Discussion
DISCUSSION

Mosquitoes are the vector of several diseases, malaria, filariasis, dengue and yellow fever are still causing chink in mans armor. Malaria carried by Anopheles sps. is one of the most important of all the mosquito borne diseases taking millions of life annually all over the world. Mosquito vector control is an important part of the global malaria control strategy. Malaria has been a problem in India for centuries. Chemical control of mosquitoes and other vectors, which seems to be most successful being cheaper, easier in application and fast reacting, but is a great source of environmental pollution ultimately affecting human health and also contribute towards the development of resistance in the target species. All these factors led to search for safer and more compatible alternatives among which natural products are of first importance. During recent decades the use of natural products in the control of mosquitoes has gained high priority (Murty and Jamil, 1987).

Now a days the growing use of phytochemicals for control of the insects may be attributed to fact that populations throughout the world are coming to see the dangers inherent is conventional insecticides. Natural botanicals have provided numerous sources of phytochemicals utilized in the development of effective mosquito control agents. Plant materials offer not only effective mosquito control agents, but also promise to be environmental safe (Choochote et al., 1999).
In the present study methanolic extracts of *G. pentaphylla*, *A.amara* and *O. basilicum* showed considerable effect on larvicidal, pupicidal and adult repellent properties against malarial vector *A. stephensi*. After the treatment of various concentrations (100, 200, 300, 400, 500 ppm). At 100 ppm, the mortality of GPLE showed (31.3%, 29.6%, 26.6% and 23.6%) whereas OBLE it was (28.3%, 25.3%, 20.3% and 19.0%) and AALE was showed (24.3%, 17.6%, 15.0% and 15.3%) mortality of the I, II, III and IV instars larvae, respectively. Among the different instars 1 instar larvae was most susceptible than the other instar larvae. Percentage of mortality was dose dependent.

Plant extracts and phytochemicals have potential as products for mosquito control because many of them are selective, may biodegrade to nontoxic products, and may be applied to mosquito breeding places in the same way as conventional insecticides (Sukumr *et al.*, 1991). Plant extracts and essential oils possess larvicidal activity against various mosquito species (Berenbaum, 1988). While screening plants know to display insecticidal activity, they identified tannins as the major constituent in extracts of *Taxus* spp. that caused larval mortality. Tannic acid, a commercial source of Tannin has been reported to induce gut necrosis and major histopathological effects such as lesions and degenerative epithelium (Bernays *et al.*, 1980; Steinly and Berenbaum, 1985) the conditions resemble the effects of *B. thuringiensis* (Salama and Sharaby, 1985). In the present study also after the treatment of *A.amara* extract had considerable mortality on the larvae of *A. stephensi* due to the presence of active chemical tannin present in the AALE.

The larvicidal activity of various plant extracts such as *Pendalim murax*, *Cleome icosondra* and *Dictyosa dietotoma* have been found to be promising against *C. quinquefasciatus* and *A. stephensi* (Kalyanasundaram and Das, 1985). Murugan and
Jeyabalan (1999) reported the effect of some indigenous plants on the larvicidal and ovipositional properties on *A. stephensi*. Babu and Murugan (2000) investigated that the larvicidal effect of resinous exudates from the tender leaves of *A. indica*. Vahitha et al. (2002) studied the larvicidal efficacy of *Pavonia zeylamica* L. and *Acacia ferruginea* D.C. against *C. quinquefasciatus* Say. In the present study GPLE, OBLE and AALE showed to be a potent mosquitocidal effect on *A. stephensi* and it may be due to the presence of active compounds such as (terpenoids, amides, imides, alkaoloides, coumarins, flavonoides albitocin, b- sitosterol, amyrin, quercetrin, isoquercetrin limonene, myrcene, thymol, methyl chavicol, linalool, methyl eugenol and methyl cinnamate).

Larvicidal activity of *Gliricidia sepium* against mosquito larvae of *A. stephensi, A. aegypti* and *C. quinquefasciatus*. All extracts were active causing 100% mortality at below a dose of 16,000 ppm. Mortality rate was dose dependent (Nirmal Sharma, 1998). In the present study also, GPLE, OPLE and AALE against mosquito larvae of *A. stephensi*. All the extracts were active causing 100% mortality at below a dose of 500 ppm, whereas AALE leaf extract were active and caused 50% mortality at below a dose 500 ppm. Mortality rate was dose dependent.

In recent years, emphasis has shifted from the use of synthetic chemicals insecticides towards biological agents for the control of mosquitoes. The application of entomopathogens such as bacteria is a promising approach. *B. thuringiensis* is one of the most actively researched entomopathogens and has found commercial application in several cases. *B. thuringiensis* var. *israelensis* (Bti) has been shown in laboratory and field studies to be a potent larvicide’s of mosquitoes (Mulligan et al., 1980; Molly and Jamnback, 1981). In the present study, Bti at different concentrations brought out toxicity on the various larval instars of *A. stephensi*. The Bti application in the field also considerably affected the different stage of the larval population of *A. stephensi*.
The toxin must be ingested by the susceptible host to become toxic and must transverse a number of physical and chemical barriers within the host exert its toxic effect. The peritrophic membrane of the midgut, acting as specialized lining to protect brush border midgut epithelium, is the first such barrier. Specific membrane receptors for *B. thuringiensis* have been detected on the brush border epithelium (Rie *et al.*, 1990; Indrasith and Hori, 1992; Lee *et al.*, 1992). Solubilization and processing of the toxin with an active form, occurs within the gut environment. pH and digestive activity may play a role in the conversion process (Ogiwara *et al.*, 1992). Histopathological changes include gut necrosis, degeneration of peritrophic membrane and epithelium and bacterial septicemia (Salama *et al.*, 1986). In the present study, Bt toxin have been tested on different larval instars and pupae of *A. stephensi*. Bt toxin considerably affected the larva and pupa of *A. stephensi*. Among Bti treated at the different larval instars I instar larvae of *A. stephensi* were more susceptible than the other instars. Bt toxin 20 ppm concentration the mortality was (34.3%) and higher mortality (93.0%) was noted at 100 ppm concentration.

The gram-positive endospore forming bacterium *B. thuringiensis* produces parasporal crystalline inclusion that contain polypeptides (δ-endotoxin) that are toxic to a variety of insect species. Upon ingestion by an insect larva, these inclusions are solubilized in the alkaline environment of the midgut and are activated by midgut proteases. The activated toxins then pass throughout the peritrophic matrix and subsequently bind to highly specific receptors on the midgut brush border membrane perhaps following a conformational change and/or oligomerization. The toxin induces the formation of a lytic pore in the midgut epithelial membrane that results in cell lysis,
cessation of feeding and death of the larva (Charles and de Barjac, 1983; Singh et al., 1986; Daniel et al., 1995). In the present study, Bti treated larvae showed mortality as well as lower growth rate suggested that, Bt toxins might have interfered the gut system and brought out higher mortality at high dose treatment and also inhibited growth after the treatment of Bt toxin at lower dose.

Garcia and Rochers (1979) observed that appreciable mortality only with high concentrations (1 x 10^7 cells/ml) of *B. thuringiensis* var. *israelensis*. The biocide at 1 to 10Kg/ha (0.25 to 2.5ppm) caused 18 to 88% mortality of midges during four-week evaluation period. Younger instars are more susceptible than older ones as shown by *C. quinquefasciatus*. Consequently lower rates would be required for their control and over all control at a given rate would depend a great deal upon percent composition of the various instars of the target species. However, late fourth instars that have ceased feeding will not be killed by application of this pathogen. Another consideration in the efficacy of *B. thuringiensis* var. *israelensis* against chironomids is the length of exposure. In the field trials, midges were exposed to the pathogen for longer periods of time than the 48hrs laboratory exposures. Exposure periods longer than 48hrs in the laboratory may produce better activity results of the *B. thuringiensis* var. *israelensis* formulations against the midge’s species (Ali, 1981). In the present study, after the treatment of Bti at different concentration on various larval instars of *A. stephensi* showed higher mortality. The effect was also dose dependent. Bti caused considerable mortality on the various larval instars of *A. stephensi* in the laboratory. It is interesting to note that the late fourth instar larvae were less affected because of feeding nature of a particular instar.
Addition of Bti with the plant extracts had adverse effect upon the larval mortality. When combined with plant extracts, Bti increased the percentage of larval mortality and decreased the times to kill when compared with treatment contain only Bti. The addition of Bti with plant extracts caused a significant mortality due to avoidance of treated diet and also may be due to increased toxicity (Gould et al., 1991). Bt provides an attractive alternative to chemical insecticides, totally substituting Bt for the use of chemicals would be a mistake. Use of the chemical arsenal in combination with Bt would probably enable a more judicious use of both and would also delay the onset of insect resistance (Gill et al., 1992). The advantage of adding these plant extracts to bacterial formulations is the potential decrease in both the pathogen dosage required to kill larvae. Ludlum et al. (1991) have reported that aromatic compounds and plant allelochemicals increase Bti activity.

In the present study, GPLE, OBLE and AALE enhance the B. thuringiensis subsp. israelensis activity, it may be an effective alternative to conventional synthetic insecticides for the control of A. stephensi, the use of plant extracts as an additive to the Bti may play a more prominent role in Integrated Pest Management Programme. Moreover, the active compounds such as (terpenoids, amides, imides, alkoloides, coumarins, flavonoides albitocin, b- sitosterol, amyrin, quercetrin, isoquercetrin limonene, myrcene, thymol, methyl chavicol, linalool, methyl eugenol and methyl cinnamate) present in the G. pentaphylla, A. amara and O. basilicum may be responsible for the additive or synergistic action on Bt toxin and further caused mortality of mosquito larvae.
In the present study, the results showed higher mortality in the combined treatment of GPLE + Bti than OBLE + Bti and AALE + Bti. It can therefore be concluded that Bti and plant compounds caused swelling of the gut epithelial cells (Nasiruddin and Mordue (Luntz), 1993). At naturally occurring concentrations, allelochemicals produce midgut lesions, reduce feeding, growth and increase mortality (Lindroth et al., 1988). In the present study, potent toxicity leading to high larval mortality exhibited by the extracts could be attributed to the group of toxic biomolecules possessing insecticidal properties in the plant extracts.

Plants contain many chemicals, which are important in their defence against insects. The phytochemicals derived from various botanical source have provided numerous beneficial uses ranging from pharmaceuticals to insecticides. The phytochemicals derived from plant sources, which is, not only act as larvicidal and also involved many biological activities. Neem products are capable of multiple effects in insects such as antifeedant, growth regulation, fecundity, suppression and sterilization, oviposition, repellency and changes in biological fitness (Mulla and Yun, 1999).

Zebitz (1986) observed that mosquito larvae treated with neem seed kernel extracts produced deformities including larval-pupal intermediates. This would imply a similar mode of action for M. volkensii bioactive compounds to that of azadirachtin, the principal active compound in the neem seeds (Mwangi and Rembold, 1988a; Mwangi and Mukia, 1988b). In the present study also GPLE, OBLE and AALE produced various larval, pupal and adult deformities. The active chemicals at lower dose also posses growth regulatory activity on mosquito, A. stephensi.
Prolongation of developmental period and induction or morphogenetic abnormalities of mosquito larvae treated with plant extracts are generally attributed to interference with the active ingredients of the extracts with the endocrine system (Zebitz, 1984; 1986). Saxena and Saxena (1992) observed such prolonged larval and pupal periods while using plant extracts for the control of mosquito larvae. In the present study, also similar effect has been noted after the treatment of GPLE, OBLE and AALE. Treated larvae showed extended larval and pupal durations and it may be due to the interference in the endocrine regulation of insects.

Solvent used in the extraction process also affected growth and reproductive inhibition effects from phytochemicals. Only methanol-eluted fraction exhibited insect growth inhibitory activity and various abnormalities in closing A. aegypti and C. quinquefasciatus (Dhillon et al., 1982). The petroleum ether fraction of Solanum trilobatum and the ethyl acetate fraction of Leucas aspera were tested on the mosquito larvae and showed less toxic to the larvae. The active fractions of the plant extracts disrupted moulting and metamorphosis, induced malformation, extended larval duration and inhibited adult emergence (Muthukrishnan et al., 1997). In contrast to the earlier work, in the present study, the methanolic extract of GPLE, OBLE and AALE showed significant toxicity and growth regulatory effects on, A. stephensi.

Reduced pupa weight only when larvae were continuously exposed to (contaminated Bti) diet; when removed from exposure, they recovered fully and developed into normal size pupae (Ramachandran et al., 1993). Larvae of C. quinquefasciatus treated with B. thuringiensis var. israelensis exhibited reduced weight gain and delayed development compared with control and result in a delayed (or) inhibition of moulting.
In the present study also at lower dose level of GPLE, OBLE and AALE with Bti applied individually and in combination showed retarded larval growth, delayed moulting and reduced weight gain and development compared with control.

In the present study GPLE, OBLE, AALE and Bti treatment also reduced the post-embryonic development and totally inhibited the adult emergence. Smaller pupae resulted in fewer eggs ovoposited because the average number of eggs per female was directly proportional to her weight as pupa. Changes in fecundity after treatment with Bti have been reported for other species (Afify and Matter, 1969; Mc Gaughey, 1978).

Reduction in egg hatching was also observed when untreated males (or) females mated with treated individuals of the opposite sex. Egg hatchability decreased more than 30 and 60% by the lower (0.0001 mg/ml) and higher (0.000039 mg/ml) treatment concentrations, respectively (Gaaboub and Hayes, 1989). In the present study methanolic extract of GPLE, OBLE and AALE showed significant effect on fecundity and egg hatchability of A. stephensi.

Repellents have an important place in protecting man from the bites of insect pests. An effective repellent will be useful in reducing man-vector contact and in the interruption of disease transmission. Repellent compounds should be non-toxic, non-irritating and long lasting (Kalyanasundaram and Das, 1985).

In the present study repellent activity of OBLE against A. stephensi was considerably greater than the GPLE and AALE extracts. This is also may be due to presence of active compounds at the leaves of OBLE. The strong repellency may be due
to the presence of volatile compounds such as limonene, myrcene, thymol, methyl chavicol, linalool, eugenol and methyl cinnamate etc present in the O. basilicum. Chemosensory effects of OBLE, either olfactory or gustatory, presumably cause this reduction.

Many plant extracts and essential oils with high volatility, such as alkanes, terpenoids, alcohols and aldehydes act on mosquitoes in the vapour phase (Browne, 1977). These volatile compounds were effective against mosquitoes for a relatively short period, typically 15 min to 10 h (Rozendaal, 1977; Barnard, 2000). Sukumar et al. (1991) studied that the most promising botanical mosquito control agents are in the families Asteraceae, Cladophoraceae, Labiate, Meliaceae, Oocystaceae and Rutaceae. The repellent constituents are mainly monoterpenoids such as geraniol, citronellol, linalool, terpineol and (-) carvone (Curtis et al., 1990; Vartalc and Sharma, 1993; Hwang et al., 1985).

Sharma et al. (1995) reported that the repellent action of neem oil was evaluated against different mosquito species in field trials. Neem oil mixed with coconut oil provided 96 – 100% protection from Anophelines, 85% from Aedes and 37.5% from Armigera where as, it showed a wide range of efficacy (61-94%) against C. quinquefasciatus. The repellent activity of methanolic extract of Ferronia elephantum leaves against A. aegypti. The repellent activity at 1.0 and 2.5mg/cm2 concentrations gave 100% protection for 10 hrs (Venkatachalam et al., 2001). Similar to above study the methanolic extract of G. pentaphylla, A.amara and O. basilicum gave considerable protection against A. stephensi for 4 hrs, when the three extracts applied over the skin of human volunteers.
Plant oils were screened for their repellent action against *A. aegypti* mosquitoes. The oils tested were citronella oil, lemon grass oil, eucalyptus oil, tulsi oil and neem oil individually or in combination with coconut oil, and those giving the most protection at dawn and dusk were neem oil followed by tulsi oil (Pandian and Devi, 1998). Hati *et al.* (1995) reported that neem seed oil in an appropriate amount when smeared on the surface of the hand showed excellent repellent action against *A. aegypti* mosquitoes. The repellent action of neem seed oil was directly proportional to the hour of exposure to the oil smeared skin, a sizeable proportion of mosquitoes were not able to take blood meal. Neem seed oil was non-irritating to the skin volunteers. Similar to above studies the methanolic extract of GPLE, OBLE and AALE showed protection against mosquito, *A. stephensi*. These plant extracts was non-irritating to the skin volunteers.

Smoke is a common method of repelling biting insects used throughout the world. Fresh or dried plants are frequently added to fires to enhance the repellent properties of the smoke. Other methods are hanging the plants around the house or sprinkling leaves on the floor. Mosquito coils made from dried plants and combustible materials such as saw dust are also a cheap and often an effective method of repelling mosquitoes. Hindus, Buddhist and the followers of Confucius probably derive them from the incense use in religious ceremonies. In Java today, the same incense used in ceremonies to honor ancestors is also used on a daily basis to repel mosquitoes (Sangat-Roemanty, 1990).

The smoke of the two plants *V. negundo* and *L. aspera* showed their repellent activity in moderate degrees against *C. quinquefasciatus* (Kalyanasundaram and Das, 1985). In the present study *G. pentaphylla*, *A. amara* and *O. basilicum* exhibited similar toxic effect against *Anopheles stephensi*. In the laboratory, powdered preparation of the
leaves of *Adhatoda vasica*, *A. indica* and *Ocimum sanctum* were combined with burning charcoal to produce smoke which repelled *Armigeres subalbatus* and *C. quinquefasciatus*. All the plants' smoke was more toxic to *C. quinquefasciatus* (Selvaraj Pandian et al., 1995).

In the present study, smoke from *G. pentaphylla*, *A. amara* and *O. basilicum* had much repellent property against *Anopheles stephensi*. Among the three plants, *A. amara* showed higher repellency than the *G. pentaphylla*, and *O. basilicum*. This is because of the presence of active compounds present in the *A. amara*.

Continued incentive for production and use of *B. thuringiensis* (H-14) is provided by its efficacy, specificity, biodegradable nature, absence of resistance development in vectors and long shelf life. Further improvements in formulations are still warranted. The continued search for more potent strains or the genetic improvement of existing ones will further encourage and expand the operational use of *B. thuringiensis* (H-14) for suppression of susceptible vector Nematocera. By adding plant extract with Bti we can improve the efficacy of Bt toxin (Murugan et al., 2003).

Biological control with entomopathogenic bacteria has been increasingly used as a larvicide to control populations of various medically important Diptera of the genera *Culex* and *Aedes*. Like chemical larvicides, these agents can cause drastic density dependent mortality, killing all larvae within 24 – 48 hrs, after breeding site treatment.

Field trial have been conducted to test on the efficacy of neem seed kernel extract, *Pongamia* seed kernel extract, *L. aspera* leaves extract with Bt toxins at different breeding habitats of *C. quinquefasciatus* in and around of Bharathiar University Campus, Coimbatore. Dipper sampling and counting of larvae has been monitored on the larval
density before 24 hrs, 48 hrs and 72 hrs after treatment. Among the plant extract treatment, the neem and pongamia seed extract showed better result than the *L. aspera* leaf extract. Combined treatment of NSKE, PSKE, LALE and Bti brought out higher mortality within shorter span of time suggest the interactive effect of plant extract with Bt toxins. The thin film of oily layer of plant extracts spread on the water surface which cuts the oxygen supply to mosquito larvae come up to the water surface to breathe. Further plant principle dissolves into the water and penetrates the larvae through the respiratory tube and kills it either by suffocation or by poisoning (Vahitha *et al.*, 2003).

Similarly in the present study, the field trials were conducted by using GPLE, OBLE and AALE with Bti treatment in different breeding habitats of *A. stephensi* in Coimbatore City, India. For the field trial the quantity of plant residues and Bti required (Based on the laboratory of LC$_{90}$ values) for each treatment was determined by calculating the total surface area of the each habitat. After the combined treatment of GPLE, OBLE, AALE with Bti brought out higher mortality with in shorter span of time suggest the interactive effect of plant extract with Bt toxins. The percentage of larval reduction also showed the variations among the different breeding habitats. This may due to the impact of geographical effect and environmental pollutions on *A. stephensi*, at the breeding sites.

Rao *et al.* (1995) reported that the field-tested relatively stable lipid-rich fractions of neem products, which were as effective as good quality crude neem products in the control of culicine vectors of Japanese encephalitis and produced a slight but significant reduction in population of anopheline pupae.
The leaf extract of *Polyalthia longifolia* evaluated against larvae and puape of *C. quinquefasciatus*. Three habits chosen were (i.e) cesspit, cement tank and ‘U’ drain. It was found to be toxic to larvae and significantly inhibited the emergence of adults. Laboratory samples were found to be more susceptible than those of field origin. Among field samples ‘U’ drain samples showed more resistance to the extract (Murty *et al.*, 1997). In the present study, the trials were conducted by different breeding habits of *A. stephensi*. The GPLE, OBLE, AALE with Bti applied on breeding place were spread on the water surface and form a thin layer which cuts of oxygen supply to mosquito come up to the water surface to breathe, the oil penetrates the larvae through the respiratory tube and kills it either by suffocation or by poisoning. All the GPLE, OBLE, AALE with Bti treatment showed higher percentage of larval reduction against *A. stephensi*.

The plant extracts contain a number of compounds, which can biologically represent to additional stress on the insects system, which allows enhanced pathogen performance. The advantage of adding these plant extracts to bacterial formulations is the potential decrease in both the pathogen dosage required to kill larvae. The use of plant extracts as an additive to the *Bti* may be promising approach because larval feeding and subsequent defoliation would be reduced greatly without interference with bacterial activity. Salama *et al.* (1986) and Lud lum *et al.* (1991) have reported that aromatic compounds and plant allelochemicals increase *Bti* activity. The result of this study indicate that leaf extracts *G. pentaphylla, A.amara* and *O. basilicum* enhance the *B. thuringiensis* subsp. *israelensis* activity, it may be an effective alternative to conventional synthetic insecticides for the control of *A. stephensi*. The use of plant extracts as an additive to the Bti may play a more prominent role in Integrated Pest Management Programme in future.