Parasitol Res (2012) 111:423–432
DOI 10.1007/s00436-012-2857-2

ORIGINAL PAPER

The effect of sublethal exposure to temephos and propoxur on reproductive fitness and its influence on circadian rhythms of pupation and adult emergence in *Anopheles stephensi* Liston—a malaria vector

Deepak Sanil · Nadikere Jaya Shetty

Received: 6 May 2011 / Accepted: 7 February 2012 / Published online: 1 March 2012

Abstract The present study was undertaken to investigate the effects of organophosphate and carbamate insecticides namely, temephos and propoxur respectively, on the life history of *Anopheles stephensi* Liston (Culicidae) under laboratory conditions. The late third instar larvae of the mosquito were exposed to sublethal concentrations of temephos and propoxur at LC_{10}, LC_{30} and LC_{50}, respectively, and adult survivors were evaluated for fitness parameters. Sublethal effects were also evaluated in subsequent generations. Fecundity, egg hatchability, sex ratio, adult longevity and morphology of gonads were the end points studied and compared to the untreated control. Adverse changes in developmental traits were mainly observed in fecundity, egg hatchability and sex ratio. However, significant differences in adult longevity were observed in the insecticide-exposed population. Pleiotropic effects through prolonged larval duration and enhanced longevity of adults were observed. Morphology of gonads in the insecticide-exposed population was severely affected and is represented by rudimentary and atrophied testes, and the size of the vas deferens was very much reduced when compared to that of the control. In another set of experiments, circadian rhythm (for pupation and adult emergence) of LC_{10}, LC_{30} and LC_{50} values to abovementioned insecticides exposed to late third instar larvae was studied. Pupation and adult emergence rhythms were found to be disturbed with an increase in concentrations of insecticides when compared to that of untreated control.

D. Sanil · N. J. Shetty (✉)
Centre for Applied Genetics, Bangalore University, J. B. Campus,
Bangalore 560 056, India
e-mail: shetty_nj@yahoo.co.in

N. J. Shetty
Janardhana Foundation,
Nagadevanahalli, Jnanabharathi Post,
Bangalore 560 056, India

Introduction

Disease-causing malarial parasites are transmitted to humans by anophelines. *Anopheles stephensi* is one such important carrier of urban malaria in the Indian subcontinent and roughly accounts for 15% of the total malaria incidence in India (Shetty 2002; Zin and Shetty 2008). Insecticides continue to be the mainstay for the control of these infections since they can kill a great number of vectors within a short time and can reduce their densities sufficiently to suppress the vector population (Saelim et al. 2005). Chemical-based control represents a key strategy in the management of populations of insect vectors of medical and veterinary importance (Curtis and Davies 2001; Zaim and Jambullngam 2007). During the past two decades, considerable progress has been made in the development of natural and synthetic compounds that are capable of interfering with the growth and development process of the target insects. However, the control of insect pests and vectors of disease has become increasingly difficult for various reasons, including the emergence of insecticide resistance (Shetty 1987, 1997; Gayathri and Shetty 1992; Beard et al. 1998). Insecticide resistance in vector-borne diseases has become a major public health concern.

Treatment of a target mosquito species with toxic insecticides however results in an uneven field distribution of the insecticide material as a result of complex and dynamic interaction between abiotic and biotic factors such as rainfall, wind speed and direction, vegetation cover so that some larvae/adults receive sublethal doses of the toxin (Ali et al.

2006). Since insect behaviour is affected by both the nervous system and hormones, insecticides that attack the nervous system and disrupt the hormonal balance and/or metabolic process in insects can affect behaviour and physiology at levels that do not lead to direct mortality. Therefore, insecticides decrease the production of offspring because of behavioural modifications in mate location, courtship and oviposition, or due to physiological effects on egg fertilization, oogenesis, ovulation, spermatogenesis, sperm motility and overall affecting population structure (Haynes 1988; Ali et al. 2006).

Temephos and propoxur have been commonly used for mosquito control under public health programmes worldwide including India (National Vector Borne Diseases Control Programme and Directorate General of Health Services 2007). Temephos has also regularly been used as a mosquito larvicide worldwide and is one of the few organophosphates registered to control mosquito larvae (USEPA 2001). Propoxur, however, is the most commonly used insecticide in vector control, particularly against vectors resistant to the organochlorines and organophosphates (WHO 2005a). Hence, these insecticides were selected for the present investigation. This study aimed to reveal the sublethal effects of temephos and propoxur on *A. stephensi*, focusing on effects on life history traits. The endpoints selected are fecundity, hatchability, sex ratio, adult longevity, morphology of gonads and circadian changes in pupation and adult emergence.

Materials and methods

Mosquito used

Subhashnagar, Bangalore (SBN) and Vector Control Research Centre, Pondicherry (VCRC) strains of *A. stephensi* from South India were used for the experiments. Larval bioassay revealed that SBN and VCRC strains were sensitive among the strains tested for temephos and propoxur, respectively (Shetty et al. 2007; Hariprasad and Shetty, unpublished data). Hence, VCRC and SBN form ideal strains for monitoring the life history trait changes occurring due to insecticidal exposure that may eventually lead to resistance.

The adult mosquitoes were maintained at 25±1°C and 75 ±5% relative humidity during 10-h photoperiods according to the procedure of Shetty (1983). The adults were fed on 10% sucrose in 8×8×8 in. metal cages covered with cotton net cloth. Females were provided with mice and pigeons for blood meals. Plastic cups (3 in. in diameter) lined with filter paper containing clean water were placed inside the cage for oviposition. The eggs were kept for 72 h to ensure complete hatching. The hatched larvae were transferred to an enamel tray and reared. A powdered mixture of fish food and dog biscuits was used as larval food.

Insecticides

Temephos (Abate) is an organophosphate insecticide [phosphoric acid,O,O′-(thiodi,1,4-phenylene) O, O,O′,O′-tetramethyl ester] with chemical formula of $C_{16}H_{20}O_6P_2S_3$. Propoxur (Baygon) is a carbamate insecticide [*O*-isopropoxyphenyl methyl carbamates] having a chemical formula of $C_{11}H_{15}NO_3$; both were used for this study.

Temephos (50% TC) and Propoxur (20% EC) were obtained from the Greater Bangalore Municipal Corporation. Different concentrations (LC_{10}, LC_{30} and LC_{50}) in milligrams per litre of the said insecticides were prepared by using denatured alcohol (98 ml of absolute alcohol+2 ml of methyl ethyl ketone) as a diluting agent. Stocks and working solutions were prepared as recommended by the WHO (1981, 2005b).

Method of exposure

Batches of 25 third instar larvae (in three replicates) of the SBN strain of *A. stephensi* were exposed to sublethal concentrations of temephos, whereas the VCRC strain was used to study the effect of propoxur. The three sublethal concentrations (LC_{10}, LC_{30} and LC_{50}) used in this study (Table 1) were derived from probit analysis results generated by Shetty et al. (2007); Hariprasad and Shetty, unpublished data.

Experiments were carried out according to the procedure of WHO (1981, 2005b). Twenty-five larvae were released into 500-ml glass beakers containing 250 ml distilled water. The larvae were exposed to different concentrations (LC_{10}, LC_{30} and LC_{50}). The experiments were carried out at 26°C± 2°C. Three replicates of each concentration were run under the same conditions along with an untreated control. The control larvae were given the same food as the experimental larvae. Mortality was assessed at 24-h post-treatment. The survivors were isolated and washed with tap water and reared in separate enamel pans for subsequent study on life traits for two generations. The process of getting F_1 and F_2 from parents is based on a selection process of surviving adults (parent) from previous generations to each sublethal concentration of insecticides. Observations on changes in life history parameters were done as described by Aguilera et. al. (1995) and Zin and Shetty (2008).

Table 1 Sublethal concentration of temephos and propoxur at which the larvae were exposed

Insecticides	Strains	Sublethal concentration of insecticides (mg/l)		
		LC_{10}	LC_{30}	LC_{50}
Temephos	Subhashnagar (SBN)	0.00030	0.00130	0.00310
Propoxur	Pondicherry (VCRC)	0.00027	0.00056	0.00094

Fecundity

The eggs laid by blood-fed females were collected from the ovitrap lined with filter paper. The eggs were counted using a dissecting microscope (×20) and allowed to hatch.

Hatchability

The hatched larvae were counted and the percentage hatchability was calculated for each of the isofemales using the formula:

$$\%\ \text{hatchability} = (\text{number of larvae}/\text{number of eggs}) \times 100.$$

Sex ratio of offsprings

Immediately after emergence from pupae, the adults were scored for sex ratio. The ratio of male/female from the offspring produced by each female was calculated for three generations.

Longevity

Freshly emerged adults whose larval stages were exposed to sublethal concentrations of the insecticides were isolated/reared in separate cages, and the number of days they survived was recorded for longevity of the adult fly.

Morphology of gonads

Five-day-old adult male and female mosquitoes (n=150) exposed at LC_{30} and LC_{50} concentrations were used for studying gonad morphological variations. Live males and females of same age were separated into a test tube and were incapacitated by tapping the lower end of the tube against the palm. Once disabled, males were placed in a drop of insect saline and examined with a dissecting microscope (×40). Their reproductive system was removed by using needles to detach the last segment of the abdomen. Slow excision removes the whole male reproductive system including testes, accessory glands and ejaculatory duct. In females, the ovaries were removed by anchoring the anterior part of the abdomen with one needle and the penultimate segment with the other and then slowly pulling the posterior to excise the last two segments. The abdomen was then gently pressed, exposing the ovaries posteriorly. The ovaries were separated from debris and examined with the dissection microscope.

Experiment on circadian pupation rhythms of larval development and pupal eclosion

Circadian rhythms were studied as described by Nayar and Sauerman (1970). Batches of 100 same age larvae were selected for studying the effect of temephos and propoxur exposure on their development until eclosion. Larvae of the SBN strain were selected, and their late third instars were exposed to a sublethal concentration of temephos, whereas late third instar larvae of VCRC strain were exposed to propoxur. The exposed larvae were washed with tap water and reared in separate enamel pans. The total number of hours taken for pupation was recorded among the insecticide treated and untreated control. Pupae thus formed were collected in wide-mouth bottles, and upon emergence, the adults were released to designated cages. The time interval from pupae formation to eclosion was also documented from the batches of larvae exposed to different sublethal concentrations and the untreated control. Similarly, rhythms of adult emergence (eclosion) maintained under strict light–dark condition were used in the regular maintenance of mosquitoes in our laboratory, i.e. 10 h of light and 14 h of darkness (10L:14D) (Shetty 1983) was recorded to correlate toxicological effect in its eclosion pattern, if any.

Statistical analysis

Mortality data recorded after 24 h of treatment of the larvae were subjected to probit analysis (Finney 1971) for calculating LC_{10}, LC_{30} and LC_{50}. Data were analysed by the Kruskal–Wallis one-way analysis of variance procedure of Minitab® statistical package for Windows, version 16 (Minitab 2010). Groups were considered significantly different at a family-wise error rate of α=0.05. In all analyses, treatments were compared to the untreated control.

Results

Sublethal effects of exposure to temephos

Effects of sublethal exposure of temephos on selected biological endpoints on the SBN strain of *A. stephensi* for subsequent generations (parental, F_1 and F_2) are illustrated in Table 2. Fecundity declined with an increase in the concentration of the insecticide, and was statistically found to be nonsignificant in the parent (Kruskal–Wallis value (H)= 1.89, df=3, P=0.596), F_1 (H=3.17, df=3, P=0.294) and F_2 (H=0.84, df=3, P=0.840) when compared to the untreated control. Significant differences in hatchability representing less fertile eggs were observed in F_1 (H=10.15, df=3, P= 0.017) and F_2 (H=17.51, df=3, P=0.001) generations, whereas nonsignificant differences were observed in the parental generation (H=5.70, df=3, P=0.127) compared to the control. The statistical values thus obtained clearly depict that the eggs were found to be less productive with an increase in sublethal concentrations. No significant difference was observed in the sex ratio of parental (H=3.956, df=3, P= 0.2662) and F_2 (H=0.8493, df=3, P=0.8376) generations,

Table 2 Effects of sublethal doses of temephos on the life history traits in SBN strain of *A. stephensi*

Generations/ conc.	No. of females	Total eggs	Mean (eggs/females) (±SD)	Ratio	Total larvae	Mean (larvae/female) (±SD)	% Hatchability	Ratio	No. of males	No. of female	M/F ratio	Mean (females/female) (±SD)	Ratio
Parent													
Control	15	1,089	72.60 (±18.17)	1.00	982	65.47 (±19.25)	90.17	1.00	409	435	0.94	29.00 (±11.12)	1.00
LC_{10}	15	978	65.20 (±25.11)	0.90	843	56.20 (±26.03)	86.20	0.96	437	401	1.09	26.73 (±15.78)	0.92
LC_{30}	15	963	64.20 (±16.11)	0.88	784	52.27 (±17.05)	81.41	0.90	399	376	1.06	25.07 (±09.54)	0.86
LC_{50}	15	970	64.67 (±18.96)	0.89	712	47.47 (±20.18)	73.40	0.81	323	378	0.85	25.20 (±11.52)	0.87
F_1													
Control	15	1,126	75.07 (±19.56)	1.00	916	61.07 (±20.20)	81.35	1.00	353	438	0.81	29.20 (±11.34)	1.00
LC_{10}	15	995	66.33 (±23.05)	0.88	780	52.00 (±23.48)	78.39	0.96	392	331	1.18	22.07 (±11.70)	0.76
LC_{30}	15	967	64.47 (±22.84)	0.86	730	48.67 (±19.09)	75.49	0.93	276	427	0.65	28.47 (±13.78)	0.97
LC_{50}	15	895	59.67 (±21.30)	0.79	523	34.87 (±18.34)	58.44	0.72	242	211	1.15	14.07 (±07.07)	0.48
F_2													
Control	15	977	65.13 (±17.73)	1.00	938	62.53 (±18.54)	96.01	1.00	412	509	0.81	33.93 (±12.30)	1.00
LC_{10}	15	923	61.53 (±20.11)	0.94	631	42.07 (±19.25)	68.36	0.71	334	276	1.21	18.40 (±02.01)	0.54
LC_{30}	15	916	61.07 (±23.84)	0.94	529	35.27 (±18.47)	57.75	0.60	239	269	0.89	17.93 (±09.90)	0.53
LC_{50}	15	874	58.27 (±19.60)	0.89	471	31.40 (±17.07)	53.89	0.56	212	225	0.94	15.00 (±08.20)	0.44

whereas a significant shift in sex ratio distortion towards males was observed in the F_1 (H=12.41, df=3, P=0.0061) generation compared to the control. Adult longevity was found to be significantly prolonged in parent (H=26.50), F_1 (H=40.92) and F_2 (H=28.19) generations when exposed to LC_{10}, LC_{30} and LC_{50} concentration of insecticides. The mean number of days adults survived (±SE) when exposed to the sublethal concentration of temephos is presented in Fig. 1.

Sublethal effects of exposure to propoxur

The effects of propoxur on the VCRC strain of *A. stephensi* for subsequent generations (parental, F_1 and F_2) are shown in Table 3. Though fecundity was marginally reduced with an increase in the concentration of the insecticide exposure, it was found to be statistically nonsignificant in the parent (H=1.15, df=3, P=0.765), F_1 (H=1.68, df=3, P=0.640) and F_2 (H=4.98, df=3, P=0.173) in contrast to the untreated control. Significant differences in hatchability were observed in F_1 (H=9.47, df=3, P=0.024) and F_2 (H=14.95, df=3, P=0.002) generations, whereas no significant difference was observed in the parental generation (H=4.51, df=3, P=0.211) compared to the control. No significant difference was observed in the sex ratio of parental (H=1.732, df=3, P=0.6299), F_1 (H=2.071, df=3, P=0.5578) and F_2 (H=2.254, df=3, P=0.5214) generations when compared to the untreated control. The mean number of days adults survived (±SE) when exposed to the sublethal concentration of propoxur is presented in Fig. 2. A significant increase in adult longevity was observed in the parent (H=40.04), F_1 (H=36.92) and F_2 (H=12.47) generation when exposed to LC_{10}, LC_{30} and LC_{50} concentration of propoxur.

Effect of exposure of temephos and propoxur on morphology of gonads

Adult males from temephos-treated larvae showed distinct structural changes in the testes in 30% of the population of the F_1 generation at LC_{50}. However, morphological distortion in testes was observed in 20% and 40% of the population in F_2 generations at sublethal concentrations of LC_{30} and LC_{50}, respectively. Frequently observed changes in male gonads were atrophied testes, reduced accessory glands and short, disintegrated, thin vas deferens (Fig. 3). Among females, 10% of the population showed size differences in ovaries at LC_{50} in the F_2 generation when exposed to temephos.

No morphological changes in ovaries were observed in females treated with sublethal concentrations of propoxur. In males, a short vas deferens and rudimentary testes were commonly recorded in 30% of the population in F_1 generation at LC_{30}. At LC_{50} concentration, 20% and 30% of the population were affected with structural deformation of testes in males in F_1 and F_2 generations, respectively. Adults from the temephos-treated group showed maximum structural distortions when compared to that of those subjected to propoxur treatment.

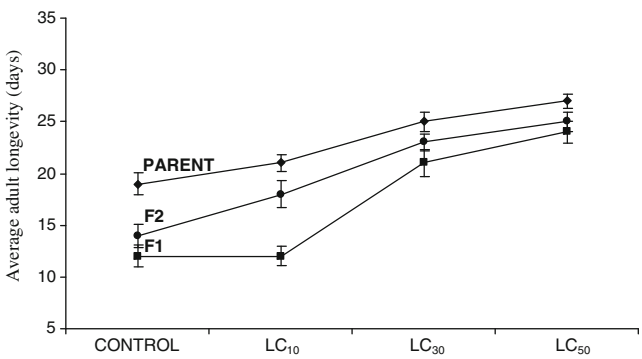

Fig. 1 Effect of sublethal exposure of temephos on the adult longevity (mean no. of days, ±SE) of parent, F1 and F2 generations of *A. stephensi* as compared to that of the control

Effect of temephos and propoxur on circadian pupation rhythms of larval development and pupal eclosion

Studies on light regime effects on pupation rhythms in larval population of *A. stephensi* treated with different concentrations of temephos and propoxur insecticides are shown in Fig. 4a, b. The first peak, indicating the maximum average pupation time occurred at 48 h, was followed by a second peak at 60 h among both the larval population treated with LC_{10} and LC_{30} of the insecticides. However, in the larval batches exposed to LC_{50} of the insecticides, the highest pupation peak occurred at 72 h followed by a peak at 60 and 40 h., indicating delayed/prolonged larval ecdysis with increase in the concentration of insecticides.

Demonstration of eclosion (adult emergence) from pupa whose larval stage was treated with the insecticides temephos and propoxur is presented in Fig. 5a, b. The results clearly depict that the eclosion peaked during early morning hours and late evening in the LC_{10} exposure of the insecticides, which was similar to that of the untreated control. Pupal eclosion into adults from the LC_{30} and LC_{50} treated batch for both the insecticides showed synchronous peaks from dusk to dawn indicating a non-rhythmic pattern of adult emergence on exposure to insecticides, compared to that of discontinuous peaks of control (Fig. 5a, b) showing a rhythmic pattern of eclosion.

Discussions

Sublethal exposure of *A. stephensi* to temephos and propoxur affected changes in life history traits. Statistically nonsignificant differences in the number of eggs produced

Table 3 Effects of sublethal doses of propoxur on the life history traits in VCRC strain of *A. stephensi*

Generations/conc.	No. of females	Total eggs	Mean (eggs/females) (±SD)	Ratio	Total larvae	Mean (larvae/female) (±SD)	% Hatchability	Ratio	No. of males	No. of female	M/F ratio	Mean (females/female) (±SD)	Ratio
Parent													
Control	15	1,037	69.13 (±22.14)	1.00	975	65.00 (±22.03)	94.02	1.00	466	456	1.02	30.40 (±10.35)	1.00
LC_{10}	15	988	65.87 (±20.28)	0.95	902	60.13 (±17.61)	91.30	0.97	396	413	0.96	27.53 (±10.36)	0.91
LC_{30}	15	964	64.27 (±16.18)	0.93	783	52.20 (±21.32)	81.22	0.86	296	367	0.81	24.47 (±09.01)	0.80
LC_{50}	15	937	62.47 (±21.17)	0.90	784	52.27 (±15.01)	83.67	0.89	312	345	0.90	23.00 (±09.86)	0.76
F_1													
Control	15	976	65.07 (±15.66)	1.00	943	62.87 (±14.84)	96.62	1.00	382	468	0.82	31.20 (±09.14)	1.00
LC_{10}	15	897	59.80 (±19.17)	0.92	735	49.00 (±16.29)	81.94	0.85	324	411	0.79	27.40 (±10.86)	0.88
LC_{30}	15	966	64.40 (±21.16)	0.99	769	51.27 (±16.17)	79.61	0.82	304	321	0.95	21.40 (±08.53)	0.69
LC_{50}	15	865	57.67 (±16.81)	0.89	652	43.47 (±17.04)	75.38	0.78	299	338	0.88	22.53 (±10.52)	0.72
F_2													
Control	15	876	58.40 (±16.48)	1.00	789	52.60 (±14.28)	90.07	1.00	354	335	1.06	22.33 (±06.37)	1.00
LC_{10}	15	843	56.20 (±20.37)	0.96	619	41.27 (±12.56)	73.43	0.82	289	290	1.00	19.33 (±06.95)	0.87
LC_{30}	15	757	50.47 (±19.74)	0.86	538	35.87 (±17.01)	71.07	0.79	238	276	0.86	18.40 (±09.44)	0.82
LC_{50}	15	687	45.80 (±17.04)	0.78	465	31.00 (±14.21)	67.69	0.75	231	231	1.00	15.40 (±06.91)	0.69

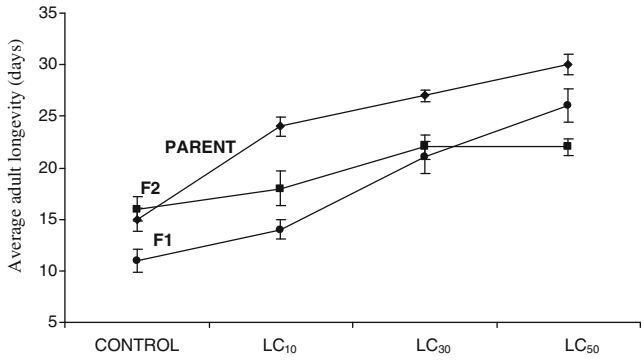

Fig. 2 Effect of sublethal exposure of propoxur on the adult longevity (mean no. of days, ±SE) of parent, F1 and F2 generations of *A. stephensi* as compared to that of the control

by blood-engorged females were observed in the control and treatment groups. However, a marginal decrease in fecundity was observed with an increase in insecticide concentrations in the following generation. These results indicate that temephos and propoxur cause reproductive disadvantages in exposed groups. Similar findings in *A. stephensi* were also recorded from sublethal exposures of malathion, fenthion, methyl parathion (Rao and Shetty 1992), neem and bifenthrin (Zin and Shetty 2008). Analogous conclusion in *Aedes aegypti* showed that females exposed to higher concentration of abate (temephos), malathion and alphamethrin during their larval stage exhibited a decrease in egg production numbers (Reyes-Villanueva et al. 1990; Minn and Shetty 2008). A decrease in fecundity on propoxur exposure has been reported in *Musca domestica* by Georghiou (1965). While many reports showed that insecticides reduced the fecundity of the affected insects, there have been some contradictory findings. In *A. aegypti*, Sutherland et al. (1967) observed an increase in basal follicles upon treatment with sublethal doses of DDT, dieldrin and malathion.

The present study on hatchability confirms that the eggs of females whose larval stages were exposed to temephos and propoxur were significantly less fertile. However, temephos had a greater influence in reduced egg hatchability compared to that of eggs from propoxur-treated females in all three generations. This indicates that both insecticides may have a direct toxic effect which may have been left unmetabolized in the body of the parent and may have been transmitted to the eggs. Insecticides acting on the central nervous system can cause a disturbance in the neurosecretory system, which results in disrupted reproductive physiology in the parent. Since reproduction is neurohormone regulated, neurohormonal imbalance from insecticide poisonings may affect normal function (Maddrell and Reynolds 1972; Lee 2000). Reduced hatchability is also reported in *A. stephensi* exposed to sublethal concentrations of alphamethrin (Hariprasad and Shetty, unpublished data). Sex ratio distortion (males/females) were found to be negligible in the parental and F_1 generation at LC_{10} and LC_{30}, but a radical decline toward male sex ratio in the F_2 generation at LC_{50} was observed when subjected to temephos. Propoxur-treated population showed small sex ratio distortion towards male in all the three generations. These results may suggest that insecticides may be nonselective towards

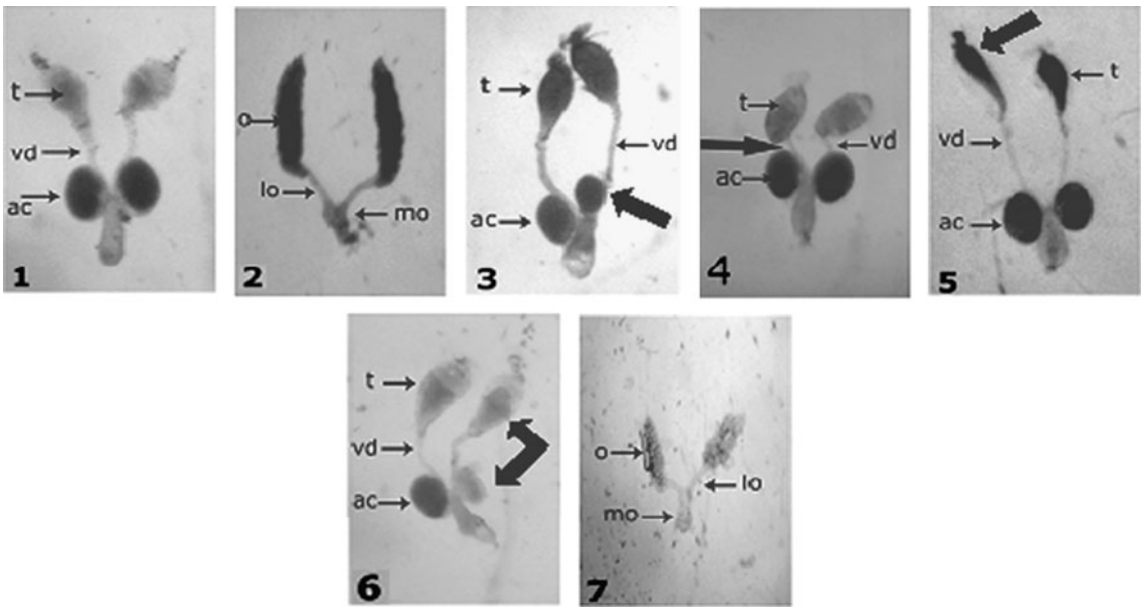

Fig. 3 Plates *1* and *2* show normal testis and ovary, respectively; *3* reduced vas deferens (*vd*); *4* short vd; *5* depicts atrophied/rudimentary test (*t*); *6* shows disintegrated testis and accessory gland (*ac*) with reduced vd; *7* poorly developed ovary (*o*) compared to that of normal as shown in *2*

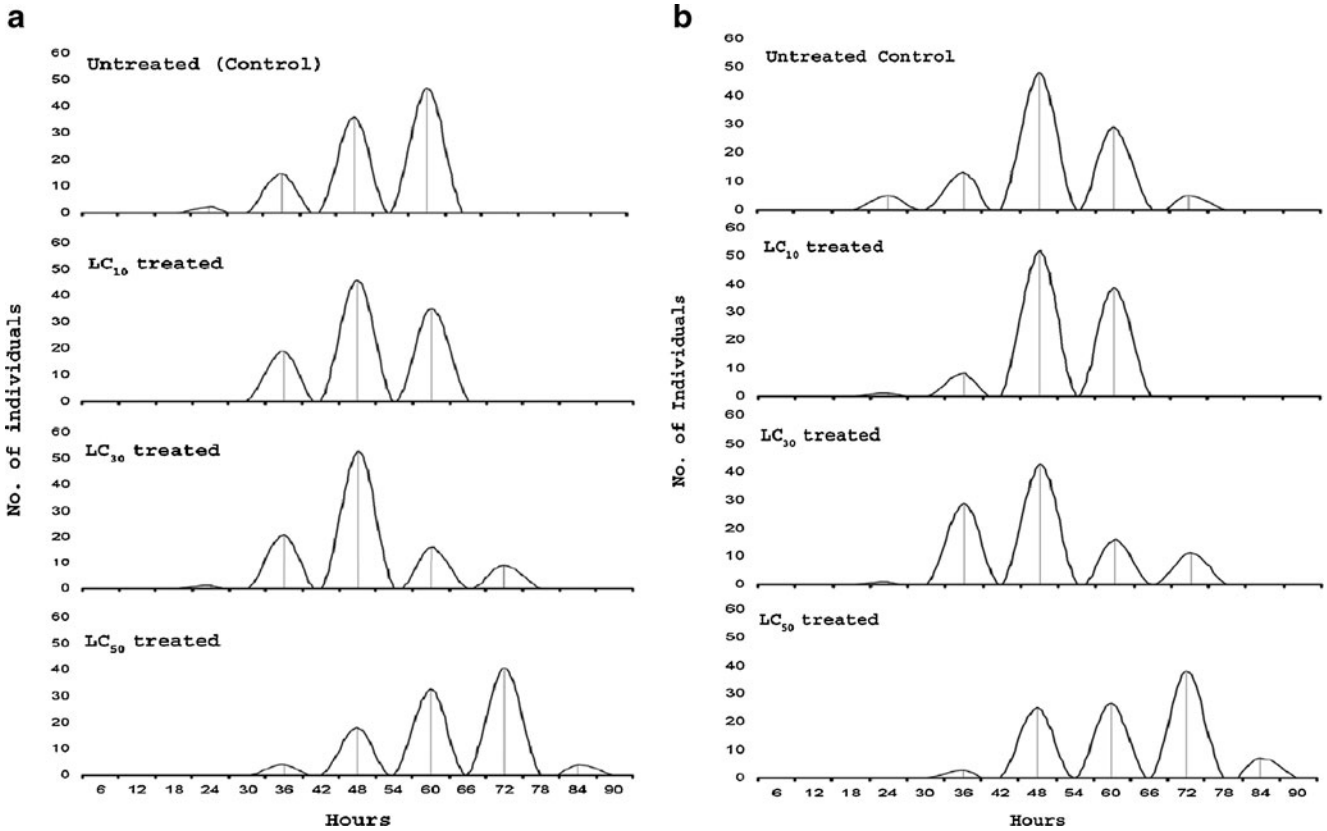

Fig. 4 Circadian larval rhythms of pupation in the population of *A. stephensi*. **a** Temephos-treated batch. **b** Propoxur-treated batch

developing larvae, females and males. Sex ratio distortions toward males were also reported in Indian strains of *A. stephensi* treated with fenitrothion, cypermethrin and deltamethrin (Priyalakshmi et al. 1999). The insignificant impact of insecticides on the progeny sex ratio was also previously reported in *Culex quinquefasciatus* using malathion, chlorpyriphos, methyl-pirimiphos, propoxur and resmethrin (Aguilera et al. 1995) and other insect species (Suh et al. 2000).

Sublethal concentrations of temephos and propoxur had affirmative effects on the adult longevity of *A. stephensi*. The average longevity of adults whose fourth instar larval stage was exposed to the temephos/propoxur was significantly increased compared to the control. The influence of temephos in increasing mosquito longevity has been previously reported by Reyes-Villanueva et al. (1990) for *A. aegypti*. An increase in adult longevity may increase opportunities for mating in males, while long-lived females tend to have more gonotrophic cycles and thus more progeny. These changes could increase the number of blood meals taken and increase the risk of pathogen transmission by insecticide-exposed individuals. Lengthened larval and pupal duration was recorded in populations that were exposed to LC_{30} and LC_{50} concentrations of temephos and propoxur, resulting in increase in average longevity compared to populations of controls. Similar findings were also observed in *A. aegypti* and *C. quinquefasciatus* exposed to temephos (Shian 2007).

Morphological effects of insecticides on reproductive organs revealed that atrophied/rudimentary testes, short vas deferens and reduced accessory glands were frequent in males exposed to increased concentrations, i.e. LC_{30} and LC_{50} of temephos and propoxur. Few structural differences in ovaries were recorded from the adult female treated with the insecticides.

Our investigation also revealed that larvae exposed to temephos and propoxur at LC_{30} and LC_{50} concentrations displayed disturbed rhythms of larval development and eclosion from puparium compared to that of the control. The fewest variations in circadian rhythms of pupation and eclosion were observed from pupae whose larval stages were exposed to LC_{10} of the insecticides. Circadian rhythms in mosquito sensitivity to toxicants were studied in *A. aegypti*, the larvae of which were most susceptible to chlorpyrifos (Dursban) 1 h after lights off (Roberts et al. 1974). Circadian changes of insecticide sensitivity were reported in both larvae and adults of three strains of *A. aegypti* (Bainbridge 1983). The occurrence of day/night ecdyses is species-dependent. It is thought that the trigger to molt is switched on and off based on a daily rhythmic activity

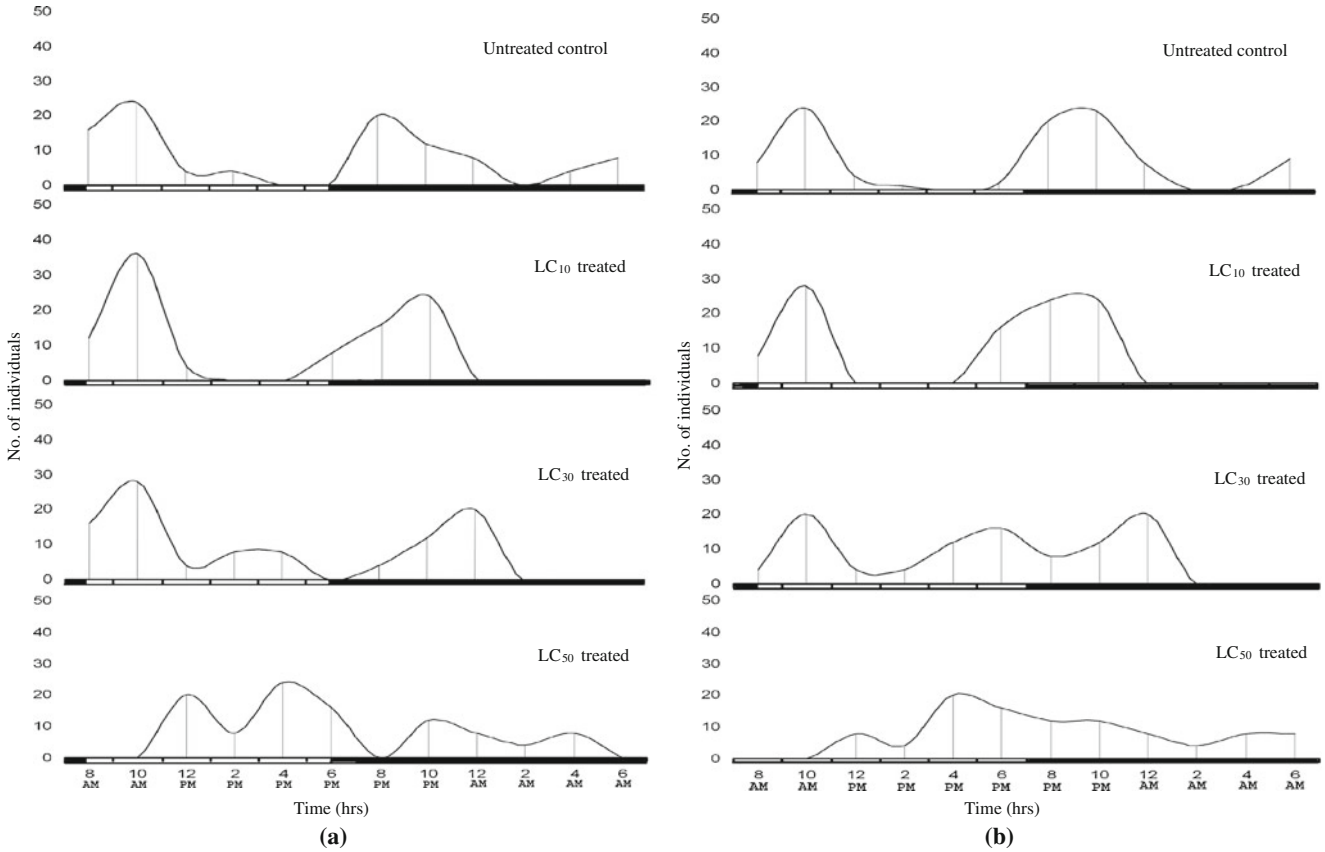

Fig. 5 Circadian pupation rhythms of eclosion in the population of *A. stephensi*. **a** Temephos-treated batch. **b** Propoxur-treated batch

cycle of 24-h intervals that is exhibited by many organisms, or a circadian rhythm. This is not universal among all mosquitoes. Examples of some found not to have such a rhythm are *Anopheles quadrimaculatus* (Nayar and Sauerman 1970) and *A. aegypti* (Haddow et al. 1959). The circadian rhythms of insect sensitivity to toxicants have been related to rhythmic biochemical processes. Thus, behavioural and physiological rhythms might be considered as marker rhythms for the rhythmic patterns of sensitivity to toxicants. However, the circadian peaks of sensitivity may not always be correlated with activity and metabolism (Eesa and Cutkomp 1995).

Survival of insect individuals exposed to sublethal level of insecticides is the consequence of an increase in the selection pressure towards resistance. This is due to physiological changes resulting in an increased number of copies of genes, coding for more protective enzymes that break down the toxins into less toxic ones. Such enzymes include esterases, glutathione transferases and mixed microsomal oxidases (Daly et al. 1998). Alternately, the number of biochemical receptors for the chemicals may be reduced, or the receptor may be altered, reducing the insect sensitivity to the toxin compounds (Daly et al. 1998). Mutation in a single gene only leads to the development of a resistant organism (Chaube and Pundhir 2005; Marchetti and Moyle 2010). In other cases, multiple genes are involved. Resistant genes are usually autosomal. As a result, resistance is inherited similarly in males and females. Also, resistance is usually inherited as an incompletely dominant trait (Alyokhin et al. 2008). When a resistant and a susceptible individual mate, their progeny has an intermediate level of resistance (Rajashree and Shetty 1998; Sanil and Shetty 2009, 2010). Adaptation to pesticides usually decreases relative fitness of organisms in the absence of pesticides. Resistant individuals often have a reduced reproductive output, life expectancy, mobility etc. (Stenersen 2004).

The significance of insecticides on reproductive potential depends upon the mosquito species, inherent toxicity, selection pressure (dosage) and behaviour of mosquitoes towards the insecticide application in the field population (Zin and Shetty 2008). The application of insecticides in the field would result in a decline of the population not only by killing the susceptible ones but also by reducing the reproductive potential among the resistant strains (Rao and Shetty 1992). Knowledge of these sublethal effects of insecticides could be important in the decision-making process in integrated pest management programmes (Robert and Olson 1989). Therefore, the present study underlines the need to maintain concentrations of insecticides at lethal levels in larval habitats with better planning of insecticide application.

Acknowledgement This work has been supported by financial assistance from University Grants Commission (UGC), New Delhi to Prof. N. J. Shetty.

References

Aguilera L, Marquetti CM, Navarro A, Bisset J (1995) Effects of three organophophorus insecticides in the reproductive potential of *Culex quinquefasciatus*. Mem Inst Oswaldo Cruz 90:411–413

Ali A, Xue RD, Barnard DR (2006) Effects of sublethal exposure to boric acid sugar bait on adult survival, host-seeking, bloodfeeding behavior, and reproduction of *Stegomyia albopicta*. J Am Mosq Control Assoc 22:464–468

Alyokhin A, Baker M, Mota-Sanchez D, Dively G, Grafius E (2008) Colorado potato beetle resistance to insecticides. Am J Potato Res 85:395–413

Bainbridge CA (1983) Factors affecting insecticide resistance in the yellow fever mosquito, *Aedes aegypti* (L.). Thesis, Liverpool Polytechnic.

Beard CB, Durvasula RV, Richards FF (1998) Bacterial symbiosis in arthropods and the control of disease transmission. Emerg Infect Dis 4:581–591

Chaube HS, Pundhir VS (2005) Chemical control. In: Crop diseases and their management. Prentice-Hall, New Delhi

Curtis CF, Davies CR (2001) Present use of pesticides for vector and allergen control and future requirement. Med Vet Entomol 15:231–235

Daly H, Doyen JT, Purcell AH III (1998) Introduction to insect biology and diversity, 2nd edn. Oxford University Press, New York, pp 279–300, Chapter 14

Eesa NM, Cutkomp KL (1995) Pesticide chronotoxicity to insects and mites: an overview. Jour Islm Acad Sci 8:21–28

Finney DJ (1971) Probit analysis, vol 3. Cambridge University Press, Cambridge

Gayathri DK, Shetty NJ (1992) Chromosomal translocation and inherited semisterility in the malaria vector *Anopheles stephensi* Liston. J Commun Dis 24:70–74

Georghiou GP (1965) Effects of carbamates on house fly fecundity, longevity and food intake. J Econ Entomol 58:58–62

Haddow AJ, Gillet JD, Corbet PS (1959) Laboratory observations on pupation and emergence in the mosquito *Aedes* (*Stegomyia*) *aegypti* (Linnaeus). Ann Trop Med Parasitol 53:123–131

Haynes KF (1988) Sublethal effects of neurotoxic insecticides on insect behavior. Ann Rev Entomol 33:149–168

Lee CY (2000) Sublethal effects of insecticides on longevity, fecundity and behaviour of insect pests—a review. J Biosci 11:1–6

Maddrell SHP, Reynolds SE (1972) Release of hormones in insects after poisoning with insecticides. Nature 236:404–406

Marchetti MP, Moyle PB (2010) Variation, natural selection and evolution. In: Protecting life on earth—an introduction to the science of conservation. University of California Press, Berkeley

Minitab (2010) Minitab statistical software. Version 16. Minitab Inc., State College, PA

Minn ZM, Shetty NJ (2008) Toxicological effect of malathion and alphamethrin on reproductive potential in *Aedes aegypti*, a yellow fever mosquito. Pestology 32:39–43

National Vector Borne Diseases Control Programme & Directorate General of Health Services (2007) Guidelines for integrated vector management for control of dengue/dengue haemorrhagic fever. Ministry of Health & Family Welfare, New Delhi, pp 1–8

Nayar JK, Sauerman DM (1970) A comparative study and development in Florida mosquito. I. Effects of environmental factors on ontogenetic timings, endogenous diurnal rhythm and synchrony of pupation and emergence. J Med Entomol 7:163–174

Priyalakshmi BL, Rajashree BH, Ghosh C, Shetty NJ (1999) Effect of fenitrothion, deltamethrin and cypermethrin on reproductive potential and longevity of life cycle in *Anopheles stephensi* Liston, a malaria mosquito. J Parasit Dis 23:125–128

Rajashree BH, Shetty NJ (1998) Genetic study of deltamethrin resistance in the malaria mosquito *Anopheles stephensi* Liston. J Parasit Dis 22:140–143

Rao DEG, Shetty NJ (1992) Effect of insecticide resistance on reproductive potential in *Anopheles stephensi* Liston, a malaria mosquito. Int J Occup Env Health 1:48–52

Reyes-Villanueva F, Juarez-Eguia M, Flores-Leal A (1990) Effects of sublethal dosages of Abate® upon adult fecundity and longevity of *Aedes aegypti*. J Am Mosq Control Assoc 6:739–741

Robert LL, Olson JK (1989) Effects of sublethal dosages of insecticides on *Culex quinquefasciatus*. J Am Mosq Control Assoc 5:239–246

Roberts DR, Smolensky JA, Bartholomew PH, Scanlon JE (1974) Circadian pattern in susceptibility of *Aedes aegypti* (L.) larvae to Dursban. In: Scheving LE, Halberg F, Pauly JE (eds) Chronobiology. Igaku Schoin Ltd, Tokyo, pp 612–616

Saelim V, Brogdon WG, Rojanapremsuk J, Suvannadabba S, Pandii W, Jones WJ, Sithiprasasna R (2005) Bottle and biochemical assays on temephos resistance in *Aedes aegypti* in Thailand. Southeast Asian J Trop Med Publ Health 36:417–425

Sanil D, Shetty NJ (2010) Genetic study of propoxur resistance (*pr*)—a carbamate insecticide in the malaria mosquito, *Anopheles stephensi* Liston. Malaria Research and Treatment 2010:6. doi:10.4061/2010/5028242010

Sanil D, Shetty NJ (2009) Genetic study of temephos resistance (*tr*), an organophosphate insecticide in the malaria mosquito, *Anopheles stephensi* Liston. J Cytol Genet 11:15–22

Shetty NJ (1983) Chromosomal translocations and semisterility in the malaria vector *Anopheles fluviatilis* James. Indian J Malariol 20:45–48

Shetty NJ (1987) Genetic sexing system for the preferential elimination of females in *Culex quinquefasciatus*. J Am Mosq Control Assoc 3:84–86

Shetty NJ (1997) Genetic control of mosquito vectors of diseases. J Parasit Dis 21:113–121

Shetty NJ (2002) The genetic control of *Anopheles stephensi*—a malaria mosquito. In: Raghunath D, Nayak R (eds) Trends in malaria and vaccine research: the current Indian scenario. Tata McGraw-Hill, New Delhi, pp 44–79

Shetty NJ, Vasanth SN, Sanil D (2007) Insecticide susceptibility studies of fenthion and temephos in thirty strains of *An. stephensi* Liston, a malaria mosquito. Pestology 31:33–39

Shian LC (2007) The effect of sublethal concentration of abate on *Aedes aegypti* (linnaeus) and *Culex quinquefasciatus* (Says); Universiti Sains Malaysia.

Stenersen J (2004) Chemical pesticides: mode of action and toxicology. CRC Press, Boca Raton

Suh CPC, Orr DB, Van Duyn JW (2000) Effect of insecticides on *Trichogramma exiguum* (Hymenoptera; Trichogrammatidae) preimaginal development and adult survival. J Econ Entomol 93:577–583

Sutherland DJ, Beam FD, Gupta AP (1967) The effects of mosquitoes of sublethal exposure to insecticides DDT, dieldrin, malathion on the basal follicles of *Aedes aegypti* (L.). Mosq News 27:316–323

USEPA (2001) Prevention, pesticide & toxic substance (7508C), United State Environmental Protection Agency, EPA 738-F-00-018.

WHO (1981) Instruction for determining the susceptibility or resistance of mosquito larvae to insecticide. WHO/VBC/81.807.

WHO (2005a) Propoxur. WHO specifications and evaluations for public health pesticides. WHO, Geneva. pp. 1–25.

WHO (2005b) Guidelines for laboratory and field testing of mosquito larvicide. WHO/CDS/WHOPES/GCP.

Zaim M, Jambullingam (2007) Global insecticide use of vector-borne disease control, 3rd edn. WHO, Geneva

Zin T, Shetty NJ (2008) Sublethal effect of bifenthrin and neem on fecundity, hatchability and sex ratio of *Anopheles stephenesi* Liston, a malaria mosquito. Pestology 32:39–44