INTRODUCTION
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1. Mosquitoes

Mosquitoes are insects under the class Insecta, order Diptera known to act as vectors for many dreadful diseases. The important diseases transmitted by mosquitoes are filariasis, dengue, yellow fever, and Malaria. Different genus of mosquitoes serves as potential vectors for the diseases. In view of eradicating these diseases, control of respective vectors play a major role. Steps to control mosquitoes involve mass application of insecticides and use of insecticidal formulations at household level. The former is aimed at community level and the later is aimed at personal protection. The household formulation include coils, vaporisers, mats, Liquid vaporisers, mosquito nets etc., however, practice of using nets to prevent mosquito bites is an age-old practice. The present strategy is to use insecticide treated nets with an aim not only to evade the mosquito but also to control them.

2. Protection afforded by untreated nets

Bednets have been used traditionally to protect people from the nuisance caused by nocturnally biting insects. An attractive alternative option for malaria protection was the use of untreated nets, as was practiced for many centuries (Lindsay and Gibson, 1988). The behaviour of mosquitoes around untreated nets has shown that considerable protection is afforded provided the nets are tucked in, maintained in good condition and sufficiently large so that the sleepers do not make contact with the net (Lindsay et al., 1989). Where untreated nets have been used in village trials, they resulted in a reduction of malaria morbidity compared with areas without nets, but the protection was significantly lower compared with treated nets (Magesa et al., 1991; Maxwell et al., 1999).
3. **Insecticide treated nets**

Insecticide treated nets are mosquito nets whose surface is coated with an insecticide so as to give adequate protection against mosquitoes to the persons sleeping inside. In the 1980s, insecticides began to be applied to bednets, which greatly increased their effectiveness even when torn (Curtis, 2005). To treat net with insecticide, required amount of suitable insecticide is calculated based on the net size and the same is mixed with the amount of water required to wet the net. Net is treated by dipping in this insecticide mixture and dried.

4. **Advantages of using Treated nets**

Use of mosquito nets is a conventional method adopted both in rural and urban areas to combat mosquitoes. The efficacy of insecticide-impregnated nets was so much greater than of untreated ones (Snow *et al.*, 1988 b). It was reported that untreated bed nets did not provide adequate protection, presumably because the mosquitoes could bite the occupants through the netting, or nets would often be torn because of excessive use, thus giving mosquitoes easy access to a blood host (Burkot *et al.*, 1990). Treatment of nets with a small deposit of a long-lasting insecticide could overcome these problems, because mosquitoes landing on the net would be killed before having taken a blood meal, or they would be repelled by the insecticide, as is the case with synthetic pyrethroids (Lengeler and Snow, 1996). The dual advantage of insecticide impregnated net is that, it prevents the mosquitoes from reaching the human beings and if mosquitoes happen to come in contact with these nets, they get knocked and thus an additional protection is assured. The use of insecticide-treated bednets (ITNs) has been widely adopted as an important method for malaria control (Takken, 2002). It was found that ITNs caused a significant reduction in malaria
attributable morbidity and mortality, especially in young children (Graves et al., 1987; Alonso et al., 1991)

The advantages of using insecticide treated nets are threefold – no negative effects of insecticide use, the avoidance of toxic chemicals at household level and financial savings (Guyat and Snow, 2002). The use of ITN provides effective protection from a major source of nuisance, that of nightly disturbance by mosquitoes (Shiff, 1998), as well as destroys bed bugs and other pests (Temu et al., 1999).

The World Health Organization has adopted the use of ITNs as one of the main strategies for malaria control in their Roll Back Malaria programme (RBM, 1999, Carter et al., 2000). At present ITNs are being applied in many malaria-endemic regions worldwide, and their use has replaced by indoor house spraying with insecticides in many countries.

5. Insecticides for net treatment

Pyrethroids are the only insecticides currently recommended by the World Health Organization for treatment of mosquito nets owing to their strong insecticidal activity at low concentrations and their low mammalian toxicity (Zaim et al., 2000). Their mammalian toxicity is low but their effect on arthropods, including crustaceans, is generally serious (Takken et al., 1978). Because of their high toxicity for mosquitoes, coupled with a long-lasting residual activity on textiles, they are considered safe for use on mosquito nets (Barlow et al., 2001). The insecticides recommended include Permethrin, Deltamethrin and Lambda-cyhalothrin (Table 1). They share the property of a relatively long residual activity when kept out of daylight but break down rapidly under influence of UV-radiation.
Pyrethroid-treated nets are effective in reducing malaria morbidity and mortality (Greenwood \textit{et al.}, 1993; Choi \textit{et al.}, 1995), and may also provide community protection through mass impact on vector mosquito populations, when used at a high coverage rate. Bed nets without insecticide can never provide complete protection against blood-questing mosquitoes. However, when treated with pyrethroids, they reduce the number of mosquitoes entering houses and further reduce the blood-feeding rates of those entering. Torn impregnated nets have been shown still to reduce host/vector contact, (Nevill \textit{et al.}, 1996; Henry \textit{et al.}, 1999).

Darriet \textit{et al.} (1984) reported the successful use of pyrethroid-treated mosquito nets for malaria control and Curtis and Lines (1985) compared the efficacy of different insecticides available for this specific purpose.

On treated nets, the pyrethroids work in three ways: first, they act as killing agent when the insect makes contact with the insecticide by landing on the net; secondly, pyrethroids have an irritating (excito-repellent) effect and the insect rests only briefly on the treated fabric and thirdly, the formulation in which the pyrethroid is presented contains volatiles that cause deterrence, leading to fewer mosquitoes entering a room where an ITN is present (Lindsay \textit{et al.}, 1991; Chandre \textit{et al.}, 2000).

6. \textbf{Long-lasting insecticide treated net}

There is an increasing trend to use insecticide impregnated bednets in malaria control programs. During the past decade, pyrethroid-treated mosquito nets have become established as an important defense against malaria transmission (Curtis,1990a; Carnevale \textit{et al.}, 1991; Lengeler, 1998). Moreover, the use of conventionally pre-treated nets is not recommended because, concentrations of insecticide in the nets are extremely variable and would be rejected under normal quality controls and users do
not recognize the need for re-treatment. It is almost impossible to ascertain if re-
treatment has been undertaken with an insecticide that meets WHO specifications.
(UNICEF, Technical Bulletin No.7). Periodic re-impregnation, low treatment rates
and repeated washing affects the application of insecticide-treated nets in longer
duration. To overcome these problems, long-lasting insecticidal nets (LLIN) have
been developed. To avoid the need for periodic re-treatment, it would be
advantageous for nets to be able to retain insecticidal efficacy for years and withstand
repeated washing (Guessan et al., 2001).

Use of ITNs treated conventionally predisposes individuals to acute pesticide
poisoning. Acute toxicity refers to the adverse effect that may result from single or
multiple exposures to a chemical over a relatively short period of time (WHO, 1999). 
Acute toxicity may occur through treatment and handling of insecticides for treatment
of nets. As would be expected, individuals involved in dipping large numbers of nets
are most at risk, whereas those who occasionally treat their own nets are less exposed
to this level of risk (WHO, 1999). These risks could be overcome by using long
lasing insecticide treated bed nets, in which the nets are factory treated and yield as
ready to use. The bio availability of insecticide on the net surface is just adequate to
act on insects and is maintained for quite longer period of time. The dual advantage of
no periodic re-treatment of net and minimum exposure to the insecticide could be
realized by the LLIN usage.

7. Insecticide Resistance and Use of ITN

Mosquitoes have developed resistance to almost all major groups of
insecticides used for agriculture and in household. Indiscriminate use of pesticides can
be attributed as an important reason for the resistance development in mosquitoes.
The ability of insects to develop cross resistance is another important factor for resistance development in mosquitoes. Hence, the protection offered by ITN against the resistant mosquitoes is important and little information is available in this regard.

8. Safety of ITN

Synthetic pyrethroids have become the most significant class of household insecticides and used extensively in different formulations for the control of flies and mosquitoes. Even though the residual concentrations of these molecules in the environment are very low, their significant presence cannot be neglected (Ramesh et al., 2002).

Coils and vaporizers are widely used insecticide formulations in household against mosquitoes with pyrethroids as active. Mosquito coil is widely known as an efficient mosquito repellent. The major active ingredients of the mosquito coil are pyrethrins, accounting for about 0.3–0.4% of coil mass (Lukwa and Chandiwana, 1998). Though the active is very less and hardly one coil is used per day, effect on health due to prolonged use of coil cannot be neglected. Weili et al. (2003) reported the acute and chronic health risks due to smoke produced by mosquito coils. Similarly, ITN as a part of insecticide formulation used in household scenario, human are knowingly exposed to minimum insecticide concentration. Hence, the safety of LLIN is a major concern.
OBJECTIVES

Though, malaria is a globally addressed public health issue, the potential use of LLIN against *Culex* and *Aedes*, which are prime vectors of lymphatic filariasis and Chikungunya cannot be under estimated as their damage is also viewed seriously against mankind. The predominant mosquito species in the south India being *Culex* and *Aedes*, the efficacy of LLIN against these vectors has its significance.

Hence, to assess the advantage of LLIN against the major mosquito species and their safety to human, the present investigation was constructed with following objectives and Olyset net being the only LLIN recommended by WHO during 2003 (UNICEF, 2003), the same was chosen for the present investigation.

The objectives are:

- To evaluate the efficacy of nettings treated with synthetic pyrethroids, *viz.* Bifenthrin, Deltamethrin, Etofenprox, Lambda Cyhalothrin & Permethrin by conventional method and long lasting insecticide treated net (LLIN).
- To study the wash resistance of conventionally treated nettings and long lasting insecticide treated net (LLIN).
- To explore the impact of storage on treated nets in terms of efficacy.
- To develop the Permethrin resistant colony of vector *Anopheles stephensi* through selection pressure in adults.
- To check the efficacy of conventionally treated nettings and long lasting insecticide treated net against Permethrin resistant mosquitoes.
- To assess acute toxicity effect of long lasting insecticide treated net using animal models.
- To assess the effect on human health of long lasting insecticide treated net.