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Laboratory and field comparisons of pyriproxyfen, polystyrene beads and other larvicidal methods against malaria vectors in Sri Lanka

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Abstract

Hand-dug gem pits are important breeding sites for larvae of malaria vectors in Sri Lanka. Therefore, studies were carried out to help to select an effective, economic and convenient method that could be used to control malaria vector mosquito breeding in gem pits in a mining area. The effectiveness of four types of floating layers of polystyrene was compared in the laboratory and it was found that 2 mm expanded beads were the most effective for suffocating *Anopheles* larvae and pupae. The insect growth regulator, pyriproxyfen at dosages of 0.01 and 0.1 mg/l were tested in the laboratory and complete inhibition of emergence was found at both concentrations. A small-scale field trial was carried out for over a year to assess the efficacy of two concentrations of pyriproxyfen, 2 mm diameter expanded polystyrene beads, temephos, used engine oil and filling pits with soil. Pyriproxyfen only required re-application twice a year, whereas temephos or oil require 12 applications per year. Due to re-excavation by gem miners, polystyrene beads and filling of pits were not as permanent solutions as was expected. Calculations based on all available data showed that two annual treatments with pyriproxyfen at 0.01 mg/l would be the most cost-effective method with oil only slightly more expensive. However, the reduced required frequency for visiting every pit made the pyriproxyfen method the one of choice. The same low concentration of pyriproxyfen also effectively inhibited emergence of adults from river-bed pools.

Keywords: Anopheles larval control; Sri Lanka; Pyriproxyfen; Expanded polystyrene

1. Introduction

After near eradication of malaria in Sri Lanka in the 1960s, this disease is once again one of the major public health problems in this country. Shallow pits left by the gem miners which accumulate water during the rainy season are major breeding places of *Anopheles culicifacies* and *A. subpictus* in Matale and Polonnaruwa District (Wickramasinghe, 1991; Yapabandara, 1997). These two mosquito species, as well as *A. varuna*, were shown by ELISA tests to be vectors of

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Plasmodium vivax and *P. falciparum* in these areas (Yapabandara, 1997).

There are practical difficulties with the use of larvivorous fish because they would have to be re-stocked when the numerous pits refill after a dry spell. Filling of pits by bulldozers is difficult because of limited access. Oil and temephos have long been use in Sri Lanka to control *A. culicifacies* larvae and pupae and their cost effectiveness in gem pits was calculated on a theoretical basis (Wickramasinghe, 1981, 1991), but no attempts had been made to determine this operationally.

Data are available on long term control of anopheline larvae after a single application of a granular formulation of the insect growth regulator pyriproxyfen at concentrations ranging from 0.01 to 0.1 mg/l (Kawada et al., 1988; Suzuki et al., 1989; Okazawa et al., 1991). Pyriproxyfen is a juvenile hormone mimic to which different instars of larvae and species of mosquitoes vary in sensitivity (Okazawa et al., 1991; Schaefer et al., 1988). Pyriproxyfen has a high margin of safety for fish, mammals and most non-target organisms including dragon flies, which are significant mosquito predators (Mulla et al., 1986; Schaefer et al., 1988; Mulla, 1995). In the present study the effectiveness against *A. culicifacies* and *A. subpictus* when exposure began early or late in larval life was evaluated.

Floating layers of polystyrene beads were suggested as a long lasting means of suffocating mosquito larvae in stagnant water in confined spaces such as pits which do not flood (Reiter, 1978). This method has been shown to be effective against C*ulex quinquefasciatus*, and also anophelines, in stagnant water confined within walls such as cess pits, pit latrines, wells and overhead tanks (Curtis and Minjas, 1985; Chandrahas and Sharma, 1987; Maxwell et al., 1990). Some of the gem pits fit the description of stagnant water confined within walls and were not subject to flooding and it seemed logical to test expanded polystyrene layers on these pits. To reduce the cost and increase availability, use of layers of broken pieces of polystyrene packing material has been tested (Nathan et al., 1996) and polystyrene in pits has been integrated with pyriproxyfen in more open sites which were unable to retain polystyrene beads layers because of flooding (Chavasse et al., 1995).

The present study was carried out to investigate the relative effectiveness, economy and convenience of these methods for controlling malaria vector breeding in gem pits. In particular, studies were carried out on (i) the effectiveness against *A. culicifacies* of the four available types of polystyrene in the laboratory; (ii) the relative efficacy of 0.01 and 0.1 mg/l of pyriproxyfen (Adeal 0.5% G) against all instars of larvae and pupae of *A. culicifacies* and *A. subpictus* in the laboratory; and (iii) to determine the effect of the selected type of polystyrene, two concentrations of pyriproxyfen, temephos, used engine oil and the closure of gem pits on anophelines breeding in gem pits; (iv) to evaluate the cost effectiveness of these methods taking into account that polystyrene has the potential to be very economic in the long term, but only provided that it remains undisturbed; and (v) to test the effect of two concentrations of pyriproxyfen on larvae in river-bed pools which make an important contribution to the breeding sites of the above mentioned vector species, as well as *A. varuna* in the gem mining area (Wickramasinghe, 1991; Yapabandara, 1997).

Pyriproxyfen inhibits the normal development of mosquitoes, but does not cause rapid killing of larvae. Therefore, its effect cannot be tested by observing larval density: the number of adults which emerge is the only criterion for measurement of its impact (Mulla et al., 1974; Kawada et al., 1988). Attempts were made to use emergence traps set over gem pits to monitor natural emergence but these were unsuccessful. Therefore, in the present trials, the water of pyriproxyfen treated pits was bioassayed for its impact in preventing completion of development from the larval to the adult stage. These bioassays were carried out (i) in situ by placing larvae in floating cages in the pits; and (ii) by bringing water samples from the pits to the laboratory. Floating cages were also used to evaluate temephos, oil and polystyrene.

2. Materials and methods

2.1. General procedure for rearing of A. culicifacies and A. subpictus

Wild *A. culicifacies* and *A. subpictus* were collected from cattle baited huts from various parts of Sri Lanka and were colonised in a hut in the study area, Elahera. This hut was made of woven *cadjan* (palm thatch) walls and roof and was prevented from becoming too hot and dry by dampening the walls. The adult mosquitoes in the colony were blood fed twice a week using 1–2-week-old chickens. Larvae were fed with Farex baby food (Glaxo laboratories, India) once a day. The water in the trays was changed every other day. Larvae produced were sufficient for the laboratory and field bioassay experiments described.

2.2. Efficacy of two concentrations of pyriproxyfen when applied to different stages in the development of A. culicifacies and A. subpictus

Pyriproxyfen in the form of 0.5% Adeal sand granules provided by the Sumitomo Chemical Co. Ltd., Osaka, Japan was used for this study. Twenty 1st, 2nd, 3rd and 4th instar larvae and pupae of colonised *A. subpictus* and *A. culicifacies* were introduced into 500 ml jars containing 0.01 or 0.1 mg/l pyriproxyfen in 250 ml of water and covered with a nylon net and kept in the field laboratory. Each species and instar was tested separately. Larvae were fed with 'Farex' baby food on alternate days. Controls were held without pyriproxyfen. Each treatment and control was replicated four times. Mortality was recorded at 24-h intervals till all had emerged or died.

2.3. Relative efficacy of four types of polystyrene against 3rd and 4th instars of A. culicifacies larvae

Four types of polystyrene were used:
(a) Polystyrene Grade R 543FE obtained from Shell International Chemical Company, London and expandable to 2 mm diameter.
(b) A mixture of different types of expanded polystyrene beads of 2 mm to 1 cm diameter from Richard Peiris and Co., Sri Lanka.
(c) Scrap polystyrene pieces of size 3 mm to 1 cm and of irregular shape (Richard Peiris and Co., Sri Lanka).
(d) Small polystyrene pieces less than 2 mm in size and of irregular shape were obtained by sieving the scrap polystyrene using a 2×2 mm mesh.

Ten laboratory reared *A. culicifacies* 3rd and ten 4th instar larvae were placed in a jar containing 250 ml of water. Sufficient Farex was added to the water as larval food. A total of 15 identical jars were prepared. Into three replicate jars each of the above types of polystyrene were added to form 1 cm thick layer on the water surface and controls were held without treatment. All the jars were covered with nylon netting. Observations were recorded at 1 h intervals up to 18 h and every 6 h thereafter, till all larvae died or adults emerged.

2.4. Relative efficacy of 2 mm diameter expanded polystyrene beads for control of different instars of larvae and pupae of A. culicifacies and A. subpictus

Twenty 1st, 2nd, 3rd and 4th instar larvae and pupae of colonised *A. culicifacies* and *A. subpictus* were placed in jars containing 250 ml of water. The species and instars were kept separately and were fed with Farex baby food on alternate days. A layer of 2 mm diameter expanded polystyrene beads were placed on the water surface at a thickness of 1 cm and controls were held without polystyrene beads. Each treatment was replicated four times. Mortality and emergence were recorded at 1 h intervals up to 18 and 6 h thereafter till all larvae and pupae died or adults emerged.

2.5. Effectiveness of 2 mm expanded polystyrene beads in the control of A. culicifacies and A. subpictus oviposition

Batches of ten blood fed *A. culicifacies* or *A. subpictus* females collected from cattle baited huts were placed in $30 \times 30 \times 30$ cm cages. Into each cage an oviposition bowl containing 250 ml of water with a floating 1 cm thick layer of 2 mm diameter expanded polystyrene beads was introduced. Controls were held with the same number of blood fed females and with oviposition bowls without polystyrene beads. Treatments and controls were replicated four times. After 1 day, the laid eggs were collected and counted.

2.6. Trial of six methods for control of breeding of *A. culicifacies* and other anophelines in gem pits

A total of 150 gem pits in a village in the Elahera gem mining area in Sri Lanka were investigated for anopheline breeding. From these, 34 pits containing *A. culicifacies* and *A. subpictus* aquatic stages and approximately similar in size and depth of the water level, were selected. The 34 pits were numbered and randomly assigned for the following treatments, each treatment being replicated in four pits. There were also untreated control pits. The treatments were the following.

2.6.1. Expanded polystyrene beads

Polystyrene granules (Shell grade R 543FE) were expanded to produce 2 mm beads in boiling water over a wood fire. The expanded beads were collected using a sieve and beads were applied to the gem pits to form a 1 cm thick layer. This required 10 l (ca. 400 g)/m^2 of water surface.

2.6.2. Temephos

Temephos was sprayed with a Hudson hand compression sprayer. The dose was equivalent to that recommended by WHO (1984) of 0.111 kg a.i./ha but this recommendation assumes a mean water depth of 10 cm. The gem pits are much deeper than this and the application rate was increased proportionately. About 20 ml of temephos 50% EC was added to 9 l of water in the tank and the necessary volume for each pit was applied by a timed application calculated for the surface of the pit and taking into account the pump emission rate of about 750 ml/min.

2.6.3. Pyriproxyfen

The weight of the pyriproxyfen granules (S-31183, Adeal 0.5% G) to be applied to the gem pits was calculated taking into account the measured depth of the water and diameter of the pits. Pyriproxyfen was applied at targets of 0.01 or 0.1 mg a.i./l (i.e. 2 or 20 g granules/m^3) using a spoon, which applied a measured quantity of granules.

2.6.4. Oil

On the basis of the recommendation of WHO (1973), dosage of 80 l/ha of a mixture of 70% waste engine oil + 30% diesel + 0.5% teepol was applied per hectare of water surface. The amount for treatment of a gem pit was calculated from its water surface area. This mixture of oil was applied using a Hudson compression sprayer.

2.6.5. Closure of gem pits

Four gem pits were filled with soil using hand tools.

2.7. Bioassay of effects of pyriproxyfen and other treatments

Field bioassays were by observing the development and adult emergence from *A. culicifacies* 4th instar larvae, which were introduced into pit water in cages made from 5 l capacity buckets floating in the pits. A series of 7×5 cm square holes were drilled about 2 cm from the bottom of each bucket and they were covered with 100-mesh nylon strainer cloth to allow water circulation between the bucket and the pit, but to prevent escape of the larvae; the top of the bucket was covered with a net. A hole was cut in the middle of a $25 \times 25 \times 3$ cm polystyrene board and the bucket was inserted to a depth of 15 cm and floated in the water of a gem pit. The bucket handle was tethered to a pole at the side of the gem pit to prevent the bucket being carried away in the event of overflowing of water from the gem pits. Ten laboratory reared 3rd–4th instar larvae of *A. culicifacies* were put into the bucket. The larvae were fed daily with Farex baby food. The bucket was removed from the gem pit daily and any emerged adults were collected. Then the net was removed and dead and live larvae and pupae were counted in the 2 cm of water remaining in the bottom of the bucket. These observations were made daily until all the larvae had died or developed into adults. Similar tests were conducted in control pits. In the polystyrene treated pits, a 1 cm thick layer of 2 mm diameter polystyrene was added to a bucket and larval survival was checked after removing the polystyrene beads.

Additional bioassays were conducted in the laboratory by bringing 500 ml of water from pyriproxyfen treated and control pits. Laboratory reared *A. culicifacies* 3rd–4th instar larvae were introduced and Farex baby food was given. The jar was covered with a netting cloth and observed till all larvae had died or developed into adults.

New field and laboratory bioassays were initiated every 10 days. If any adults emerged in the field bioassay of a treated pit, that pit was retreated. In the absence, at the time of the test, of a ready supply of *A. culicifacies*, larvae of the secondary vector species, *A. subpictus* or *A. varuna*, were used.

2.8. Persistence of expanded polystyrene beads layers in the gem pits

Forty gem pits with a similar depth and diameter were selected and 2 mm expanded polystyrene beads, at the rate of 10 l/m^2 of water surface, were added to each pit. Observations were made monthly for 14 months of the persistence of the polystyrene layer in the pit whether it was wet or dry.

2.9. Re-excavation of gem pits filled with soil

All 20 gem pits with similar depth and diameter were selected and filled with soil using hand tools. Observations were made monthly for 14 months for any re-excavation of filled pits by gem miners.

2.10. Small scale field trial on the effect of two concentrations of pyriproxyfen on A. culicifacies larvae in river bed pools

Twelve river bed pools positive for anopheline larvae were selected. Four pools were chosen randomly from the 12 pools for application of pyriproxyfen at 0.01, 0.1 mg/l and as controls. Field and laboratory bioassays of the ability of *A. culicifacies* larvae to develop to adult emergence in the pool water were carried out as described above.

2.11. Cost comparison of six treatments

Estimates were made of the costs of the six treatments, i.e. expanded polystyrene beads, temephos, used engine oil, 0.01 and 0.1 mg/l pyriproxyfen and earth filling of gem pits. The labour cost per treatment using the first five methods was calculated by counting the number of gem pits treated by a person within 4 h. For calculation of the cost of employing labourers for earth filling of gem pits, ten pits were selected and the time taken by ten labourers to fill the pits using hand tools was recorded.

In addition to labour costs, the cost of transport, consumables (oil, polystyrene beads, pyriproxyfen, temephos) and for hiring hand tools for filling of gem pits were considered. The cost for each treatment was calculated for frequently re-excavated and for abandoned gem pits and these costs were compared.

3. Results

3.1. Relative efficacy of two doses of pyriproxyfen applied at different stages of development of A. culicifacies and A. subpictus

When larvae were treated with pyriproxyfen the body colour whitened, whereas the controls remained brown. Almost all treated larvae continued development to pupae and died at that stage. Two adults were produced but they could not detach their legs from the pupal case and died. Pyriproxyfen exposure was begun at each larval instar and continued for the rest of the larval stages. The times elapsed before 100% mortality of the pupae were approximately 11, 8, 5 and 3 days for treatments initiated at the 1st–4th instars, respectively, at each pyriproxyfen dose.

With *A. culicifacies* and *A. subpictus*, there was little difference in these times for 100% mortality with doses of 0.1 or 0.01 mg/l. When treatment was not begun until the pupal stage, the pupae emerged normally. In the controls there was only 2.5–5% mortality at the 1st and 2nd instars and all the others developed to pupae and emerged successfully.

3.2. Relative efficacy of four types of polystyrene against 3rd and 4th instar A. culicifacies larvae

The time taken for 100% mortality of larvae after application of 2 mm diameter polystyrene beads, a mixture of different sizes of polystyrene bead and small polystyrene pieces were 30, 96 and 108 h, respectively. One adult in one replicate managed to penetrate through the layers of irregular shaped large pieces of polystyrene. However, all remaining larvae were dead 120 h after this treatment. About 6.6% mortality was observed in the controls.

3.3. Relative efficacy of expanded polystyrene beads for control of different instars of larvae and pupae of A. culicifacies and A. subpictus

The times taken for 100% mortality of first, second, third and fourth instars and pupae of *A. culicifacies* and *A. subpictus* were 180–158, 120, 72, 48 and 13–6 h, respectively. The mortality in control jars was 12–25%.

3.4. Effectiveness of 2 mm expanded polystyrene beads in the control of A. culicifacies and A. subpictus oviposition

A. culicifacies and *A. subpictus* females, presented with water covered with expanded polystyrene beads layers and no other oviposition sites, did not lay any eggs. However, mosquitoes from the same batches presented with uncovered water surfaces laid thousands of eggs.

3.5. Small scale trial on six methods for control of breeding of A. culicifacies and other anophelines in gem pits

Attempts to monitor natural emergence from pits in traps set over the pits were unsuccessful—only 13 mosquitoes were caught from 464 nights of trapping on untreated pits. Therefore, all results to be reported came from bioassays. Fig. 1 shows results, over a period of more than one year, of bioassays of the ability of *A. culicifacies* to complete development to the adult stage in pit water treated with pyriproxyfen at 0.01 and 0.1 mg/l. The bioassays were carried out both in floating cages in the pits and in water samples taken to the laboratory. The pits became dry about 150 days after the pyriproxyfen treatments and remained so for over 100 days until the next monsoon season.

All the pits treated with either of the concentrations of pyriproxyfen showed zero adult emergence in the bioassays in floating cages up to 280 days, except for one emergent from one pit 60 days after initial treatment with 0.01 mg/l pyriproxyfen. This one was probably due to heavy rain diluting the pyriproxyfen (Fig. 1) and this pit was retreated. When the pyriproxyfen treated pits re-filled with water after the dry season, the bioassays recommenced and in all four pits at each concentration of pyriproxyfen, there was zero emergence in the first set of field bioassays, showing persistence of active pyriproxyfen residues over the dry season. However, some of the test larvae completed development to emergence in the field bioassays at days 290–300. The pits were, therefore, all retreated with pyriproxyfen at the same concentration, which had been used in them originally. This restored the situation in which no larvae could complete development in the pits.

In contrast to the bioassays in floating cages in the pits, the bioassays of pyriproxyfen treated water brought to the laboratory showed that successful development and emergence occurred in water collected 40 days after the initial treatment, and 60–70 days after the second treatment (Fig. 1).

Control pits showed 95–100% emergence in field and laboratory bioassays throughout the trial period, except just before and just after the dry season when emergence rates were only 60–75%. These low values were probably due to contamination of the pit water with leaves and debris, which made conditions unfavourable to *Anopheles* larvae.

Bioassays in floating cages with polystyrene bead layers showed that the layers completely prevented adult emergence in all tests carried out while the pits remained wet. When the water level of the pits decreased, polystyrene beads in the pits were stranded on the sides. However, with the rise in water level the beads re-floated. On day 280 after initial treatment two pits flooded and lost their bead layers. Those two pits were retreated with polystyrene beads.

The histories of the pits treated with used engine oil are shown in Fig. 2. Three of them remained wet until day 100–140 after treatment, as in the pit trials described above, but one of the oiled pits went dry much earlier. Bioassays in floating cages in these pits showed that the oil penetrated into the cages but did not cause pro-

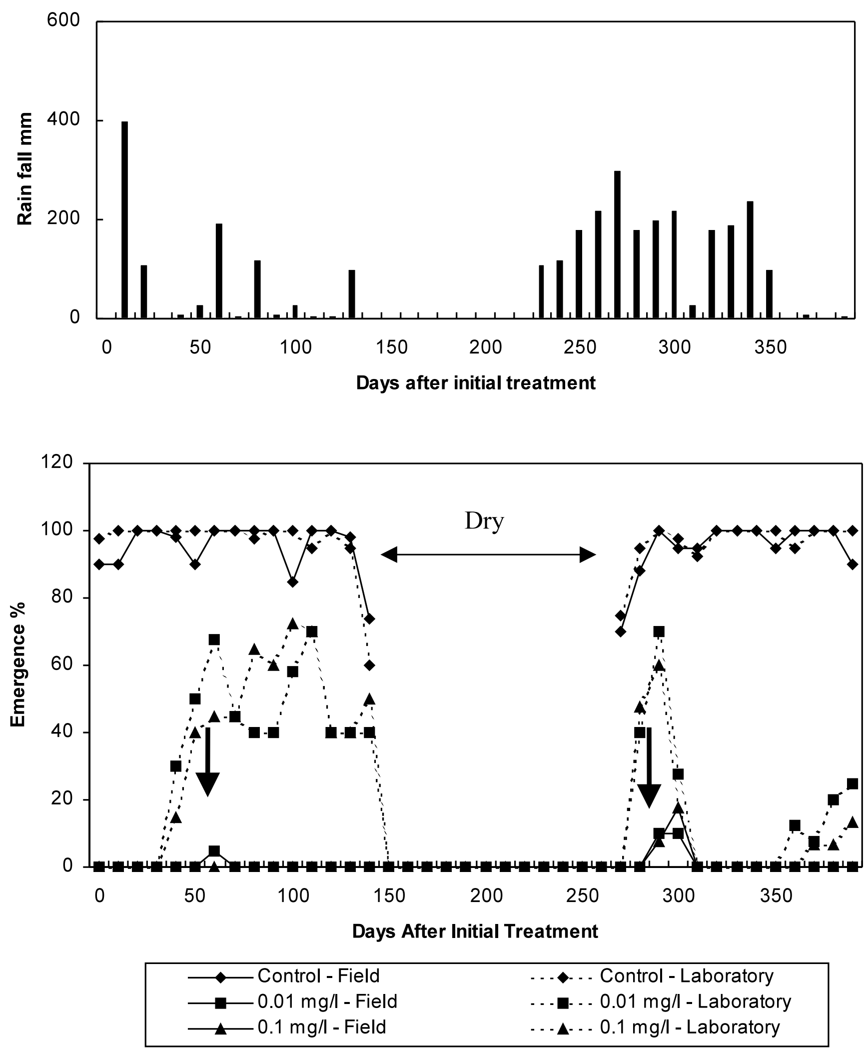

Fig. 1. Rainfall and percentage emergence in field and laboratory bioassays of *A. culicifacies* in control pits and after application of 0.01 or 0.1 mg/l pyriproxyfen (0.5% granular formulation) to the gem pits. Arrows indicate when adults emerged in field bioassays and re-treatment was, therefore, applied.

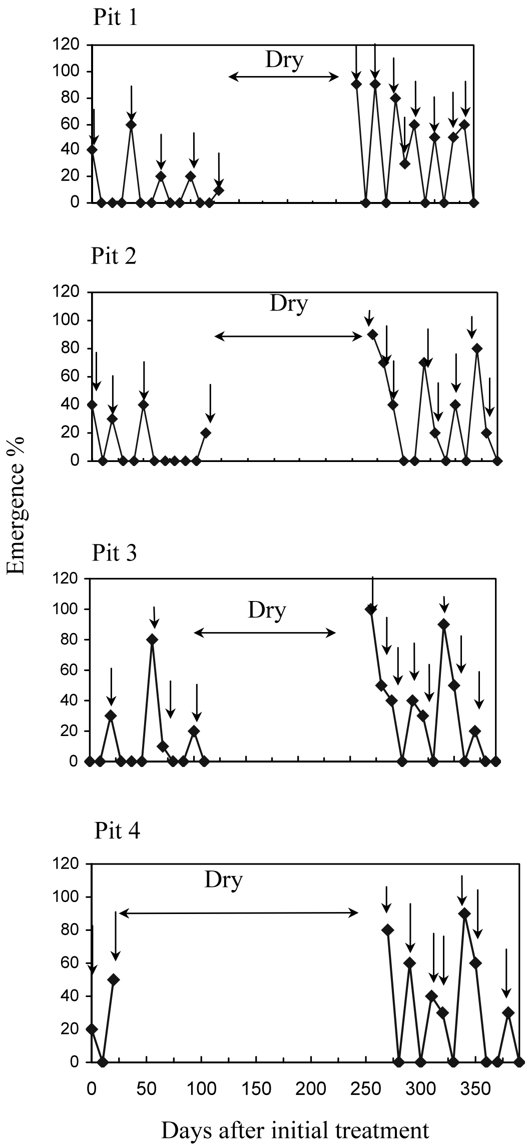

Fig. 2. Percentage emergence in field bioassay of *A. culicifacies* larvae after application of 70% used engine oil + 30% diesel oil + 0.5% teepol into four gem pits. Arrows indicate when adults emerged in field bioassays and re-treatment was, therefore, applied.

longed and reliable larval mortality and had to be repeatedly re-applied to the pit to restore larval control (see arrows indicating re-treatment in Fig. 2). In three of the pits the first of these re-treatments with oil was required only five days after the initial treatment due to breaking up of the oil layer by rainfall (Fig. 2). Two pits (pit numbers 1 and 4) close to streams had to be treated frequently before the dry period as the oil lost its efficacy due to rainfall and percolation of water from the streams causing a rise in the water level and hence enlargement of the water surface. All the oil treated gem pits had to be treated frequently after the dry period due to rainfall. Dead fish, damsel-fly larvae and water boatman were found in oiled pits.

The histories of the temephos treated pits are shown in Fig. 3. There was a dry period of 100–150 days in the middle of the trial. The first two field bioassays in floating cages showed 100% lethality to the test larvae of the temephos treated pit water. However, thereafter there were frequent failures to give 100% larval mortality and a consequent need to re-treat at 10–20 day intervals (Fig. 3). As with oil, re-application of temephos was required an average of 12 times to each pit during the wet months of the one year trial.

3.6. Persistence of expanded polystyrene beads layers in the gem pits

Out of 40 pits treated with polystyrene, 37 retained their layers for 4 months, from November to February during the wet season. Three pits flooded two months after treatment and these were re-treated. However, a re-survey 14 months after the initial treatment showed that, while the pits were dry, all had been re-excavated by gem miners or filled with soil, and the polystyrene beads had been buried in the soil.

3.7. Re-excavation of gem pits filled with soil

Re-excavation of the gem pits by gem miners started 4 months after we had arranged for the pits to be filled. After a year all had been re-excavated, but three pits had subsequently been refilled with soil during excavation of adjacent pits.

3.8. Cost comparisons for frequently re-excavated gem pits

A total of 3100 gem pits in four villages were scheduled for anti-larval treatment in 1995 (Yapabandara et al., 2001). Table 1 shows the calculated total costs for a year of control of mosquito breeding in these 3100 gem pits using various different anti-larval agents, the unit costs of which in 2001 are shown in the footnote to the table.

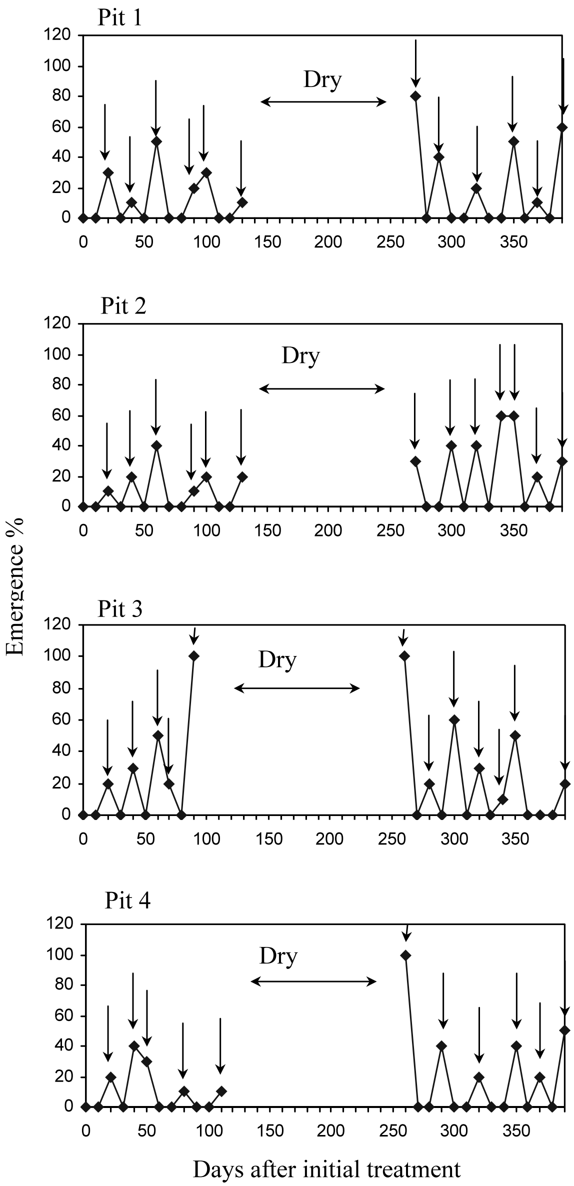

Fig. 3. Percentage emergence in field bioassays of *A. culicifacies* larvae after application of temephos 50% EC to four gem pits. Arrows indicate when adults emerged in field bioassays and re-treatment was, therefore, applied.

From the small scale trial it was considered that pyriproxyfen would have to be re-applied twice per year (Fig. 1) whereas temephos and oil would need to be re-applied 12 times during the 8 wet months when the gem pits are filled with water (Figs. 2 and 3). From the history of persistence of polystyrene layers and soil filling of pits, it was considered that those two treatments would have to be applied once a year at the beginning of the monsoon to many of the pits which were re-excavated by the gem miners during the dry season. Total costs per year shown in Table 1 are based on costs of labour, transport, chemicals and other consumables for each type of treatment. The labour cost for closure of the 3100 pits with soil would amount to over US$50000 and the cost for polystyrene would not be much less. The costs for 12 annual treatments with temephos would be less than $6000. A similar number of treatments with very cheap waste oil is estimated at $3149, mainly represented by the cost of labour. As indicated in Table 1, two annual applications of 0.01 mg of pyriproxyfen/l at the price of US$15 per kg of granules which prevailed in 2001, would cost about US$3050 i.e. slightly less than the annual cost of oil treatments.

3.9. Costs of treating abandoned gem pits

The total cost per year if polystyrene layers or filling the pits with soil persists for up to 10 years are shown in Fig. 4. The initial cost for application of polystyrene or closure of a pit is high but, as shown in Fig. 4, the cost per year would decrease the longer the polystyrene or closure of a pit with earth lasted. There would be break-even points for polystyrene with temephos, if a polystyrene application lasted for 7 years. However, even if polystyrene lasts for 10 years, the cost for application of oil or 0.01 mg/l of pyriproxyfen would be less than the cost for polystyrene (Fig. 4).

3.10. River bed pools

Bioassays in floating cages in the pools and laboratory bioassays of pool water are summarised in Fig. 5. There was no emergence of *A.*

Table 1
Cost evaluation for six possible methods of preventing mosquito breeding in 3100 gem pits

Treatment	Rate	Treatments per annum	Cost in US $			
			Consumables[a]	Labour	Transport	Total
Pyriproxyfen	0.01 mg/l = 0.01 g/m^3	2	2640	324	45	3009
Pyriproxyfen	0.1 mg/l = 0.1 g/m^3	2	26 400	372	45	26 817
Polystyrene beads (2 mm diameter)	10 l = 0.4 kg/m^2	1	36 825	321	1602	38 748
Temephos	0.111 g a.i./m^3	12	2628	3270	44	5942
70% used engine oil 29.86% diesel, 0.14% teepol	80 l/ha	12	565	2562	22	3149
Closure of pits		1	20	51 048	–	51 068

All 3100 pits have water volume of approximately 43 843 m^3 and water surface area of approximately 21 921 m^2. Costs: 0.5% Pyriproxyfen 1 kg = $15; Polystyrene beads 1 kg = $4; 50% Temephos 1-l = $22, Used engine oil 1-l = $0.16, Diesel 1-l = $0.31; teepol 1-l $4; spraying machine at the rate of $67 with 10 year life—13 required for oil application, 10 required for temephos application; 6263 sacks for polystyrene $1753; labourers $3 per day; supervisor for every 5 labourers $6 per day.

[a] Chemicals, hire of hand tools and annual cost of spray machines.

culicifacies in the bioassays in floating cages up to 45 days after initial treatment with 0.01 or 0.1 mg/l pyriproxyfen. All the tested river and stream bed pools dried up 45 days after treatment and refilled with water after 165 days. However, the river did not flow at that time and all the bioassays in floating cages showed zero emergence in pools which had been treated with either concentration of pyriproxyfen, i.e. residues had persisted over the dry period. However, soon afterwards bioassays in floating cages started to show successful completion of development and the pools were re-treated. Not long afterwards, as the rains continued the river started to flow slowly and all bioassays in floating cages in pools treated with 0.01 or 0.1 mg/l showed adult emergence. Soon after that, faster river flow would inevitably have washed away the pyriproxyfen. As expected, therefore, adult mosquitoes emerged in all the bioassays in floating cages.

As with the bioassays of the treated gem pits, the laboratory bioassays showed emergence of adults much sooner than the bioassays in floating cages in the field.

The emergence percentage in the control bioassays in the field or laboratory using water of untreated river bed pools ranged from 95 to 100%.

4. Discussion

In the laboratory, pyriproxyfen at rates of 0.01 and 0.1 mg/l was equally effective against *A. culicifacies* and *A. subpictus* whether treatment began early or late in larval life, but not when pupae were treated. Deaths of treated larvae occurred at the pupal stage. Effectiveness of similar concentrations of pyriproxyfen has been reported in other anophelines by Kawada et al. (1988), Suzuki et al. (1989) and Okazawa et al. (1991). We observed a colour change to white in pyriproxyfen treated larvae as earlier reported in various anophelines by Hemingway et al. (1988), Suzuki et al. (1989) and Okazawa et al. (1991).

The bioassays in floating cages in the field indicated that single treatments of pyriproxyfen at the rates of 0.01 or 0.1 mg/l effectively inhibited emergence of adult mosquitoes in the gem pits and river bed pools for a period of 60–140 days until the pits or pools dried out. When water returned 140 days later, activity of pyriproxyfen

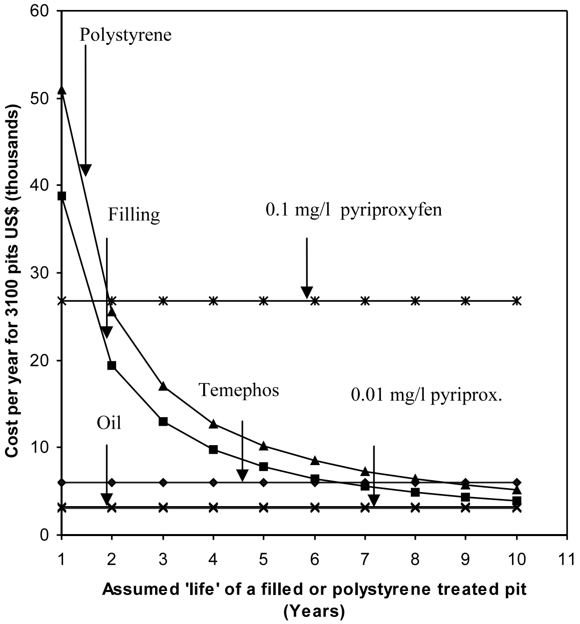

Fig. 4. Annual cost for application of polystyrene, 0.01 and 0.1 mg/l pyriproxyfen 0.5% granules, temephos 50% EC, used engine oil and filling of 3100 gem pits. The annual cost for polystyrene and filling is shown in relation to a range of assumptions about the 'life' of these treatments.

was still detectable for a short time, but then re-application was required (Fig. 1). These observations are in agreement with Okazawa et al. (1991) who reported that pyriproxyfen at the rate of 0.1 mg/l inhibited emergence of *A. punctulatus* completely for 150 days, even after a dry period of 50 days.

It was remarkable that laboratory bioassays with water brought from treated gem pits or river bed pools showed much shorter persistence of the prevention of adult emergence (Figs. 1 and 5) than in the bioassays in floating cages in the field. Our results agree closely with those of Kawada et al. (1988) who reported that the duration of effectiveness of pyriproxyfen, with floating cage bioassays in treated water was three times that obtained by the water collection method. It appears that pyriproxyfen does not persist for long dissolved in water but is retained in the mud sides and bottom of pits and pools and, from there, the active dosage in the water is constantly replenished. Thus, removal of water from a pit or pool would remove it from this source of pyriproxyfen from which the rapidly decaying dissolved material could be replenished. Pyriproxyfen was reported to be adsorbed onto organic matter in an animal waste water lagoon and it was effective on mosquito aquatic stages over a 2-month period (Schaefer et al., 1988; Mulligan and Schaefer, 1990; Schaefer and Miura, 1990).

Our observation of long persistence of the effects of pyriproxyfen in the floating cage bioassays in the field were repeated in several pits and pools, whereas controls in untreated pits and

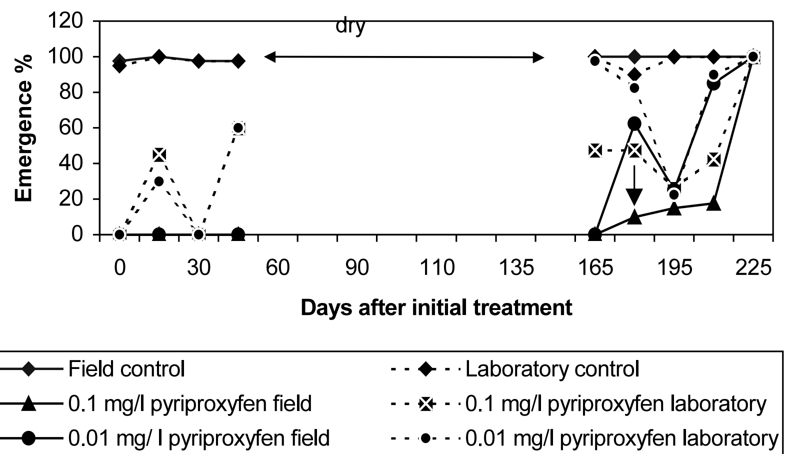

Fig. 5. Emergence percentage from field and laboratory bioassays of *A. culicifacies* in control pools and after application of 0.01 or 0.1 mg/l of pyriproxyfen into river bed pools. Arrows indicate when adults emerged in field bioassays and re-treatment was, therefore, applied.

pools confirmed that it was the applied pyriproxyfen which was causing inhibition of adult emergence—not any other feature of our bioassays in floating cages. Therefore, we consider that these field bioassays gave a more reliable indicator than the laboratory bioassay of how long the effect of pyriproxyfen would persist in preventing effective breeding in a pit or pool.

Similar bioassays with floating cages in pits showed that, to retain control of breeding, oil or temephos needed to be re-applied at approximately 20 day intervals in the wet season, i.e. 12 times in the year (Figs. 2 and 3). This result with temephos agrees with WHO (1984) which states that with emulsifiable concentrate or sand granules applied at 56–112 g a.i./ha (mean water depth 10 cm) one can expect persistence of 10–20 days. Similar persistence of temephos in river bed pools has been reported in Sri Lanka (Anti-Malaria Campaign, 1985).

Among the four types of polystyrene tested, 2 mm beads killed mosquito larvae the most quickly. The mixtures of irregular small pieces and beads, as used by Nathan et al. (1996), were also effective but took about three to four times longer to kill all larvae, and one adult from one of the replicates managed to penetrate through the layer of irregular shaped large pieces of polystyrene. The quick death of larvae with 2 mm expanded polystyrene beads is presumably due to the closer and more compact arrangement of small uniform beads than the larger and irregular sizes of bead and pieces tested. The pupae were most rapidly killed by polystyrene; the younger instars required longer to be killed, presumably because they could absorb almost enough oxygen for their needs through their cuticle, given the relatively large surface area to volume ratio of small larvae.

We found that a layer of 2 mm expanded polystyrene beads completely prevented oviposition whereas Sharma (1984) reported that blood fed *A. culicifacies* females laid a few eggs on the wet beads at the edges of a laboratory beaker.

Expanded polystyrene beads are biologically inert (Reiter, 1978; Iyer et al., 1973) and are used for potting mixtures to improve soil (Cook and Dunsby, 1978). Thus there is no reason to think that they could be environmentally harmful, though they are unsightly when scattered on the ground if they are carelessly applied to breeding places or after flooding of a treated site.

In bioassays in floating cages polystyrene beads completely prevented emergence of adults throughout the trial period of 390 days. Polystyrene bead layers are long lasting provided the site is undisturbed by flooding or human activities, and the beads re-float if the site becomes dry and then water returns (Tiwari and Tyagi, 1989; Maxwell et al., 1990). Filling of gem pits with soil was also an effective method if pits are not re-excavated by gem miners. However, in many cases polystyrene treated or soil filled pits were re-excavated. Thus only in a minority of abandoned pits could the potential for long term cost effectiveness of these two methods (Fig. 4) be realised.

For pits where re-excavation in the dry season is likely, the most cost effective method was shown to be two annual treatments of 0.01 mg/l pyriproxyfen, which would cost only about US$1 per pit treated (Table 1). The oiling method was only slightly more expensive but has the disadvantages of killing non-target organisms and of requiring six times as many pit visits to all the pits each year. Supervision of so much field activity is difficult and these activities expose field staff to appreciable danger. Therefore, pyriproxyfen at 0.01 mg/l with its need for only two applications per year, was clearly the method of choice for a multi-village trial. This trial showed clear evidence for control of adult vector populations, prevalence of malaria infection and incidence of malaria attacks in four villages in which all pits and pools were treated, in comparison with four villages in which pits remained untreated (Yapabandara et al., 2001).

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