lengthy duration of time (up to four years). EPS beads represent an excellent advantage to other mosquito control methods like insecticides in that they have less of an environmental impact [15].

Petroleum based mineral oils have been used for insect pest control for over a century [16]. Initially used as 'ancestral insecticides', petroleum products (PP), such as petrol, kerosene, engine oil and waste oil, have in the recent past produced spectacular results for larviciding in several communities and, were advocated for vector control by several National Malaria Control Programmes [17]. This traditional vector control method is still in use in communities where the populations are poor and do not have the financial resources to employ conventional insecticides.

Materials and methods

Study site selection

An experimental study on Field evaluation of efficacy of expanded polystyrene beads and diesel oil for mosquito's emergence reduction was carried out from March to September 2015 at different localities of district Lahore, Pakistan. Larvae breeding sites from following areas namely Babu Sabu, PWD colony, Muslim Town Mor and Miyani Qaberistan of district Lahore were selected for implementations of experiment. About Ten larvae rich breeding sites (high larval populations and evidence of continued oviposition) were selected including cemented water reservoirs, fountains and standing or slow moving water.

Application of Expanded polystyrene breeds and Diesel oil

Mosquitoes larvae breeding sites were divided into three groups on the basis of experiment application i.e. (1) control group, (2) expanded polystyrene beads and (3) diesel oil group.

(1) In control group no treatment was applied and was observed under natural population fluctuations. (2) In expanded polystyrene beads group (EPB), it was applied at quantity100g/m^2 using a 2 liter capacity plastic bowl containing 40 g of expanded polystyrene beads by volume [18]. (3) In diesel oil group, diesel oil was added on the water surface. The control group was remained uncovered while the breeding sites in the expanded polystyrene group and diesel oil group were covered with the emergence traps (1 m^3 made of net with 156 holes/$inch^2$) to record the daily number of adult emerging.

Before adding the polystyrene beads and diesel oil to the water surface, information on mosquito species in each breeding sites and the larval density was calculated (density per 10 dippers) and adult emergence (by mouth aspirator) over 3 days. Then on the 3rd day polystyrene beads and diesel oil was added to the water surface of breeding sites, and the densities of larvae and adults emerging was calculated and recorded daily for up to about 2 weeks.

Data analysis

Percentage of reduction in Adult emergence was calculated using the formula [19].

%reduction= 100-(C1×T2)/T1×C2) ×100 Where

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

Mean values were compared using statistical test ANOVA using software (SPSS version 17)

Results

In current study Table: 1, shows that mosquito's natural breeding habitats treated with expended polystyrene beads (EPB) and Diesel oil showed little variations in adult emergence at localities named, Babu Sabu (Larvae source; Fountain, Genus; Culex), PWD colony (Cemented water, Genus; Culex), Muslim town mor (Fountain, Genus; Aedes), Miyani Qaberstan (Cemented water tank, Genus: Aedes & Culex at site No. 4,5&6, Culex at site 7 and Aedes at site 8,9& 10) (Table 1).

Our result demonstrate that recorded % reduction in emergence of mosquitoes in expanded Polystyrene beads (EPB) treated sites were 98.72%, 88.3%, 100%, 95.5%, 100%, 94.5%, 97%, 100%,100% and 100% respective to the serial vise source (Table1). The % reduction in emergence of adult mosquitoes in Diesel oil treated sites were 90.24%, 75.23%, 88.5%, 72.8%, 58.4%, 84.6%, 88.6%, 76.63% and 88.4% respectively (table1).

In Table 1 statistical analysis validate that there was a significant reduction in adult emergence where the breeding places were treated with Polystyrene beads (EPB). Significant reduction in adult emergence found in fountain ($p=0.01$), cemented water reservoir at site No. 4, 5, 6, 8, 9 and 10 with p value ($p=0.02$), ($p=0.04$), ($p=0.01$), ($p=0.04$), ($p=0.02$) and ($p=0.01$). Result shows that there was No any significant reduction observed ($p>0.05$) in adult mosquito emergence where site were treated with diesel oil groups.

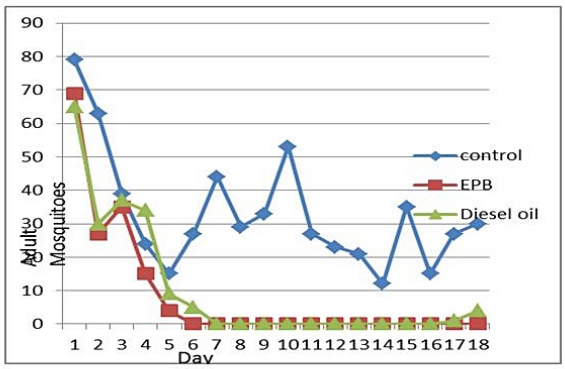
In our study each site where expended polystyrene beads (EPB) and diesel oil was applied is graphically represented. In our findings graphs Number 1 to 10, display adult mosquitoes emergence after site treatment. It could be clearly perceived from the graphs that the mosquito's population in control groups showed fluctuations during 18 days of field trials but adult mosquito emergence remained persistent. Whereas, expended polystyrene beads EPB group showed drastic reduction in emergence of adult mosquitoes in each site, it started decreasing adult emergence from day 4 after one day of site treatment and attains zero emergences and fixed the status until trials ends. The diesel oil group showed reduction in emergence of adult mosquitoes from day 5, after two days of diesel application and remained effective and maintained the zero emergences for only average 10 days.

Table 1: Percent (%) Reduction in emergence of Adult Mosquitoes after application of Expanded Polystyrene Beads (EPB) and Diesel oil

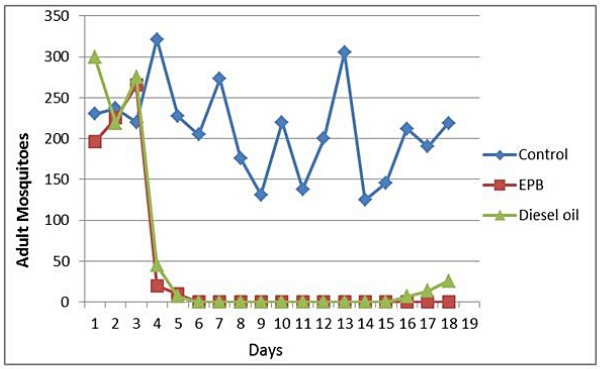
Location	Larval Habitat/ Source	Site No	Genus	% Reduction in EMG		P Value	
				EPB	Diesel oil	EPB	Diesel oil
Babu Sabu	Fountain	1	Culex	98.72	90.24	0.07	0.118
PWD colony	Cemented water reservoir	2	Culex	88.3	75.23	0.08	0.144
Muslim town mor	Fountain	3	Aedes	100	88.5	0.01*	0.12
Miyani Qaberstan	Cemented water reservior	4	Aedes & culex	95.5	72.8	0.02*	0.09
		5	Aedes & culex	100	58.4	0.045*	0.064
		6	Aedes & Culex	94.5	84.6	0.010*	0.119
		7	Culex	97	88.6	0.091	0.094
		8	Aedes	100	88.4	0.048*	0.096
		9	Aedes	100	78.9	0.025*	0.105
		10	Aedes	100	76.63	0.010*	0.281

Graph 1-10: Showing the Adult mosquitoes emergence over time after sites treated with Expanded Polystyrene Beads (EPB) and Diesel oil

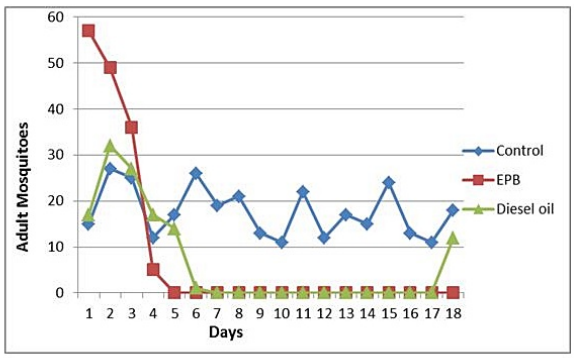
Graph.1. Site no 1. Fountain



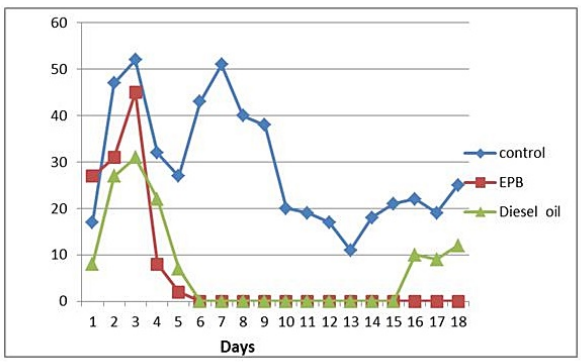
Graph.2. Site no 2. Cemented water reservoir



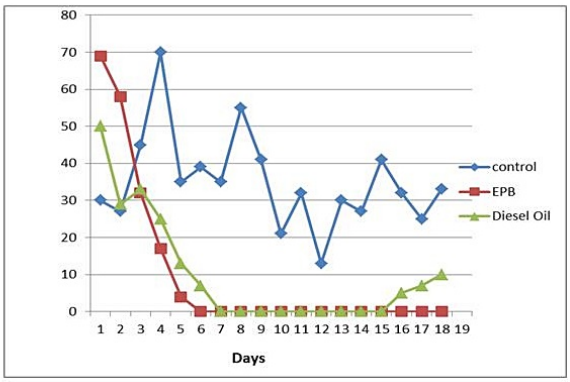
Graph.3. Site no 3. Fountain



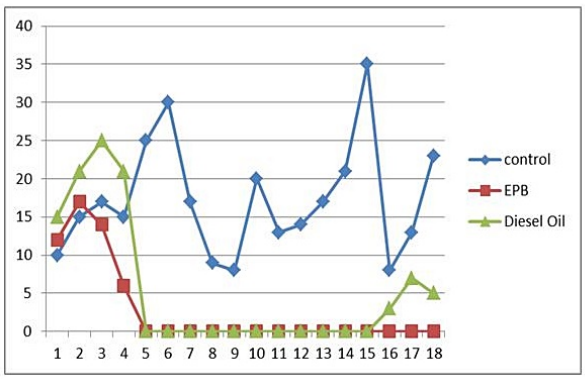
Graph.4. Site no 4. Cemented water reservoir



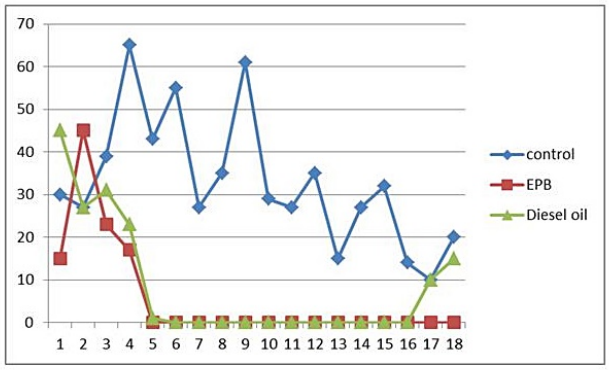
Graph.5. Site no 5. Cemented water reser-



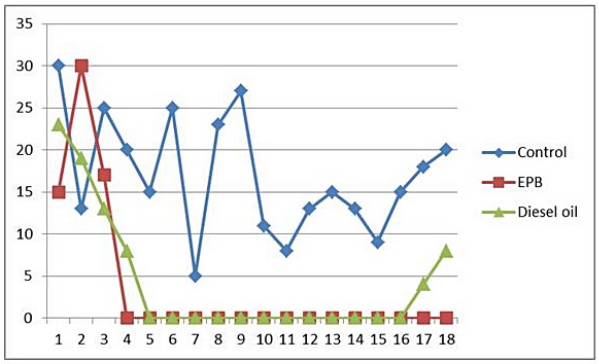
Graph.6. Site no 6. Cemented



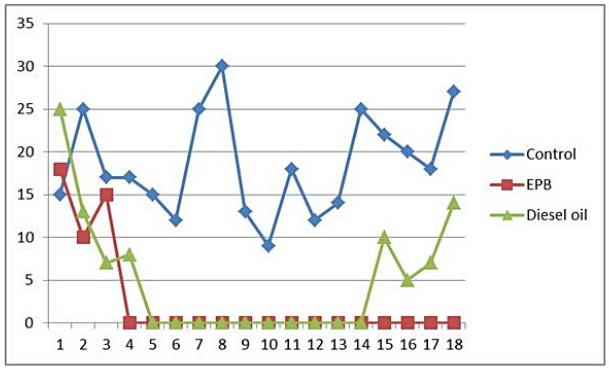
Graph.7. Site no 7. Cemented water reservoir



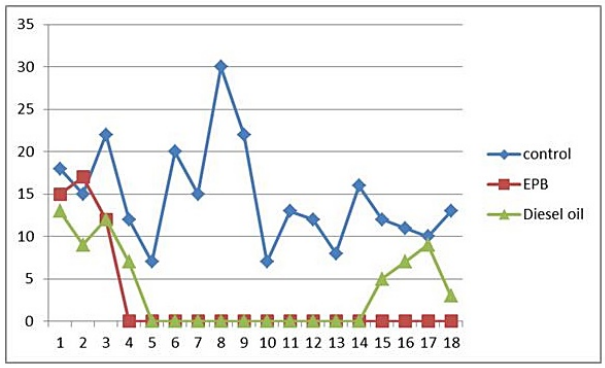
Graph.8. Site no 8. Cemented water reser-



Graph.9. Site no 9. Cemented water reservoir



Graph.10. Site no 10. Cemented water reservoir



Discussion

Comparative study conducted on the efficacy of polystyrene beads and diesel oil in actual field conditions reveals the superiority of polystyrene beads over diesel oil. There are also several similar studies 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 [20, 21, 1, 8]. The polystyrene beads layer reduces the emergence of adult mosquitoes by suffocating the larvae, pupae and inhibiting mosquito egg laying in water bodies [18]. Our results showed that EPB are effective and practical method for the reduction of Culex, Aedes and other mosquitoes, under actual field conditions, while diesel oil is only effective for 1-2 weeks. Similar study compared the oil and expanded polystyrene beads for the control of mosquito breeding in Egypt and results revealed the superiority in effectiveness of beads over oil for the control of mosquito breeding [22]. In 2014, similar kinde of experiment was conducted in the insectaries of Londonn School of Hygiene and Tropical Medicine. Larval mortality and adult emergence of *Cu. quinquefasciatus* were monitored in bowls with four treatments, EPS beads, PLA beads, corn starch shreds and a control. EPS and PLA beads resulted effective with 100% mortality after 10 days [23]. Field trials of the use of EPB to control the breeding of mosquito's larvae in artificial pools were also done in South-east Islamic Republic of Iran, where the application of expanded polystyrene beads (EPB) to pool surfaces produced a significant difference between pre-treatment and post-treatment density of mosquitoes within two weeks [24]. In average, EPS was able to kill 98.9% of larvae under laboratory condituons [24]. A field trial conducted on the use of EPS to control the breeding of mosquito larvae in household septic tanks in 1995 showed that one week after treatment, the breeding of *Cu. quinquefasciatus* and *Ae. albopictus* was reduced by 100% and 68.7% respectively and no any adult was caught in emergence trap one month after the treatment [25]. Through statistical analysis it was confirmed that there was a significant reduction in adult emergence, where the breeding places were treated with expanded polystyrene beads and there was no significant reduction observed in adult mosquito emergence in diesel oil treated group [25]. Similar scenario was also witnessed in South-east Republic of Iran [24]. Similar study conducted at Egypt also indorses our findings by revealing the superiority in effectiveness of expanded polystyrene beads over oil for the control of mosquito breeding [22].

Conclusion and recommendations

The pioneer study in Pakistan on such experiment concluded that the use of Polystyrene beads as a component of integrated vector management coupled with other supportive control measures could assist in elimination of mosquitoes borne diseases in Pakistan.

The cost effective appropriate vector control methods need to be directed in the right place and the right time. Such control measures should not be simply prescribed; instead site specific control methods should be developed and implemented with a partnership approach. An expanded polystyrene bead requires effective coverage and should be applied according to the nature of the habitats. It can be applied in area where water resource is restricted and water supply systems are non-piped.

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