DISCUSSION
6. DISCUSSION

*An. stephensi* has been used as model mosquito in our laboratory to carry out the regular research on genetic basis of insecticide resistance, biochemical studies on the resistant strain, chromosomal translocations and inversions, susceptibility to various malaria parasites and formal genetics involving isolation and characterization of mutant markers, allelism tests and linkage study etc. (Shetty, 1997; Gayathri and Shetty, 1990; 1992a; Rao and Shetty, 1994; Rajasree and Shetty, 1998a; Ghosh and Shetty, 1999, 2002; Madhyastha and Shetty, 1999, 2002).

Classification of the *An. stephensi* strains was done as this was basic requirement for carrying out the present investigation. This mosquito is classified into three variants viz. type (14-21 ridges), intermediate (12-17 ridges) and var. *mysorensis* (9-15 ridges) based on the number of ridges on egg float (Sweet and Rao, 1937; Subbarao *et al.*, 1987). The significant variations in egg length and width were also reported earlier (Rao *et al.*, 1938).

In the present investigation, nine strains of *An. stephensi* were analyzed for egg float ridge number (Table 2). The egg measurements of these strains were also recorded (Table 3). Marked differences were observed in ridge number and measurement of eggs. Based on this, the strains were categorized into three variants. Seven strains namely CLO, MGNa,
DELa, AGB, HRN, PDY and CHNb were grouped into type form (14-21 ridges); WCR was intermediate (12-19 ridges) and CUB was var. *mysorensis* (10-13 ridges).

The average length of eggs for type form varied between $585.5 \pm 0.34 \mu m$ and $560.5 \pm 0.54 \mu m$, for intermediate $532.3 \pm 0.23 \mu m$ and $478.7 \pm 0.33 \mu m$ for *mysorensis* respectively. Similarly, the average breadth for type form ranged between $208.7 \pm 0.23 \mu m$ and $200.2 \pm 0.75 \mu m$, while in intermediate $187.7 \pm 0.54 \mu m$ and in *mysorensis* $106.4 \pm 0.23 \mu m$. The average length of float on one side for type varied between $296.2 \pm 0.23 \mu m$ and $280.6 \pm 0.54 \mu m$; in intermediate $245.6 \pm 0.38 \mu m$ and in *mysorensis* $216.3 \pm 0.43 \mu m$. The present observation corresponded to those observed by Sweet and Rao (1937) and Rao *et al.* (1938). The average measurement of intermediate eggs was observed closer to the size of type form. The other six strains namely CHNa, MGNb, RJN, KNG, YLH and DELb were type forms already classified earlier in our laboratory (Shetty *et al.*, 1999).

The marked difference in the habitats of *An. stephensi* in many parts of India reported by earlier observers led to the postulation of the existence of biological races, where the type form, an efficient vector of malaria was reported to be prevalent in the urban areas whereas the *mysorensis* variety, a poor vector was present in rural areas (Rao, 1984). In the present study as mentioned above, the type and intermediate forms, which were
predominantly found in the urban areas, are in agreement with the earlier report. But the *mysoresensis* variety (CUB strain from Bangalore) also collected from urban locality which is not consistent with the earlier report (Sweet and Rao, 1937). So the present investigation revealed that all the three varieties were present in urban localities.

Siddiqui and Aslam Khan (1973) reported the presence of inversion in polytene chromosomes of *mysoresensis* variety. However, Suguna (1981b) found no such correlation while Subbarao et al. (1987) reported such inversion from the females of all the three variants.

Earlier report on breeding experiments between different varieties of *An. stephensi* indicated no post copulatory barriers between the populations. Genetic crosses and backcrosses between the variants revealed that variation in ridge number is controlled by more than one genetic factor (Curtis et al., 1985). Mahmood and Sakai (1984) found rural and urban *An. stephensi* populations to differ in chromosome inversion frequencies where eight autosomal paracentric inversions were found floating in these populations.

In the present study, the baseline susceptibility status of fifteen strains of *An. stephensi* to fenitrothion was carried out for selection of resistant strains under laboratory condition. In this study it was observed that all the strains were highly susceptible to fenitrothion (Table 4, Figs. 1a, 1b
and 1c) and there were no significant differences between the least and most sensitive strains. CHNb was the most susceptible strain which showed lowest LC50 value of 0.0023 ppm (95% CL – 0.0021 to 0.0025) while KNG was the least susceptible strain showing highest LC50 value of 0.0041 ppm (95% CL – 0.0039 to 0.0044). The response slopes ranged from 1.4 to 2.6 (Ghosh et al., 2002).

Due to its specific mode of action against insects, fenitrothion has been used in vector control programme both as a larvicide as well as an adulticide (WHO, 1986). Fenitrothion has been extensively used for mosquito control in many countries including India.

Two colonies of the taxon An. culicifacies Giles (one from Maharashtra and other from Gujrat, India) produced 28% and 64% mortality respectively on 1% fenitrothion for two hours exposure. Six hour exposures were required to achieve 96% mortality in both colonies (Herath et al., 1981).

Susceptibility tests on the adults of five species of mosquitoes vectors of JE viz. Culex tritaeniorhynchus, Cx. vishnui, Cx. pseudovishnui, Cx. gelidus and Cx. fuscoccephala were reported from Kolar district, Karnataka during 1990 to 1991 against organochloride and organophosphate compounds. Cx. tritaeniorhynchus was found resistant to fenitrothion
while Cx. vishnui, Cx. pseudovishnui, Cx. gelidus and Cx. fuscocephala were susceptible to fenitrothion. (Kulkarni et al., 1992).

Larval populations of Cx. tritaeniorhynchus and Cx. fuscocephala from Mysore city were tested for their susceptibility against fenitrothion (Vijayan et al., 1993). The investigation revealed that Cx. tritaeniorhynchus were highly tolerant against fenitrothion showing 6.5 times more tolerant than Cx. fuscocephala in terms of the LC$_{50}$ value. Susceptibility tests were conducted on the adults of two species of Japanese Encephalitis (JE) vectors viz. Cx. pseudovishnui and Cx. tritaeniorhynchus against diagnostic doses of fenitrothion (1%) at different exposure duration and both the species were found susceptible to fenitrothion (Bansal and Singh, 1995).

Studies on the current response of An. stephensi to fenitrothion and other insecticides were carried out in three districts (Barmer, Jodhpur and Pali) of the Thar Desert (Singh and Bansal, 1996a). The species was found susceptible to fenitrothion.

A laboratory trial was carried out by Ganguly et al. (1994) at Pune to evaluate the effectiveness of five types of larvicides including fenitrothion against Culex quinquefasciatus larvae. The study revealed that out of five, fenitrothion was the most effective. The estimated LC$_{90}$ for fenitrothion was the lowest and was 0.007 mg/l. Susceptibility of Phlebotomus
papatai to fenitrothion was also reported from Pali and Barmer districts of Rajasthan (Singh and Bansal, 1996b).

Routine susceptibility tests of the female *An. gambiae* complex have been carried out with fenitrothion in Senegal. *An. gambiae* showed normal susceptibility to fenitrothion but resistance was observed in Dakar and Kolda (Faye et al., 1991; 1992). Larvae of twenty six strains of *Aedes albopictus* (13 from USA, 5 from Brazil, 3 from South East Asia and 5 from Japan) were tested for susceptibility to fenitrothion. All strains were susceptible to fenitrothion while three Japanese strains showed tolerance or low level resistance (Wesson, 1990).

A range of dosage that gives a low to complete mortality in susceptible strains will provide the baseline with which the response of any other population may be compared (WHO, 1970). In view of the existing differences and the increasing resistance level under varied circumstances in vector species, it is rather essential to undertake susceptibility tests especially in disease prone areas.

It is believed that the use of larvicides is an appropriate adjunct to source reduction and offers the advantages of killing mosquitoes in the innocuous larval stage before the adults develop and disperse (Metcalf, 1994). This also provides a quick and relatively inexpensive method of
preventing and incipient attack by the adults in view of the fact that larval tests are more sensitive than adults.

As the most successful group of animals from the evolutionary point, insects surpass other animals in their genetic diversity and biochemical versatility as a result of which they successfully develop resistance to insecticides (Pillai, 1996). Resistance is defined “as an inherited characteristic that imparts an increased tolerance to an insecticide or a group of insecticides such that the resistant individuals survive a concentration of the compound (s) that would normally be lethal to the species” (WHO, 1986). Studies on genetics of insecticide resistant genes date back to late 1950s. Davidson (1956) reported dieldrin resistance in *An. gambiae* to be of mono-factorial nature. DDT resistance in *An. sundaticus* from Java was found to be due to a single recessive gene (Davidson and Jackson, 1961). Singh and Mohan (1965) reported DDT resistance in *An. stephensi* from India to be due to a single recessive gene but suggested that expression of gene depended on the genetic background.

Dieldrin resistance in *An. stephensi* and *An. culicifacies* was found to be controlled by a single incompletely dominant gene (Davidson and Mason, 1963).
Malathion resistance in *An. stephensi* has also been found to be controlled by a single gene, autosomally inherited and incompletely dominant (Rathor and Toquir, 1981; Herath and Davidson, 1981; Hemingway, 1982; Rao and Shetty, 1994). Backcrosses data in *An. culicifacies* indicated the involvement of more than one genes for malathion resistance. Synergistic studies also showed the involvement of carboxylesterase and mixed function of oxidases in resistance. The mode of inheritance of fenitrothion resistance in *Culex pipiens* L. was studied and it was reported that fenitrothion resistance was due to monofactorial inheritance with partial dominance and slight cytoplasmic effect (Shoukry and Hamed, 1990).

The genetic basis of fenitrothion resistance in *An. stephensi* has not been reported earlier. In the present study, based on the data of the genetic crosses between the homozygous fenitrothion resistant and susceptible individual (F₁), backcrosses and F₂ crosses revealed that the gene for fenitrothion resistance (*Fnr*) was a single gene. It is incompletely dominant and autosomal in *An. stephensi* (Table 6). The dosage-mortality (d-m) line for F₁ hybrids were found to be closer to that of resistant strains (Fig. 2). The F₁ individuals were significantly more resistant than susceptible parental strains, but less resistant than the resistant parental strains.
*An. stephensi*. In fenitrothion resistant strains, decreased fecundity by 31%, 24% and 20%, reduced egg hatchability by 35%, 31% and 38%; sex ratio distortion (males: females) by 0.82:1, 0.62:1 and 0.83:1 and duration of life cycle prolonged by 4, 4 and 5 days for parental, F1 and F2 generations respectively were observed against their corresponding control strain. However, a tendency to gain normalcy in F2 generation was noticed. The treated strains and their F1 and F2 progeny required longer duration to complete their life cycle. These are important factors which would be useful in the mosquito control programme.

Rao and Shetty (1992) have reported reduced fecundity (28% and 27%), egg hatchability (40% and 43%) and sex ratio distortion towards males (19 and 21) in parental (treated) generations for fenthion and methyl parathion treated larvae of *An. stephensi* at sub lethal doses of 0.0001 and 0.001 ppm respectively while malathion treated ones did not show any considerable changes.

*An. stephensi* treated at the sub lethal doses of 0.0005 and 0.00175 ppm resulted decreased fecundity (23% and 27%), egg hatchability (18% and 26%), sex ratio distortion (males: females) by 0.86:1 and 0.79:1 and duration of life-cycle by 2 and 3 days in parental generations for deltamethrin and cypermethrin respectively (Priyalakshmi *et al.*, 1999).
In other study, DDT and malathion showed 40% and 50% decreased fecundity, but did not affect the hatchability in *Culex pipiens* (Gaaboub and Darwood, 1973). Similarly the resistant strain was less fecund to temephos in *Culex quinquefasciatus* (Ferrari and Georgiou, 1981). The fecundity was decreased by 67.20% in *Cx quinquefasciatus*, 43.30% in *Aedes aegypti* and 40.60% in *An. stephensi* when treated with pyrethroids (Verma, 1986).

Saxena and Kaushik (1986) have reported that in *An. stephensi* chitin inhibitors perfluron at 0.001 ppm and furyltriazine at 10 ppm reduced the fecundity by 78.30% and 23.50% respectively. They also observed a dose dependent reduction in average number of eggs per female.

It is evident from the available literature that the results of the present study were consistent with the earlier reports and the effects of fenitrothion in relation to fecundity, hatchability, sex ratio and duration of life cycle have not been reported earlier in *An. stephensi*.

The quantitative changes of protein were estimated among the fenitrothion resistant and susceptible strains. The resistant strain showed increase of protein by 8.71%, 61.59%, 40.31%, 12.92% and 13.44% in eggs, larvae, pupae, adult males and females respectively when compared to the susceptible strains. The protein content was maximum in larval stage and declined in adults (Table 8; Fig. 3). The increased protein content in the OP
resistant strains of *An. stephensi* correlated with detoxification or dehydration (Rao and Shetty, 1996).

Variation was observed in protein profiles of resistant and susceptible strains during different development stages (Table 15). In both the resistant and susceptible strains, some bands were common; six (1, 4, 8, 10, 15 and 16) in eggs; twenty two (1, 2, 3, 4, 6, 8, 10, 12, 13, 15, 16, 17, 19, 20, 23, 25, 26, 27, 28, 29, 30 and 31) in larvae; fourteen (1, 2, 3, 4, 10, 11, 12, 15, 16, 17, 20, 23, 25 and 27) in pupae; eleven (1, 3, 5, 7, 9, 14, 15, 17, 18, 19 and 23) in adult males and eleven (1, 3, 5, 7, 9, 14, 15, 17, 18, 19 and 23) in adult females. Maximum bands were observed in larvae when compared to the other stages. One specific band 21 (Rm 0.56) was found in all the developmental stages of resistant strains except in eggs. Another specific band 24 (Rm 0.63) were detected in resistant adult males and females. In susceptible adult males and females, three bands 4, 22 and 26 having Rm values of 0.06, 0.59 and 0.69 were found. These were absent in the resistant strains.

Similar reports of increased protein levels in malathion, fenthion, methyl parathion (OP compounds) and in deltamethrin resistant strains of *An. stephensi* have been studied (Rao and Shetty, 1996; Rajasree, 1998b). Increased protein level due to fenitrothion resistance in *An. stephensi* has not been reported earlier.
The protein profiles in the resistant strain reflects the increase in protein levels during denaturation of proteins by insecticides and utilization of them for detoxification. Insecticide resistance is believed to develop largely not entirely as a result of natural selection of preadaptive mutants that possess genetically controlled mechanisms for detoxification, target-site insensitivity or other means of survival in the presence of insecticides (Oppenoorth, 1985). Metabolic pathways in mosquitoes are modified either to detoxify the insecticide or prevent metabolic conversion of insecticide into the active form (Mullen and Scott, 1992). Enzyme dependent detoxification is catalyzed by microsomal monooxygenases, in particular Cytochrome P450 and esterases (Dauterman and Hodgson, 1978). These enzymes may exist in multiple isoenzymic forms differing in substrate spectra. Induction of these enzymes by insecticides often result in the synthesis of more proteins in the resistant insects (Soderland and Bloomquist, 1990). The protein synthesis in larval stage was high because of its high activity in developmental stages which are used for the synthesis of adult organs (Agosin, 1978). Usually, the adult females showed higher protein content than males because of its high enzyme activity and requirements of specific proteins for egg production (Bianchi et al., 1983). The changes of protein content in different stages of life cycle reflected specific differences in protein synthesis, release and uptake during metamorphosis (Ferrari, 1996).
were found only in resistant adult males and females. These bands were absent in susceptible strains.

The β-esterase bands that were common in both resistant and susceptible strains include three (12, 13 and 14) in eggs; ten (1, 2, 3, 6, 8, 9, 10, 12, 13 and 15) in larvae; eleven (1, 2, 3, 6, 7, 8, 9, 10, 12, 13 and 15) in pupae; six (6, 7, 10, 11, 13 and 15) in adult males and six (6, 7, 10, 11, 13 and 15) in adult females. A specific β-esterase band number 14 (Rm 0.68) was found in all the developmental stages in resistant strains except in the eggs whereas in susceptible strains this band was found only in eggs (Table 17).

Esterases are mainly responsible for detoxification of OP compounds. Among the esterases, carboxylesterase is responsible for the resistance (Pasteur and Georghiou, 1980). It was reported that apparently this enzyme first forms a complex with OP compounds that further breaks down to give a phosphorylation product of the esterase and the corresponding hydroxyl compound. The phosphorylated esterase may be gradually hydrolyzed by water and by this process the esterase activity is restored (O’ Brien, 1978). The resistance of insects against organophosphorous is associated with high esterases activity in assays using α-naphthyl acetate substrate (Dary et al., 1990). The genetic basis of high esterase activity is involved in amplification of the esterase gene (Mouches et al., 1990; Soderland and Bloomquist, 1990; Devonshire and Field, 1992).
The difference in banding pattern and varied intensity among resistant and susceptible strains clearly signifies the role of esterases (α and β) in hydrolysis of insecticides. OP insecticide resistance associated with increased esterase levels in Culex tarsalis (Prabhakar et al., 1987) and An. stephensi (Rao and Shetty, 1996) have been reported earlier. Recent studies have confirmed the involvement of Est.-2 in resistance in An. albimanus (Cordon-Rosales et al., 1996). The use of esterase bands as a marker in the identification of resistant strains in a natural population of mosquitoes has been reported in An. stephensi (Georghiou and Pasteur, 1980); in Culex pipiens (Pasteur and Georghiou, 1980; Villani et al., 1983; Maruyama et al., 1984) and in Ae. albopictus (Tadano, 1987).

In the present investigation, the activity of Aph and Acph were increased in all the stages of fenitrothion resistant strains when compared to that of control strains.

The increase of Aph was 10%, 57.20%, 52.08%, 16.67% and 32.72% in eggs, larvae, pupae, adult males and females respectively (Table 11, Fig. 6). The activity was maximum in larvae followed by pupae, adult females, males and eggs. The Aph bands that were common in both resistant and susceptible strains included two (4 and 5) in eggs; seven (1, 2, 3, 4, 6, 9 and 10) in larvae; six (1, 2, 3, 4, 10 and 11) in pupae; three (1, 4 and 6) in adult males and six (1, 2, 4, 6, 7 and 10) in females (Table 18).
The increased activity of Aph and Acph in deltamethrin resistant strains in *An. stephensi* has been reported (Rajasree, 1998c).

Reduced LDH activity was observed in fenitrothion resistant strains of *An. stephensi* during all the developmental stages when compared to the susceptible ones. The decrease was -20%, -50%, -12.90%, -25% and -13.64% in eggs, larvae, pupae, adult males and females respectively (Table 13; Fig. 8). Single band 1 (Rm 0.17) was observed in all the developmental stages of both resistant and susceptible strains of *An. stephensi*. The bands 4, 5 and 6 (Rm 0.60, 0.66 and 0.73) were found only in the susceptible strains but absent in resistant ones (Table 20). But the activity was reduced in all the developmental stages of resistant strains. Maximum activity was observed in larvae followed by adult females, pupae, adult males and eggs respectively.

In the present investigation, reduced AChE activity was noticed in the resistant strain. The decrease was -2.22%, -4.85%, -0.969%, -1.738% and -3.118% in eggs, larvae, pupae, adult males and females respectively (Table 14, Fig. 9). Resistant pupae showed highest activity followed by adult males, females, larvae and eggs.

Organophosphate insecticides are neurotoxins. These insecticides exert their effects by inhibiting the enzyme acetylcholinesterase. Many insects and other arthropods have developed resistance to these compounds
through structural modification of their acetylcholinesterase. The AChE enzyme from resistant individuals is far less sensitive to inhibition than that from susceptible individuals (Hama, 1983; Soderlund and Bloomquist, 1990; Oppenoorth, 1985).

Similar studies on reduced sensitivity of AChE among OP insecticide and carbamate resistant species of anopheline mosquitoes have been reported (Ayad and Georghiou, 1975; Hemingway and Georghiou, 1983; Hemingway et al., 1986; Rao, 1993). Changed form of AChE due to target site insensitivity was also observed in resistant strains of Culex against OP insecticide and carbamate insecticides (Priester and Georghiou, 1980; Wood et al., 1984; Thakahashi and Yasutomi, 1987; Bonning and Hemingway, 1991; Rao, 1993). Reduced sensitivity of AChE in deltamethrin resistant strains also reported in An. stephensi (Rajasree, 1998c). Explanation of biochemical mechanisms of different types of resistance in vectors has facilitated to devise innovative, recent biochemical tests like microassay techniques. These biochemical diagnostic tests can confirm resistance within minutes in a single insect either field collected, preserved or used in bioassay tests. The results can be used in estimating frequency of individuals with such resistance mechanisms in the field populations. Early detection of resistance can be further employed to assess the efficacy of methods of resistance management.
Distinct biochemical markers in the fenitrothion resistant strains were detected for the first time in *An. stephensi* in the present investigation. These specific markers can be isolated and used as diagnostic tool to find out the resistance status of important insect vectors under field conditions.

Polytene chromosome preparations were obtained from the semigravid females of fenitrothion resistant and susceptible strains and the comparison were made between them. In this study, two paracentric heterozygous inversions were observed on the chromosome 2R, in the zones of 11-14 and 12-17 (Plate Xa A and Xb) and another on the chromosome 2L in the zone of 24-26 (Plate XI A; Table 21). Significant changes were observed in the banding sequence and bifurcation were found both at the centromeric and terminal ends (zone 1 and 6) in the X – chromosome of fenitrothion resistant strain (Plate IX a A and B) when compared to the susceptible one (Plate IXa C). In addition, a large puff was observed in the zone 6 in the X - chromosome of resistant strain (Plate IX b A). Some asynaptic gaps were also observed on 3L of resistant strain in the zone of 38 and 41 (Plate XII a A and A1). The inversions and asynaptic gaps in the chromosome arms (2R, 3L and X) in resistant strains were found in the same position with high frequency (Table 22). The above mentioned inversions are new and for the first time reported from fenitrothion resistant strains of *An. stephensi*. 
In the present investigation, the crosses between the greenish black mutant \( (gbl) \) and wild clearly showed that the inheritance of mutant \( gbl \) larva in \textit{An. stephensi} is incompletely dominant and autosomal (Table 23). The mutant, \( gbl \) larva described here is altogether different from the mutant greenish brown reported earlier in \textit{An. stephensi} (Sharma \textit{et al.}, 1979). Progeny derived from each cross were analyzed. The F\textsubscript{1} progeny of reciprocal crosses 3 and 4 showed both intermediate and wild type. The colours of intermediate type were light grey with green tinge in the thorax which is clearly different from the \( gbl \) and wild types.

Intermediate adults of F\textsubscript{1} progeny were inbred to yield F\textsubscript{2} progeny (crosses 9 and 14) and resulted \textit{gbl}, intermediate and wild in 1:2:1 ratio. In addition, wild adults of F\textsubscript{1} progeny (crosses 15 and 16) were inbred and resulted only wild types. Therefore, this indicates that gene, \textit{gbl} is incompletely dominant. The backcrosses between the heterozygous intermediate larvae (F\textsubscript{1} of crosses 3 and 4) and pure \textit{gbl} larvae (crosses 5, 6, 7, 8 and 10, 11, 12, 13) gave only two classes of \textit{gbl} and wild type. As there was no intermediate phenotypic expression in the backcross progeny, it clearly indicates that the gene \textit{gbl} is not controlled by a single gene. However, the colour expression of mutant was shown in both sexes for F\textsubscript{1}, backcrosses and F\textsubscript{2} hybrids by 1:1 (Table 23). There was no deviation in sex ratio. This clearly indicates the gene \textit{gbl} is autosomal. The mutant gene
Significant variations in infection were also observed among the different strains of DELa, DELb and DELc ($\chi^2 = 12.91; d.f=3; P< 0.005$).

The comparative susceptibility studies of anopheline mosquitoes to different malarial parasites have been studied by many workers from time to time (Green and Gater, 1931; Barder et al., 1936; Russel and Mohan, 1939,1940; Jeffery et al., 1950; Eyles and Young, 1950; Humninen, 1951; Eyles, 1960; Rutledge et al., 1970b; Kaay et al., 1975; Vander Kaay and Boorsma, 1977; Waren et al., 1979; Ichimari, 1989; Rudin et al., 1991; Klein et al., 1991a; 1991b; Rastogi et al., 1992; Fleck et al., 1994).

The comparative susceptibility of An. oswaldoi and An. konderi to infection by Plasmodium vivax was based on the presence of oocysts and sporozoites (Marrelli et al., 1998; 1999). They showed that the percentage of oocysts positive for An. oswaldoi (13.8%) was higher than An. konderi (3.3%). These results indicated that An. oswaldoi was more susceptible than An. konderi in the state of Acre.

In other experiment, Collins et al. (1990a, 1990b, 1991, 1993) showed that An. gambiae s.s. can readily serve as a good vector for the development of P. brasilianum as well as for P. malariae. But, the strains of An. gambiae s.s. was less susceptible to infection than An. freeborni or An. stephensi mosquitoes.
(54.1%) with an average number of oocysts (21.6) per infected female, here the parasite yield was low. Xenobitic An. stephensi became infected (76.2%) with a higher mean number of oocyst per female (94.4). Thus gnotobiotic An. stephensi were as xenobiotic ones to support the sprogonic development of P. berghei, but were less able to support ookinete development.

This basic difference of susceptibility and its causes will guide us for the future studies on mosquito genetics with the ultimate aim to find a solution for control of malaria. Comparative susceptibility of different strains of An. stephensi to rodent malaria parasite P. yoelli nigeriensis showed varied degrees of significant infectivity and also refractoriness. Efforts should be made to isolate such refractory strain from nature and genetically analyzed their inheritance pattern which would be very useful in the vector control program.
SUMMARY
AND
CONCLUSION
7. SUMMARY AND CONCLUSION

1. Twenty two strains of An. stephensi, Liston colonized in our laboratory were used for the present investigation. Nine strains from Bangalore district (CUB, KNG, RJN, YLH, GRP, WCR, CLO, GAN and MSK), two from Mangalore (MGNa and MGNb), three from Delhi (DELa, DELb and DELc), two from Chennai (CHNa and CHNb), and one each from Pondicherry (PDY), Shimoga (SHM), Poona (PON), Aurangabad (AGN), Goa (GOA) and Haryana (HRN) were used in various experiments.

2. Depending on the ridge number on the egg float and egg measurement, nine strains of An. stephensi namely CLO, MGNa, DELa, AGB, HRN, PDY, CHNb, WCR and CUB were grouped into type, intermediate and mysorensis variants with 14-20, 12-17 and 10-14 ridges respectively. The remaining six strains namely CHNa, MGNb, RJN, KNG, YLH and DELb were type forms already classified earlier in laboratory.

3. Baseline susceptibility studies were carried out in fifteen strains by using different concentrations of fenitrothion. LC\textsubscript{50} and LC\textsubscript{90} values were calculated by log dosage–probit analysis. From the data it was observed that the above strains were susceptible to this insecticide. The strain CHNb was the most susceptible (LC\textsubscript{50} = 0.0023 ppm and LC \textsubscript{90} = 0.0049
7. In biochemical analyses, the quantity of proteins in the resistant strains was compared with susceptible (control) strains in different stages of development such as eggs, larvae, pupae, adult males and adult females. Electrophoretic analysis showed a marked variation in the number and intensity of bands in resistant and susceptible strains. Difference in protein profiles can be due to the increased activity of several detoxifying enzymes.

8. The activities of α- and β- esterases were found to be increased in the resistant strains. Electrophoretic studies showed a marked variation in the number and intensity of bands in resistant and susceptible strains. The α-esterase bands 6, 8 and 16 (Rm-0.44, 0.54 and 0.78) were specific in resistant strains. The specific β- esterase band 14 (Rm- 0.68) were observed in resistant strains. Fenitrothion resistance is associated with elevated esterase activity and this can be used as one of the parameters to detect resistant strains.

9. Elevated Alkaline and Acid Phosphatase (Aph and Acph) were observed in resistant strain. Electrophoretic analysis of Aph revealed a total of eleven zones of activity and the Acph revealed a total of ten zones of activity. Variation in number and intensity of bands for both the parameters was observed during different developmental stages of both the resistant and susceptible strains. The difference in banding pattern of
resistant could be due to phosphorylation as a compensatory mechanism to develop resistance.

10. Reduced Lactate dehydrogenase (LDH) activity was observed in resistant strain. A total of six zones of activity was observed in both resistant and susceptible strains. The band 2 (Rm- 0.33) was found common for all the stages in both the strains, but the activity were more in susceptible strains. The intensity of bands were less in resistant strains when compared to that of susceptible strains. The reduced LDH activity in resistant strain can be due to prevalence of oxidative mechanisms, to adjust with the toxic effect of insecticide.

11. Acetyl Cholinesterase (AChE) activity was found to be reduced in the resistant strains. In biochemical analysis, the activity of AChE in resistant strain was less in all the developmental stages like egg, larvae, pupae, adult male and female. The insecticide exerts their effects by inhibiting the enzyme acetylcholinesterase and mosquito develops resistance against this insecticide through structural modification of this enzyme.

12. The polytene chromosome preparations were carried out from ovarian nurse cells of semigravid females of fenitrothion resistant and susceptible strains. Two inversions were observed on chromosome arm 2R and a single inversion on 2L of fenitrothion resistant strains when
compared to the susceptible strain. In addition, a few asynaptic gaps were found on 3L and bifurcation were observed on both the centromeric and terminal ends of X- chromosome of resistant strains.

13. A larval colour mutant greenish black (gbl) has been isolated from fenitrothion resistant strain and its inheritance mechanism was studied. The mutant gene gbl is autosomal and incompletely dominant and controlled by more than one gene. This mutant is reported for the first time in An. stephensi Liston and can be used as a good marker for basic and applied genetic research.

14. Comparative susceptibility studies of twenty two strains of An. stephensi to P. yoelii nigeriensis were carried out. High, medium and low infectivity were observed among these strains. Shimoga (SHM) strain was refractory to this parasite as there was no oocyst on the gut.

15. Among the three mutants (grb, ru and grb-ru) grb showed 50% infectivity, ru with 87.5% infectivity and grb-ru showed 71.74% infectivity.

16. Among the FNS and FNR strains, FNS showed 40.29% infectivity in contrast to the FNS one having 87.18% infectivity.