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REVIEW OF THE EFFICACY AND PERSISTENCE OF PYRIPROXYFEN 0.5% GRANULES ON *ANOPHELES* SP. AND *AEDES* SP. BREEDING SITES IN SRI LANKA

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Abstract The insect growth regulator, pyriproxyfen (Sumilarv 0.5 G) at the rate of 0.01 mg a.i./L was applied to gem pits as well as river and stream bed pools as a part of a large scale trial in a gem mining area in Sri Lanka. The results indicated pyriproxyfen inhibited emergence of the primary malaria vector, *Anopheles culicifacies* adults in gem pits and river bed pools for 185 and 190 days respectively and significant reduction of incidence of malaria. A small scale trial showed that two annual treatments with pyriproxyfen at the rate of 0.01 mg a.i. /L would be cost effective. Twelve villages were determined to be the major breeding places of the primary vector and secondary vector, *An. subpictus*. Pyriproxyfen was applied at the rate of 0.01 mg a.i. /L to the vector breeding places of the treatment villages. The treatment caused significant reduction of the adult *An. culicifacies* (78%) and *An. subpictus* (72%). Similarly incidence of malaria (70%) was reduced in the treatment villages. Ground level domestic water storage cement tanks are one of the important breeding sites of *Aedes aegypti* and *Aedes albopictus*. Small scale field trial was carried out using the 1000 liter capacity unwashed tanks to assess the efficacy of 0.001 ppm, 0.1 ppm of pyriproxyfen and 1 ppm temephos 1% granules. Results indicated the inhibition of emergence of adults up to 40, 57 and 56 days respectively. Data from similar field bioassays using 0.01 ppm pyriproxyfen and 1ppm temephos, but with washing of tanks after 14 days and refilling of the tanks showed 100% emergence inhibition up to 22 days and 15 days respectively. Other breeding places were treated with a combination of temephos 50 EC and pyriproxyfen 0.5% granules at the rate of 0.111 kg a.i. /ha and 0.01mg a.i. /L respectively. The results to date indicate pyriproxyfen inhibits mosquito adult emergence for at least one month.
Key Words *Anopheles culicifacies*, *Aedes aegypti*, mosquito control

INTRODUCTION

Malaria and dengue are considered to be the most important public health problems in Sri Lanka. *An. culicifacies* Giles is regarded as the principle vector of malaria in Sri Lanka (James and Gunasekara, 1913; Carter and Jacocks, 1929; Amerasinghe et al., 1999). *An. subpictus* is considered as a secondary vector (Mendis et al., 1990 and 1992; Amerasinge et al., 1992 and 1997; Konradsen, 2002; Ramasamy et al., 1992a and b). River and stream bed pools, gem pits are among the major breeding sites of *An. culicifacies* and *An. subpictus* (Gills, 1936; Wickramasinghe, 1981, Amerasinghe et al., 1997; van der Hoek, 1988; Yapabandara, 2004). Container breeding *Aedes aegypti* and *Ae.albopictus* serve as the primary and secondary vectors of dengue and dengue hemorrhagic fever in the island (Yapabandara, 2002: Kusumawathie, 2004). Ground level domestic water storage cement tanks are among the major breeding places of these vectors where water supply is unreliable (Yapabandara, 2002).

Applications of temephos EC and temephos 1.0% granules have been widely used in Sri Lanka to limit the breeding of both *Anopheles* sp. larvae in river and stream bed pools and dengue vectors in domestic water containers (AMC admin reports 1985-87). There are reports that *Anopheles* sp. vectors have subsequently developed resistance to temephos in some parts of the country. New alternative cost-effective insecticides are therefore required to ensure the continued success of such control measures.

Gem pits are localized in only a very few areas of Sri Lanka but pools in the beds of rivers, streams and irrigation ditches are common breeding places in most of the malarious areas in the country. Studies were conducted to check whether the successful results in the "Gem Mining" trial were repeatable in a areas where pools in the beds of rivers, streams and irrigation ditches are the predominant breeding sites.

Studies were carried out to determine the effect of pyriproxyfen against malaria and dengue vector larvae in their natural environment. This paper presents the review of the efficacy and persistence of pyriproxyfen 0.5% granules on *Anopheles* sp. and *Aedes* sp. breeding sites in Sri Lanka.

MATERIALS AND METHODS

Insecticide. The insect growth regulator, pyriproxyfen (Sumilarv® 0.5% pumice granule formulation, Sumitomo Chemical Co. Ltd., Japan) inhibits the emergence of adult mosquitoes and is known to have a low mammalian toxicity and a good margin to safety to fish and other non target organisms. Data is available on the long-term control of *Anopheles* sp. larvae after a single application of the pyriproxyfen 0.5% granular formulation at concentrations from 0.01 to 0.1 mg/l (Kawada et al., 1988; Okazawa et al., 1991).

Gem Pits Site. A study was conducted in twelve villages in an irrigated settlement scheme in the dry zone of Central Sri Lanka where there are many pools in the beds of rivers, streams and irrigation ditches during the dry season of the year. These are the major breeding places of the primary malaria vector, *Anopheles culicifacies* and secondary vector, *An. subpictus*. Adult mosquito collections were carried out using six standard methods and parasitological data were collected by daily malaria clinics set up for the project and through the two government hospitals. All villages in the study area were under residual house spraying regimes with lambdacyhalothrin WP. Using the first year's base line data, the villages were separated into six with high malaria incidence and six with low incidence. Within each group, three villages were randomly assigned for larval control by treating all the pools in the beds of rivers, streams and irrigation ditches and agricultural wells with a granular formulation of the insect growth regulator pyriproxyfen at the rate of 0.01 mg a.i./l.

Gem Pits Site Treatment. Pyriproxyfen (0.5% pumice granule formulation) at the rate of 0.01 mg a.i./l was applied to gem pits as well as river and stream bed pools as part of a small scale trial in a gem mining area (Yapabandara et al., 2001). The effects on the treated and control pits were evaluated by observing the emergence of adults and death of *An. culicifacies* larvae which were introduced into each river bed pool water in 5 liter capacity buckets floating in the pits. A series of 7 x 5 cm square holes were drilled about 2 cm from the bottom of the bucket and they were covered with 100-mesh nylon strainer cloth to allow water circulation between the bucket and the pool, but to prevent escape of the larvae and the bucket was covered with a net. A hole was cut in the middle of a 25 x 25 x 3 cm polystyrene board and the bucket was inserted up to 15 cm and floated on the water of the pool. The bucket handle was tethered to a pole at the side of the river to prevent the bucket being carried away in the event of overflowing of water. Ten third and fourth instar larvae were introduced to each bucket. The larvae in the emergence traps were fed daily with fish food. The bucket was removed from the pool daily and adults were collected using an aspirator through a hole in the netting cover. Then the net was removed and dead and live larvae and pupae were counted in the 2 cm water remaining in the bottom of the bucket. These observations were carried out daily until all the larvae/pupae had died or developed into adults. In the absence at the time of the test of readily supplied *An. culicifacies*, larvae of secondary vector *An. subpictus* were used. If any adult emerged in the field bioassay of a treated pool, the pool was treated.

Water Storage Tanks Sites. Ground level domestic water storage cement tanks are one of the main breeding sites of *Aedes aegypti* and *Aedes albopictus* in Sri Lanka (Yapabandara, 2002). A small scale field trial was set up to assess the efficacy of 0.001 ppm and 0.01 ppm pyriproxyfen, applied as a 0.5% pumice granule, and 1.0 ppm temephos, applied in a 1.0 % sand granule. The emergence inhibition of *Aedes* sp. larvae in each treatment was monitored using "emergence bucket" bioassays.

RESULTS

Gem Pits Treatment. The results indicate pyriproxyfen inhibited emergence of adults for more than 185 days, which included a 4-month dry period. The trial showed that two annual treatments with pyriproxyfen at the rate of 0.01 mg a.i./l would be more cost effective than temephos, expanded polystyrene beads, used engine oil or filling the pits with soil (Yapabandara and Curtis, 2002). This large scale trial resulted in a 60% reduction of the density in adult *An. culicifacies* and significant reduction of incidence of malaria fever due to either *Plasmodium falciparum* or *P. vivax* (Yapabandara et al., 2001). This control method has continued to be implemented in the present gem mining area by the malaria control programme of Sri Lanka. Consequently, the case incidence of malaria in the study area has decreased from 121 per thousand population in 1997 to 4 per thousand population in 2002. The chemical cost for vector control in the area decreased by over 70%.

Table 1. Percentage emergence inhibition in field bioassays of *Aedes aegypti* of control water storage cement tanks and after application of 0.001 ppm or 0.01 ppm of pyriproxyfen (0.5% granular formulation) and 1.0 ppm of temephos (1% sand granules) to the tanks.

Hours/Days after treatment	**Pyriproxyfen (0.5%G) 0.001 ppm**	**Pyriproxyfen (0.5%G) 0.001 ppm**	**Temephos (1% sandG) 1.0 ppm**	**Control**
48 hours	100	100	100	0
7 days	100	100	100	0
14 days	100	100	100	5
28days	87	100	100	3
40 days	87	100	100	0
49 days	75	95	85	0
56 days	60	85	70	0
57 days	60	75	70	3
63 days	60	62	67	4
72 days	47	50	57	0

The field bioassays using the “floating emergence buckets” indicated a single treatment of pyriproxyfen effectively inhibited the emergence of adult mosquitoes in the river bed pools for a period of 190 days. The treatment resulted in a significant reduction of the adult *An. culicifacies* (67%) and *An. subpictus* (42%) in the area. Similarly, the incidence of malaria was reduced by just over 70% in the treatment villages (95% CI 58-78%) when compared with the controls. It is therefore concluded that in areas where the major breeding sites of the vector can be identified and treated, larvicide applications with pyriproxyfen can be a very effective means of reducing vector populations to a level where a significant reduction in malaria transmission is noted.

Water Storage Tanks. In unwashed tanks, 0.001 ppm pyriproxyfen gave more than 75% emergence inhibition for 40 days post application. Extended residual action resulted from 0.01 ppm pyriproxyfen and 1.0 ppm temephos, with both giving more than 75% emergence inhibition for up to 57 and 56 days respectively (Table 1). Data from similar field bioassays using 0.01 ppm pyriproxyfen and 1.0 ppm temephos, with the cement tanks washed and refilled with fresh water 14 days after the larvicide application, showed 100% emergence inhibition up to 22 days for pyriproxyfen, with temephos showing 70% emergence inhibition up to 15 days. The residual activity of pyriproxyfen under such circumstances would make this both an effective and convenient *Aedes* sp. larval control agent in the water storage containers (Fig. 1).

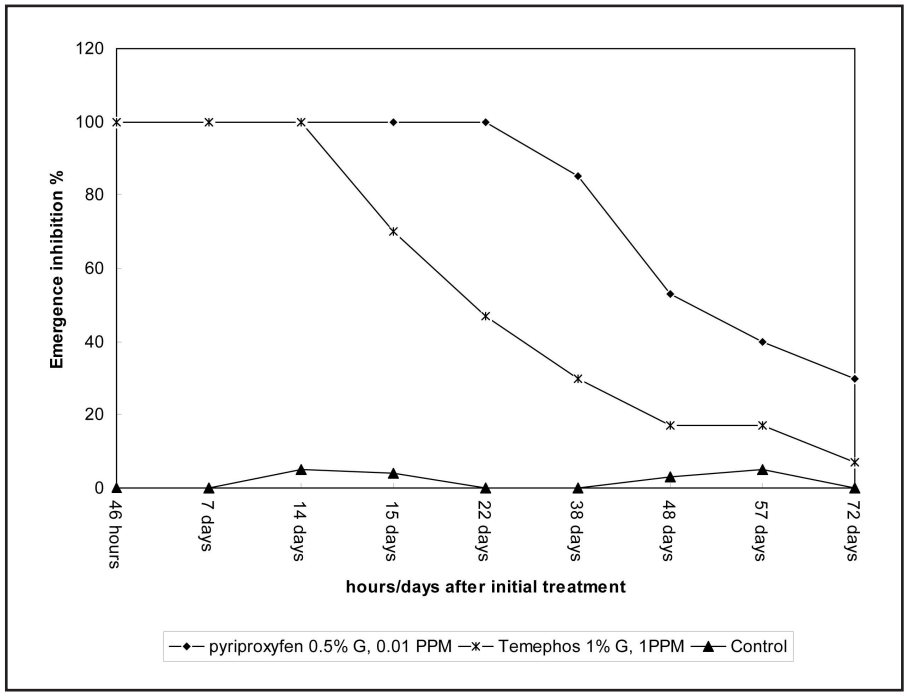

Figure 1. Percentage emergence inhibition in field bioassays of *Aedes aegypti* of control water storage cement tanks and after application of 0.01 ppm of pyriproxyfen (0.5% granular formulation) and 1.0 ppm of temephos (1% sand granules) to the tanks and 14 days after washing and refilling of the tank. Indicate the washing up of the tank.

VECTOR CONTROL IN TSUNAMI AFFECTED AREAS

Sri Lanka had been badly affected with the tidal waves on the 26th of December 2004 with about 30,959 deaths and with more than 5,000 missing people. The estimated number of displaced people is 500,688. The tsunami affected districts stretch from Jaffna in the North to Kilinochchi, Mullative, Trincomalee, Batticoloa, Ampara, Hambantota, Matara, Galle, Kalutara, Colombo, Gampaha and Puttalum on the Western coast. Out of 13 affected districts 9 districts are malaria endemic. All the districts are Dengue/DHF and Japanese Encephalitis risk areas. Risk of filariasis transmission is present in Southern and Western costs of the affected areas.

There is also a serious increased risk from vector-borne diseases such as malaria, dengue, filariasis and Japanese Encephalitis resulting from the disruption of vector control activities; the formation of new breeding places due to the destruction cause by tidal waves; an increased exposure of displaced people to vectors whilst living in partly damaged houses, tents and schools, and a shortage of logistics and man-power to conduct vector control and entomological surveillance activities.

Entomological investigations carried out by the entomological teams in the affected areas have already revealed that there is indeed an increased density of malaria, filarisis, Japanese Encephalitis vectors as well as house flies and nuisance mosquitoes. The main breeding places are burrow pits filled with water from the tsunami waves and abandoned wells. These breeding places were treated with combination of temephos 50 EC and pyriproxyfen 0.5% granules at the rate of 0.111 kg a.i./ha and 0.01 mg a.i./L respectively. The results to date indicate pyriproxyfen inhibits mosquito adult emergence for at least one month. Observation continues.

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REFERENCES CITED

***A.MC. Administrative reports* (1969-1989)**, Anti Malaria Campaign, Sri Lanka

Amarasinghe, F.P., Amerasinghe, P.H., Wirtz, R.A, Indrajith, N.G., Somapala, W., Pereira, L.R. and Ratnayake, A.M.S. 1992. Malaria transmission by *Anopheles subpictus* (Diptera:Culicidae) in a new irrigation project in Sri Lanka. J. Med. Entomol., 29(4):577-581

Amerasinghe, F.P., Kondradsen, F., Fonseka, K.T. and Amerasinghe, P.H. 1997. Anopheline (Diptera:Culicidae) breeding in a traditional tank based village ecosystem in north- central Sri Lanka. J. Med Entomol.34:290- 7

Carter, C.F. and Jacocks, J.P. 1929. Observation on the transmission of malaria by Anopheline mosquitoes in Ceylon. Ceylon J. Sci., 2:66-86

Epidemiological Bulletin, 1995-2004. Suspected dengue/dengue hemorrhagic fever cases, positives and deaths in Sri Lanka, Epidemiological Unit, Sri Lanka

Gubler, D.J. 1996. World distribution of Dengue, Dengue Bulletin, Volume 20. pp1-4

James, S.P. and Gunasekara, S.T. 1913. Report on malaria at the port of Talaimannar. Ceylon Sess. Paper No. 34.

Kondradsen, F., Amerasinghe, F.P., van der Hoek, W. and Amerasinghe, P. 2002. Malaria in Sri Lanka: Current knowledge on transmission and control. International Water Management Institute, Colombo, Sri Lanka.

Mendis, C., Gamage-Mendis, A.C., De Zoysa, A.P.K., Abhayawardana, T.A., Carter, A., Herath, P.R.J. and Mendis, K.N. 1990. Characteristics of malaria transmission in Katharagama, Sri Lanka: A focus for immuno-epidemeiological Studies. Am. J.Trop. Med. Hyg., 42(2):298-308.

Mendis, C., Herath, P.R.J., Rajakaruna, J., Weerasinghe, S., Gamage-Mendis, A.C., Mendis, K.N. and De Zoysa, A.P.K. 1992. Estimation of relative transmission efficiencies of different Anopheline species in human malaria transmission. J. Med. Hyg., 42(4):298-308

Okazawa, T. Bakote'e B., Suzuki, H., Kawada, H. and Kere N., 1991. Field evaluation of an insect growth regulator pyriproxyfen against *Anopheles punctuatus* on north Guadalcanal, Solamon Island. J.Am. Mos. Control. Assoc. 7:604-607

Rajendram, S. And Jayawickrame, S.H. 1951. Malaria in Ceylon, Part 1. Ind. J. Malariol., 5: 1-50.

Ramasamy, R., Ramasamy, M.S., Wijesunadara, A., Wijesundara, A.P.D.S., Dewit, I. Ranasinghe, C. Shrikrtiraj, K.A. and Wickramaratne, C. 1992a. High seasonal malaria transmission rates in the intermediate rainfall zone of Sri Lanka. Ann. Trop. Med. Parasitol., 86(6):591-160

Ramasamy, R., Alwis, R.D., Wijesundara, A. and Ramasamy, M.S. 1992b. Malaria transmission at a new irrigation project in Sri Lanka: The emergence of *Anopheles annularis* as a major vector. Am. J. Trop. Med. Hyg. 47:547-553

van der Hoek, W., Amerasinghe, F.P., Konradsen, F. and Amerasinghe, P.H. 1988. Characteristics of malaria vector breeding habitats in Sri Lanka: Relevance for environmental management. Southeast Asian J. Trop. Med Public Health; 29:1 168- 172.

Wickramasinghe, M.B. 1981. Malaria and its control in Sri Lanka. Ceylon Med. J., 26:107-115

Yapabandara, A.M.G.M. and Curtis, C.F. 2002. Laboratory and field comparison of pyriproxyfen, polystyrene beads and other larvicidal methods against malaria vectors in Sri Lanka. Acta Tropica. 81(3):211-223

Yapabandara, A.M.G.M. and Curtis, C.F. 2004. Malaria Transmission and Vectors of Malaria in a gem mining area in Sri Lanka. J. Vector Ecology. Dec: 264-276

Yapabandara, A.M.G.M. and Curtis, C.F. 2004. Control of malaria vectors in an irrigated settlement scheme in Sri Lanka using the insect growth regulator, pyriproxyfen. J. Am. Mos. Control. Assoc. 20 (4):395-400

Yapabandara. A.M.G.M., Curtis. C.F., Wickramasinghe, M. B. and Fernando, W. P. 2001. Control of malaria vectors with the insect growth regulator pyriproxyfen in a gem mining area in Sri Lanka. Acta Tropica, 80:265-276

Yapabandara, A.M.G.M. and Abeykoon, A.R. 2002. *Aedes aegypti* and *Ae. albopictus* breeding in the Matale Municipal area. Sri Lanka Association for the Advancement of Science, Proceedings of the58th Annual Session: Part1**:** page 20