DISCUSSION
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Mosquitoes are the most single group of insects in terms of public health significance. Despite many attempts to successfully eradicate them, they still co-exist with man and animals transmitting dreaded diseases like malaria, filariasis and dengue etc., (Service, 1983).

Phytochemicals obtained from plants with proven mosquito control potentially can be used as an alternative to synthetic insecticides. Plants product obtained either from the whole plant or from a specific part by extraction with different types of solvents such as aqueous, methanol, chloroform, hexane etc. depending on the polarity of phytochemicals. Studies have been carried out on some phytochemicals act as general toxicant (insecticide / larvicide) both against adult as well as larval stages of mosquitoes, while others interfere with growth and development or with reproduction (chemosterilant) or produce olfactory stimuli thus acting as repellent or attractant.

Most of the plant based products are not as effective as synthetic insecticides and do not produce fast results, their use for mosquito control in a large scale programme under epidemic conditions may not be acceptable. However, the use of indigenous plant based products by individual and communities can provide a prophylactic measures for protection against various mosquito-borne diseases. There is a need for promoting the use of herbal products through community based vector control programme. Some indigenous plant based products are very promising against mosquitoes and can be used as insecticides and/or repellents (ICMR, 2003).

Yet another drawback with use of chemical insecticides is the failure of many vector control campaigns resulting in the vector resurgence in epidemic zones. The use of commercially available conventional synthetic insecticides has raised serious ecological, economical and environmental problems. They 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.
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).

*Aedes aegypti* is the major vector of dengue, which is also a common pestiferous mosquito, *Bacillus thuringiensis* var. *israelensis* made possible, efficient microbiological control of Diptera vectors of diseases, such as mosquitoes. Botanicals from the different parts of the plants, the active ingredients have been isolated and identified. Neem products are capable of producing multiple effects in insects. Because of a variety of components and different mechanisms, insecticide resistant to this compound. Neem components affect different insects as well as medically important insects like mosquitoes, flies etc.

In the present study, the methanolic extract of *Canarium strictum* seed and *Plectranthus amboinicus* leaf with *Bacillus thuringiensis* var. *israelensis* showed considerable effect of larvicidal, pupicidal and adult smoke repellent properties against *Aedes aegypti*. When *Canarium strictum* seed and *Plectranthus amboinicus* leaf extracts combined with *Bacillus thuringiensis* var. *israelensis* had significant larval and pupal toxicity of *Aedes aegypti*.

Today, the environmental safe of an insecticide is considered to be of paramount importance. An insecticide does not have to cause high mortality on target organisms in order to be acceptable (Kabaru and Gichia, 2001). Phytochemicals may serve as suitable alternatives to synthetic insecticides in future as they are relatively safe, inexpensive and are readily available in many areas of the world. According to Bowers *et al.* (1995) the screening of locally available medicinal plants for mosquito control would generate local employment, reduce dependence on expensive imported products and stimulate local efforts to enhance public health.
The biological activity of the plant extract might be due to the various compound including phenolics, terpenoids and alkaloids exist in plant and these compounds may jointly or independently contribute to produce larvicidal activity against Aedes aegypti.

A total elimination of toxic pesticides is a landmark in this study and the major role played by predators, parasites, biopesticides and biocides in the suppression of the pest well below the economic injury level which are quite safe, selective and ecofriendly with environment in comparison with chemical control.

Plant extracts and phytochemicals have potential as products for mosquito control because many of them are selective, may often biodegradable to nontoxic products, and may be applied to mosquito breeding places in the same way as conventional insecticides (Sukumar et al., 1991; Hostettmann and Potterat, 1997). Many plant extracts and essential oil possess larvicidal activity against various mosquito species (Miyakado et al., 1989; Sukumar et al., 1991; Mohanty et al., 1994; Assabgui et al., 1997; Nirmal Sharma et al., 1998; Pushpalatha and Muthukrishnan, 1999; Ciccia et al., 2000; Ahmed et al., 2001; Pelah et al., 2002; Jeyabalan et al., 2003; Thomas et al., 2004; Jang et al., 2005; Murugan et al., 2006).

Certain plant-derived compounds were found to be highly effective against-resistant insect pests (Arnason et al., 1989; Ahn et al., 1997). Park et al. (2002) concluded that the Piper fruit –derived materials could be useful for managing field populations of C. pipiens pallens, A. aegypti and A. togoi.

The screening of local medicinal plants for mosquito larvicidal activity may eventually lead to their use in natural product-based mosquito abatement practices (Bowers et al., 1995).

Sukumar et al. (1991) has pointed out that the most promising botanical mosquito control agents are in the families Asteraceae, Cladophoraceae, Lamiaceae (formerly Labiatae), Meliaceae, Oocystaceae and Rutaceae. The species of Solanaceae and rutaceae families have been reported to have insecticidal properties against various pests and vectors (Jang et al., 2002). In the present study, potent larvicidal activity against
Aedes aegypti was observed with Plectranthus amboinicus in the family Lamiaceae. Various compounds, including monoterpenoids such as geraniol, citronellol, linalool, terpineol and (-) carvone exits in plants (Hwang et al., 1985; Curtis et al., 1990; Vartak and Sharma, 1993).

The differential responses of various mosquito species are influenced by extrinsic and intrinsic factors such as plant species, the part of the plant, the solvents used for extraction, the geographical location where the plants were grown and the application methods (Sukumar et al., 1991). Crude extract of Swartzia madagascariensis fruits produced higher mortality in larvae of Anopheles gambiae (Edwards) than larvae of Aedes aegypti, but was ineffective against larvae of Culex quinquefasciatus Say. (Minijas and Sarda, 1986). Sujatha et al. (1988) examined the larvicidal activity of five plants, among which the extracts of Acorus calamus and Bambusa arundanasis were the most effective against C. quinquefasciatus and A. stephensi, respectively. On the other hand, the extract of Citrus medica affected only larvae of A. stephensi and the extract of M. longifolia responses was ineffective against this species. In the present study, larvicidal responses varied according to plant species. Against larvae and pupae of Aedes aegypti, the larvicidal activity against the Aedes aegypti was more pronounced with methanolic extract of Plectranthus amboinicus leaf than with methanolic extract of Canarium strictum seed. Plectranthus amboinicus were grown in Western Ghats and Canarium strictum grown in Eastern Ghats of Kolli hills. Their efficacy is varied depending upon the geographical location of the plants were grown.

Pandian et al. (1994) have reported Mentha piperita to be highly effective in controlling the larvae of C. quinquefasciatus. The methanolic extracts of Solanumsuratense, Azadirachta indica and Hydrocotyl javanica exhibited larvicidal activity against C. quinquefasciatus (Muthukrishnan et al., 1997; Babu and Murugan, 1998; Venkatachalam and Jebanesan, 2001a; Jaswanth et al., 2002).

The leaf extract of C. asiatica is superior to various neem extracts, which are reported to be effective with LC50 values ranging from 55-65 ppm against mosquito larvae (Ascher and Meisner, 1989). The median lethal concentrations (LC50) of various
parts of *Mellia azederach* ranging from 30-40 ppm against larva of *Culex pipiens* (Al-Sharook *et al.*, 1991). The effect of various neem extracts and various parts of *Melia azederach* was slightly lower than that reported for the *Culex asiatica* leaf extract.

Seven mosquitocidal compounds isolated from *Magnolia salicifolia* show 100% mortality at the concentration of 15-100mg/l (Kelm *et al.*, 1997). The mosquitocidal compound ar-turmerone which isolated from rhizomes of *Curcuma longa* seems 100% mortality of *Aedes aegypti* larvae at 50mg/l concentration (Roth *et al.*, 1998).

Variation in mosquito responses to the extracts related to plant species has been studied. Differences in the larvicidal effects on *Aedes aegypti* among the steam distilled oils from the whole plants of *Tagetes erecta* L., *Tagetes minuta* and *Tagete patula* L., have been reported (Green *et al.*, 1991; Perich *et al.*, 1994) and suggested that *Tagetes minuta* had the most potent larvicidal activity. In the present study, the larvicidal activity against *Aedes aegypti* was most pronounced with the leaf extract of *Plectranthus amboinicus* than the seed extract of *Canarium strictum*. These results suggest that chemical composition between the two plant species (*Plectranthus amboinicus* contains thymol, carvacrol, 1, 8-cineole, p-cymene, spathulenol, terpinnen-4-ol and *Canarium strictum* contains Oleo-gum-resins) may be different.

Murugan and Jeyabalan (1999) studied the effect of some indigenous properties in *Anopheles stephensi*. Babu and Murugan (2000) investigated that the larvicidal effect of resinous exudates from tender leaves of *Azadirachta indica*. Vahitha *et al.* (2002) have studied the larvicidal efficacy of *Pavonia zeylamica* L. *Acacia ferruginea* D.C. against *Culex quinquefasciatus* say.

Effect of an insecticide and acetone leaf extracts of *Withania somnifera* and *Argemone mexicana* against *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* was studied. The insecticide effectively checked all mosquitoes population. Both *W.somifera* and *A.mexicana* were found to be more effective in controlling the mosquito’s population. The early larval instars were highly susceptible to plant extracts than later stages. When the effects of two extracts were compared, it was discernible that
the leaf extract of *W. somifera* was more effective in controlling the population of *A. aegypti* that the leaf extract of *A. mexicana* (Uma et al., 2003). In this study also indicated the same results. The first instar larvae were highly susceptible to *Plectranthus amboinicus* and *Canarium strictum* than the later stages.

The larvicidal activity of plant extracts of *Datura metel* (seed) *Cinnamomum zelaynicum* (leaf) *Acorus calamus* (rhizome), *Achyranthes bidentata* (leaf) *Baliospermuni montanum* (seed) on the *Anopheles stephensi* was determined. Methanol extracts of *Datura metel* and *C. zeylanicum* showed very high larvicidal activity. Mortality in the larval, pupa and adults produced a marked decrease in mosquito populations in laboratory experiments. When *A. stephensi* larvae in their fourth instar were exposed to a low concentrations of *D. metel, C. zeylanicum, A. calamus, A. bidentata* and *B. montanum* low mortality was observed. A higher concentration the larval mortality occurred in a dose-dependent manner. Egg hatching was also significantly lower where compared with the control (Jeyabalan and Rajkumar, 2003). In this study, the methanolic extracts of *Plectranthus amboinicus* and *Canarium strictum* showed higher larvicidal activity occurred in a dose-dependent manner.

Analysis on the efficacy of various solvent (petroleum ether, hexane, chloroform, ethyl acetate and methanol) active fractions of *Leucas aspera* leaf extract on the bioefficacy of filarial vector, *Culex quinquefasciatus*. Among the different solvent extraction, chloroform extraction (I & II) showed higher efficacy for larvicide and repellency. The nature of chemical compounds on different fractions in relation to bioactivity of mosquito has been discussed (Murugan et al., 2003).

Plant allelochemicals may be quite useful in increasing the efficacy of biological control agents because plants produce a large variety of compounds that increase their resistance to insect attack (Barbosa and Sauder, 1985, Berenbaum, 1989). While screening plants known to display insecticidal activity, they identified tannis as the major constituent in extracts of taxus spp.
While screening plants known 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 *Bacillus thuringiensis* (Salama and Sharaby, 1985).

In the present study, methanolic extract of *Plectranthus amboinicus* leaf have been brought out toxicity on first, second, third and forth larval instars and pupae and had detrimental effect upon larval growth and development of *A. aegypti*. *Plectranthus amboinicus* leaf extract (PAME), however, proved to be the most detrimental to the leaves at the concentrations tested. At the highest concentration tested (i.e. 10%) the mortality (97%) was higher in PAME treated larvae when compared to other treatments. Larval development was also delayed and mortality was increased in the plant extracts treatment at 10% and above concentrations. Moreover 79, 84, 70, 64 and 59% reduction of larval and pupal mortality was noticed after combination of *Bti* and PAME. These toxic actions may be independent of each other as suggested by adult longevity experiments, which differed in the way in which larvae were exposed to the plant extracts.

Higher mortality was evident after the treatment of methanolic extract of *Plectranthus amboinicus* leaf at various concentrations (2, 4, 6, 8 and 10%). At 2%, the mortality of *Plectranthus amboinicus* showed 46, 37, 32, 29 and 27% of I, II, III, IV instars and pupae, respectively. At 10% concentration PAME showed 97% mortality in the first instar larvae. Among the different larval instars and pupae, I instar larvae was most susceptible than the other larval and pupal stages.

After the treatment of methanolic extract of *Canarium strictum* seed extract at various concentration (2, 4, 6, 8 and 10%) in first instar stages showed 42, 59, 70, 83 and 95% mortality, respectively. At 2% concentration, the larval and pupal mortality of *A. aegypti* showed 42, 35, 30, 26 and 23% of I, II, III, IV instars and pupae, respectively.
76% to 95% mortality was noted at 10% concentration in all the larval and pupal stages. Among the different larval and pupal stages, the first instar larvae were more susceptible than the other larval and pupal stages.

In the present study methanolic extracts of *Plectranthus amboinicus* and *Canarium strictum* had its effect on the various larval instars and pupae of *Aedes aegypti*. The active chemicals such as thymol, carvacrol, 1, 8-cineole, p-cymene, spathulenol and terpinen-4-ol present in the *Plectranthus amboinicus* and Oleo-gum-resins present in *Canarium strictum* may bring the mortality on larvae and pupae of *Aedes aegypti*.

Methanolic fraction of leaves *Mentha piperita*, *Phyllanthus niruri*, *Leucas aspera* and *Vitex negundo* exhibited the LC$_{50}$ values whereas 43.65, 1819.70, 2818.38 and 3019.95 ppm against the IV instar larvae of *Culex quinquefasciatus* (Pandian et al., 1994). In the present study, the toxicity LC$_{50}$ value of methanolic extracts of PAME and CSME was 3.089 and 3.853% against the IV instar larvae of *Aedes aegypti*. Pandian et al. (1994) studied the effect of *Mentha piperita* and certain other plants against *Culex quinquefasciatus*. In the present study, the same results were shown, while studying the effect of PAME and CSME against *Aedes aegypti*. Acute toxicity caused 97% mortality at 10% was observed. Hence, plant extracts used toxic impact on mosquito larvae.

Larvicidal activity of *Gliricidia sepium* against mosquito larvae of *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*, all the extracts were active causing 100% mortality at below a dose of 16 ppm mortality at was dose dependent (Nirmal Sharma, et al., 1998). Similar larvicidal principle of PAME against mosquito larvae of *Aedes aegypti* has been proved their toxicity on the extract was active causing 97% mortality at below a concentration of 10.0%, mortality rate was dose dependent. In the present study, PAME and CSME tested against the mosquito larvae of *Aedes aegypti*. All the extracts were active causing nearly 100% mortality at below a dose of 1000 ppm, whereas *Canarium strictum* seed extract were active and caused 95% mortality at below a dose of 1000 ppm. Mortality rate was dose dependent.
The larvicidal activity of the plant *Hydrocotyle javanica* was tested against the larval mosquito *Culex quinquefasciatus*. The LC₅₀ values ranged from 189.0 to 407.3 ppm. The lowest larvicidal efficacy observed in benzene fraction and highest larvicidal efficacy was observed in ethyl acetate fraction. (Venkatachalam and Jebanesan, 2001a). In the present study the methanolic extracts of *Plectranthus amboinicus* and *Canarium strictum* against *Aedes aegypti*, the LC₅₀ values were ranged from 2.53 to 6.79%. The lowest larvicidal efficacy observed in CSME and highest larvicidal efficacy was observed in PAME.

The petroleum ether extract of *Acalypha indica* and *Ervatamia divaricata* showed toxic effect against the fourth instar larvae of *Culex quinquefasciatus*. The LC₅₀ values were 362.75 and 364.60 for *A. indica* and *E. divaricata*, respectively. The chi-square value showed highly significant. (Thilagavathy Daniel et al., 1995). The petroleum ether extracts of the leaves of *Andrographis paniculata* and *Swietenia mahagoni* were evaluated for larvicidal activity against the late third instar larvae of *Culex quinquefasciatus*. *Andrographis paniculata* was highly efficacious as shown by its LC₅₀ value of 189.91 (LC₅₀ of 810.24), followed by *Swietenia mahagoni* LC₅₀ of 521.92 (LC₅₀ of 1247.09) (Anuradha et al., 1995).

The petroleum ether extracts of the seed kernels of *Melia azadirach, Azadirachta indica* and *Pongamia glabra* were tested in the laboratory against the early fourth instar larvae of *Culex quinquefasciatus*. Observation showed that *Pongamia glabra* was efficacious after 24 hrs with an LC₅₀ value of 166.87 ppm followed by *Melia azadirach* 248.11 ppm and *Azadirachta indica* 250.95 ppm (Sakthivadivel et al., 1996). In the present study, methanolic extract of PAME and CSME were tested in the laboratory against the third and fourth instar larvae of *Aedes aegypti* and resulted that the PAME is efficacious after 24 hrs with an LC₅₀ value of 4.44 and 5.13% in third and fourth instars larvae followed by CSME which shows 5.02 and 5.86%, respectively. The methanolic extracts at different concentrations (2, 4, 6, 8 and 10%) tested in the present study on the first, second, third and forth larval instars and pupal stages had higher mortality at low dose level indicating much larvicidal activity.
The toxicity of the molluscicidal plant *Ambrosia maritima* was evaluated against *Anopheles stephensi*. In the larvicidal assay a negligible mortality was observed in both species after application of the dried leaves in water at concentration up to 2000 mg/l. When the powdered leaves were applied at the surface of the water, however, up to 38% of the larvae of *Anopheles stephensi* was killed at 2000 mg/l. Virtually no inhibitory effect on the larval growth of both species was noticed. It can be that *Ambrosia maritima* has little (or) no effect on the larvae of *Anopheles stephensi* and *Aedes aegypti* (Geerts et al., 1994). In the present study after the treatment of CSME, the mortality of *Aedes aegypti* was minimum (23%) in pupal stage at 2% treatment.

The petroleum ether extract of *Acorus calamus*, *Ageratum conyzoides*, *Annona squamosa*, *Bambusa arundaniasia*, *Madhuca longifolia* and *Citrus medica* were tested for larvicidal and juvenile hormone activity against different mosquito species viz *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi*. *Acorus calamus* extract was found to be effective with LC$_{50}$ values of 33.7, 39.4 and 48.8 mg/l against *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* respectively. *Bambusa arundanasia*, *Madhuca longifolia* and *Citrus medica* extracts were found to be effective at concentrations higher than 50 mg/l. The juvenile hormone activity of these extracts showed that only *Acorus calamus*, *Madhuca longifolia* and *Ageratum conyzoides* were found to produce significant inhibition in adult emergence at the test concentration of 5 and 10 mg/l. Similar results were reported by Sujatha *et al.* (1988). Similar to above studies in the present study also the suppression of growth of various larval and pupal stages were evident after the treatment of PAME and CSME.

Babu and Murugan (2000) investigated that the larvicidal effect of resinous exudates from the tender leaves of *Azadirachta indica*. Vahitha *et al.* (2002) have studied, the larvicidal efficacy of *Pavonia zeylania* L. *Acacia ferruginea* D.C. against *Culex quinquefasciatus* Say. Murugan *et al.*, (2003) studied the interactive effect of botanical and *Bacillus thuringiensis* subsp *israelensis* on *Culex quinquefasciatus* say. Earlier, Jeyabalan *et al.* (2003) investigated the effects of *Pelagonium citrosa* leaf extracts on
malarial vector, *Anopheles stephensi* liston. In the present study also PAME showed to be a potent mosquitocidal effect on *Aedes aegypti* and it may be due to the presence of active compounds such as thymol, cavacrol, 1, 8-cineol, p-cymene, spathulenol and terpinen-4-ol.

Sujatha *et al.* (1988) observed differential susceptibilities of larvae of three mosquito species to petroleum ether extracts of *Acorus calamus* L. *Citrus medica*. Minijas and Sarda (1986) reported that crude extract of the fruit pods from *Swartzia madagascariensis* Desvaux produced higher mortality in larvae of *Anopheles gambiae* (Giles) than larvae of *A. aegypti* but was ineffective against larvae of *Culex quinquefasciatus* (Say). Murugan and Jeyabalan (1999) studied the effect of some indigenous properties in *Anopheles stephensi*. Babu and Murugan (2000) investigated that the larvicidal effect of resinous exudates from tender leaves of *Azadirachta indica*. Vahitha *et al.* (2002) have studied the larvicidal efficacy of *Pavonia zeylamica* L. *Acacia ferruginea* D.C. against *Culex quinquefasciatus* say.

Analysis on the efficacy of various solvent (petroleum ether, hexane, chloroform, ethyl acetate and methanol) active fractions of *Leucas aspera* leaf extract on the bioefficacy of filarial vector, *Culex quinquefasciatus*. Among the different solvent extraction, chloroform extraction (I & II) showed higher efficacy for larvicide and repellency. The nature of chemical compounds on different fractions in relation to bioactivity of mosquito has been discussed (Murugan *et al.*, 2003).

Prolongation of developmental period and induction of morphogenetic abnormalities of mosquito larval treated with plant extracts are generally attributed to interference of the active ingredient of the extracts with the endocrine system (Zebitz, 1984, 1986). Saxena and Saxena (1992) and Daniel *et al.* (1995) have also observed such prolonged larval and pupal periods while using plant extracts for the control of mosquito larvae. In the present study, PAME and CSME treated larvae showed extended larval pupal durations and it may be due to the interference in the endocrine regulation of insects. Further studies to understand the chemical nature of the active ingredients of the extracts and field trails are required.
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 reports by Mwangi and Rembold (1988) and Mwangi and Mukiama (1988). In the present study PAME and CSME extracts produced various larval, pupal and adult deformities.

Senthil Nathan et al. (2005) tested the methanolic extracts of leaves from the Indian white cedar Dysoxylum malabaricum Bedd. (Meliaceae) against mature and immature Anopheles stephensi Liston (Diptera) mosquitoes under laboratory condition. The extract showed strong larvicidal, pucidal, adulticidal and antioviopositional activity. The maximum leaf extract concentration tested in this study was 4%, which produced pronounced effect.

Jang et al. (2005) examined mosqutio larvicidal activity of Chamaecyparis obtusa leaf-derived materials against the 4th-stage larvae of Aedes aegypti (L.), Ochlerotatus togoi (Theobald), and Culex pipiens pallens (Coquillett). A crude methanol extract of C. obtusa leaves was found to be active (percent mortality rough) against the 3 species larvae; the hexane fraction of the methanol extract showed a strong larvicidal activity (100% mortality) at 100 ppm. The LC50 value of beta-thujaplicin was 2.91, 2.60, and 1.33 ppm against A. aegypti, Oc. togoi and Cx. pipiens pallens larvae. In this study also indicated the same results. The methanolic extracts of PAME and CSME showed very active against Aedes aegypti and their LC50 values were 5.13 and 5.86%.

In the present study, the plant materials has been extracted by using solvents like, petroleum ether, hexane, chloroform, acetone, ethyl acetate, ethanol and methanol. The methanolic extract of plant materials had showed higher larval and pupal mortality. When methanolic extracts of two plant materials combined with each other had significant larval and pupal toxicity of Aedes aegypti. Among these two may serve as a new source for managing various mosquito larvae in field ecosystem, although their effects on non-target organisms remain unknown.
Plants contain many chemicals, which are important in their defence against insects. The phytochemicals derived from various botanical sources have provided numerous beneficial uses ranging from pharmaceuticals to insecticides. The phytochemicals derived from plant sources which is not only act as larvicidal activity 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 Su, 1999).

The bioefficacy against several orders of insects, including over 400 species, has been reported gobally (Parmar, 1998). Neem products act by intervening at several stages of the life of an insect. They may not kill the pests instantly but also incapacitate it in several other ways. The precies effect of neem extracts on insects species is often difficult to pinpoint (Vijayalakshmi and Mishra, 1991).

The highly purified azadirachtin has longer shelf life, aflatoxin free and does not have a quick knockdown effect. Treated instars may become permanent larvae which are unable to moult (Govindhachari, 1998).

In the present study, Econeem, a neem based insecticide showed larvicidal and pupicidal activity on Aedes aegypti. Similar results were obtained on the pupicidal effect against malarial vector, A. stephensi (Murugan and Jeyabalan, 1998). Murugan et al. (2003) also worked on the toxicity of Neem oil and Bti and found that the combined toxicity brought higher mortality of C. quinquefasciatus. In the present study, the toxicity of botanical insecticide, Econeem on Aedes aegypti not only affects the larval and pupal stages of mosquitoes but also reduces the growth and development.

Botanical pesticides like many other natural products showed a limited persistence under field conditions. Temperature, ultraviolet light, pH on treated plant parts, rainfall, and other environmental factor may exert a more or less negative influence on the active principle. Therefore, the residual effect of neem based products is, in general, restricted to a
few days, mostly around five to seven days. In the present study also econeem, a neem based insecticide treatment not only affected the growth of the mosquito and also prolonged larval and pupal duration and affected adult emergence of mosquito.

Neem products can be mixed with other bioproducts (for instance, insecticides based on *Bacillus thuringiensis*) or with synergists to increase their efficacy, if necessary. However, for crops with high quality demands, neem pesticides are less suitable or not suitable at all, because they are less efficient in quickly killing target insects than are synthetic products. By neem treatment due to lack of feeding, the insect starved by the way as well as the gut. Rao *et al.* (1995) 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 populations of anopheline pupae.

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).

One of the promising area of microbial control is the use of disease to increase the susceptibility infected beetles of *Brachyrhinus ligustici* (Weiser, 1956). In recent years, emphasis has shifted from the use of synthetic chemicals insecticides towards biological agents for the control of mosquitoes. This has been due to an increased awareness of the harmful effects of chemical insecticides on non-target organisms and the environment as well as the development of resistance in many species of vectors. The application of entomopathogens such as bacteria is a promising approach. *Bacillus thuringiensis* is one of the most actively researched entomopathogens and has found commercial application in several cases. *Bacillus thuringiensis* var. *israelensis* (Bti) has been shown in laboratory and field studies to be a potent larvicide of mosquitoes (Mulligan *et al.*, 1980; Molly and Jahnback, 1981).

The gram positive endospore forming bacterium *Bacillus thuringiensis* var. *israelensis* produces parasporal crystalline inclusion that contain polypeptides
(δ- endotoxin) that are toxic to a variety of insect species. Upon ingestion by an insect larvae, 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 ion the midgut epithelial membrane that results in cell lysis, cessation of feeding and death of the larvae (Charles and De Barjac, 1983; Singh et al., 1986; Daniel et al., 1995). The mode of action of Bti toxin on the guts of mosquito larvae

i) ingestion of spore toxin
ii) midgut dissolution
iii) activation of protein gut protease into active toxin
iv) binding of active toxin in specific binding receptors in midgut brush border membrane
v) internalization and excretion of toxin and finally cell lysis

Midgut cell in mosquito larvae are similarly affected after ingestion of other varieties of Bacillus thuringiensis, excepted that a selective lysing is observed in those portions of the midgut with high pH where protein absorption takes place (Lacey and Federici, 1979). Correct activation of a Bacillus thuringiensis endotoxin is likely and insufficient prerequisite for toxicity and insufficient processing (or) over digestion of a toxin many render it inactive. The battery of midgut proteases than an insect possesses is therefore likely to be a major determinant of toxin potency. The midgut lumina of Dipteran insect larvae have been shown to contain variety of alkaline proteases, mainly members of the serine protease class, that exhibit predominantly trypsin like and chymotrypsin like protease activities (Jongsma et al., 1996) such midgut processes are likely to be responsible for δ-endotoxin activation. In the present study also the toxin treated larvae showed irregular movement and finally lead to death of the larvae and it may be due to the toxic effect of Bt toxin mosquitoes.
In the present study after the treatment of *Bacillus thuringiensis* var. *israelensis*, the activity of infected larvae arrested after ten minutes showing abnormal movement. Larvae move frequently up and down. In general, *Bacillus thuringiensis* toxins breakdown the larval midgut epithelium. After ingestion of the spore crystal complex of mosquito larval the protein crystal matrix quickly dissolves in lumen of the anterior stomach (Charles, 1987) through the combined action of midgut proteases and the high pH (Charles and de Barjac, 1981).

The bacterium *Bacillus thuringiensis* var. *israelensis* produces a crystalline inclusion concomitant with sporulation that is toxic to the larval stage of many mosquito and blackfly species (De Barjac, 1978). *Bacillus thuringiensis* var. *israelensis* crystals exhibit two biological activities which occur at markedly different protein concentrations, a mosquito larvicidal activity by infact crystals (at a 0.2 to 5 μg/ml) and a general cytolytic is commonly measured via erythrocytolysis (Ellar et al., 1985) but it also causes rapid lysis of man mammalian cells in vitro.

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 *Bacillus thuringiensis* have been detected on the brush border epithelium (Van 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 and Sharaby, 1985). In the present study, the methanolic extracts of *Plectranthus amboinicus* and *Canarium strictum* have been tried to test with the toxicity of Bt toxin and moreover we could find that there was complete reduction of larval and pupal mortality, and it may be due to the interactive effect of plant and Bt toxin on the gut of mosquito larvae.
Formulation additives may enhance activity of *Bacillus thuringiensis* and could increase both the host range and the biological activity of the toxin. Some examples are already evident. Such as the use of feeding stimulants to increase ingestion of the toxic dose (Bartlet *et al.*, 1990) and the addition of protease inhibitors to protect the proteinaceous toxin (Macintosh *et al.*, 1990). Other investigations have tested chemical additive compounds (Salama *et al.*, 1986) and various plant allelochemicals (Felton and Dahlman, 1984; Salama *et al.*, 1986; Ludlum *et al.*, 1991) to increase *Bacillus thuringiensis* activity.

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, during breeding site treatment. In the present study *Bti* at different concentration, brought out laboratory toxicity on the various larval instars of *Aedes aegypti*. The *Bti* application in the field also considerably affected the different stage of the larval population of *Aedes aegypti* and it suggested that the thymol, carvacrol, terpinnen-4-ol and Oleo-gum-resins present in the plant extracts might have affected physiology of insects and thereby increases the toxicity of Bt toxins on mosquito larvae.

After ingestion of the spore crystal complex by mosquito larvae, the protein crystal matrix quickly dissolves in the lumen of the anterior stomach through the combined action of midgut proteases and the high pH. In the present study also after application of *Bti* at different concentration showed mortality against mosquito larvae and it may be due to effect of Bt toxin on the midgut protease of the mosquito larvae. *Bti* toxin completely break down the larval midgut epithlium, whereas *B. sphaericus* toxins do not. Nevertheless, midgut alteration start as soon as 15 min after ingestion of the *B. sphaericus* spore crystal complex. Midgut damages in *Culex pipiens* are the same after ingestion of spore crystals of either strain 2297. In contrast the symptoms of intoxication produced by these two strains differ in other mosquito species. Large vacuoles appear in *C. pipiens* cells, whereas large area of low electron density appears in *Anopheles stephensi* midgut cells. Generally occurring symptom is mitochondrial
swelling described for *C. pipiens* and *Anopheles stephensi* as well as for *Aedes aegypti* when intoxicated with a very high dose of spore crystals. The midgut cells, especially those of the posterior stomach and the gastric caecae, are the cells most severely damaged by the toxin, and Singh *et al.* (1986) also reported late damage in neutral tissue and in skeletal muscles.

In the present study also the application of Bt toxin possible on mosquito larvae was the implication on the death of the *Aedes aegypti* larvae may be due to the toxic spore ingestion at treated water and its effect of crystal spores on the gut epithelial cells of the midgut and subsequent death of insect.

The over all midgut pathology of Bt toxicity results in a loss of basal involutions in the columnar cells; swellings of the endoplasmic reticulum; vesticulation of the endoplasmic reticulum; loss of ribosomes; swelling of mitochondria; swelling of the cell and nucleus and subsequent rupture of nuclear organelles, and plasma membranes; and finally release of the cell content into the lumen with sloughing including increase in the number and size of nuclear pores separation of the cells from each other and from the basement membrane and nearly complete destruction of the goblet cell. Since Bt toxin is species specific, it can be used as biolarvicide for the control of mosquitoes.

By application of combinations with *Bacillus thuringiensis* var. *israelensis* additive or even synergistic effects are obtained. The use of simple and cheap neem products, such as crushed neem seed kernels seems promising for treatment of water pools in towns and villages in developing countries during the rainy season, for prevention of breeding of mosquitoes (Vahitha, 2002). Murugan *et al.* (2003) also established that the larval and pupal toxicity of Bti and Bs on larvae of *Culex quinquefasciatus*. Moreover, earlier worker have proved neem had an impact on gut physiology. In the present study, it was clearly established that Bt toxin at 3.23% concentration had a complete control of mosquito larvae at laboratory as well as in the field.

The insect immune system comprises of humoral and cellular components of haemolymph which function to retain the integrity of the individual. The two components
serve crucial physiological functions, including the capacity for wound healing and recognize and destroy foreign microbial invaders. This capacity is suppressed in various immune responses, e.g. production of antibacterial proteins and other inducible factors, phagocytes, nodule formation, encapsulation and melanisation (Nappi and Christensen, 1987).

In general, young larvae are more susceptible than elder larvae to most diseases (Vasiljevic, 1957; Saxena et al., 1981a, b). Certain insect pathogens, especially viruses and bacteria, have been combined satisfactorily with various adjuvants (Wetting and Sticking agents). Such as blood albumin, milk powder, wheat flour glycogen and several of the recent proprietary surface-active agents (Angus, 1956). These adjuvants usually increase the effectiveness of the insect pathogens after combination of neem products, since neem products coming by array of bio-active limonoids (Schmutterer, 1990; Murugan et al., 1998).

In the present study Bti caused considerable mortality on the various larval instars of Aedes aegyti in the laboratory. This is comparable to that of earlier studies Goldberg and Margalit (1977); De Barjac and Coz (1979); Garcia and Des Rochers (1979); Mulla et al. (1984).

Larvae of the Aedes aegyti treated with Bacillus thuringiensis var israelensis exhibited delayed development compared with control and result in a delayed (or) inhibition of moulting (Sieber and Rembold 1983; Schmutterer, 1984). The combination of Plectranthus amboinicus and Canarium strictum with Bacillus thuringiensis could be useful for controlling mosquito populations. Since Bacillus thuringiensis var. israelensis toxicity is primarily through ingestion of the parasporal toxic crystal, the presence of food particles most likely offers competition for ingestion of the toxic crystal. The mortality values with food should be more realistic for practical control purposes of these insects because of the occurrence of food materials in habitats of the target insects.

Mortality of Aedes aegyti after the treatment of Bti at different dose level showed considerable effect on the various larval instars dose dependent mortality were evident at Bt toxin treatment. Combination of Plectranthus amboinicus and Canarium strictum with Bacillus thuringiensis var. israelensis resulted in additive and synergistic
effects on larval mortality of the Bt-S and Bt-R strains, respectively. Alter et al. (1996) found that larval feeding behaviour of these 2 strains were different when they were exposed to potato foliage treated with cry IIIA. In the present study Plectranthus amboinicus and Canarium strictum with Bacillus thuringiensis var. israelensis applied individually or in combination showed retarded larval growth, delayed moulting and development compared with control and it proved clearly on the combined effect.

Correct activation of a Bacillus thuringiensis endotoxin is likely, and in sufficient prerequisite for toxicity, and in sufficient processing (or) over digestion of a toxin many render it inactive. The battery of midgut proteases that an insect possesses is therefore likely to be a major determinant of toxin potency. The midgut lumina of Dipteran insect larvae have been shown to contain variety of alkaline proteases, mainly members of the serine protease class, that exhibit predominantly trypsin like and chymotrypsin like protease activities (Jongsma et al., 1996). Such midgut processes are likely to be responsible for δ-endotoxin activation. In the present study also the Bt toxin treated larvae showed irregular movement and finally lead too death of the larvae, and it may be due to the toxic effect of Bt toxin on the mosquitoes.

Sharma et al. (2003) tested a new Bti formulation in the laboratory and small scale field trials against mosquito larvae in different breeding habitats of Kumaun foothill region of National and Udham singh Nagar districts, Uttaranchal state, India. Results of the field trials revealed 100% mortality of mosquito larvae after treatment with Bti @ 0.5 gm/m2 surface area. The impact was similar on different mosquito species breeding in different habitats. However, repeated treatments were required due to reappearance of larvae in the breeding habitats within a week. No side effects of Bti was observed during field trial on non- target organisms (NTOs). Environmental disturbances and man made problems affected the spray impact of Bti.

Shililu et al. (2003) evaluated the larvicidal activity of the granular formulation of Bacillus thuringiensis israelensis (Bti) serotype H-14 (Vectobac G, 200 ITU/mg) and Bacillus sphaericus (Bsph) serotype H5a5b (Vectolex CG, 670 Bs ITU/mg) against Anopheles arabiensis and other mosquitoes in breeding habitats in 3 sites, Gash-Barka,
Anseba, and Debub zones, in Eritrea. The primary objective was to determine the optimal application rate and duration of effect for Bti and Bsph in representative larval habitats as compared with the organophosphate temephos. The biolariicides were tested at 100% (high) and 50% (low) of the maximum recommended application rate. Temephos was applied at a rate of 100 ml/ha. At least 4 replicate experiments with Vectobac G (5.6 and 11.2 kg/ha), Vectolex CG (11.2 and 22.4 kg/ha) were conducted in each study site. All 3 larvicides caused significant mortality of the main malaria vector species, *An. arabiensis*, and other mosquito species (*Anopheles cinereus, Anopheles pretoriensis, Culex quinquefasciatus*). The larvicidal activity for Bti and Bsph was variable depending upon breeding habitat, mosquito species and general ecology of the area.

Lee *et al.* (2005) carried out on the bioefficacy and residual activity of *Bacillus thuringiensis israelensis* H-14 (Bti) (water-dispersible granules of VectoBac ABG 6511 and liquid formulations of VectoBac 12AS) and pyriproxyfen (insect growth regulator, Sumilarv 0.5%) as direct applications for control of larvae of *Aedes aegypti* and *Aedes albopictus*. In this study, Water-dispersible Bti granules provided effective initial control activity against *A. aegypti* and *A. albopictus* for both test designs (with replenishment and without replenishment of water). The higher dosage (570 ITU/liter) for both Bti formulations was only partially effective at the end of 1 wk after being diluted. After 1 wk, water-dispersible Bti granules provided greater larval mortality than did liquid Bti formulation against both mosquito species when integrated with pyriproxyfen. Pyriproxyfen (79.5 and 159 mg/liter) on its own showed low larvicidal activity but provided very effective control of adult emergence. In this study, integration of Bti (285 and 570 ITU/liter) with pyriproxyfen (79.5 mg/liter) extended the duration of partial larval control somewhat, but live larvae persisted throughout the 4-wk test. The integration effect was more obvious when water-dispersible Bti granules were integrated with pyriproxyfen than when liquid Bti was used. Integration of Bti with pyriproxyfen had a negative effect on adult emergence, which was completely inhibited by pyriproxyfen after day 1. Daily replenishment of water increased Bti activity and provided slightly better larval control. *Aedes albopictus* and *A. aegypti* were both completely susceptible to the higher concentration of Bti and pyriproxyfen in both test designs (with replenishment and without replenishment of water).
In the present study, *Bti* treated larvae showed mortality as well as lowered growth rate suggest that, *Bt* toxins might have interfered the gut system and the mortality. In the present study, after the application of *Bacillus thuringiensis* var. *israelensis* at 0.5, 1.0, 1.5, 2.0 and 2.5% concentrations on first, second, third and forth instar stages of larvae of *Aedes aegypti*, leads to inhibition of mosquito growth regulatory and adult emergence activity. *Bacillus thuringiensis* toxins completely breakdown the larval midgut epithelium. After ingestion of the spore crystal complex of mosquito larval the protein crystal matrix quickly dissolves in lumen of the anterior stomach through the combined action of midgut proteases and the high pH (Charles and de Barjac, 1981). In the present study after the treatment of *Bacillus thuringiensis* var. *israelensis*, the general activity have been arrested after ten minutes, the larvae showed abnormal movement and due to toxic effect larvae move frequently to keep up and down. *Plectranthus amboinicus* and *Bacillus thuringiensis* toxin specifically affected the *Aedes aegypti* and caused mortality of mosquito larvae. Since *Plectranthus amboinicus* contains thymol, carvacrol, terpinen-4-ol, p-cymene and spathulenol (Gurdip et al., 2002). Further, present study also the liquid formulation of Bti imposed to toxicity brought out significant mortality at laboratory and field level. Moreover, the plant products have active compounds and higher population reduction after combined treatment may be suggested that the active interaction of plant compounds and Bt toxins.

Keplinger and Deichmann (1967) evaluated the interactions of eight organochlorine and five organophosphate insecticides on rats and mice. The investigators concluded that the toxicity of dieldrin was synergistic with all five organophosphates, delnave, diazinon, V-C 13, malathion and parathion. In the present study, the interaction of plant extracts and organophosphorous insecticide chlorpyriphos was synergistic against the dengue vector, *Aedes aegypti*.

Kreitzer and Spann (1973) determined that dieldrin and diazilinon, an organophosphate, interacted additively in Pheasants and quail. These results do not distinguish between additively and independence, however they do not indicate the presence of synergy or antagonism between dieldrin and an organophosphate.
Chlorpyrifos is known to inhibit acetylcholine esterase by irreversibility binding to the active site of the enzyme. Synergistic interactions between mercury and the organophosphates, malathion and parathion, have been reported in Coturnix quail (Coturnix laponica) (Dieter, 1974; Dieter and Ludke, 1975).

Elliott et al. (1978) also reported that synthetic pyrethroid, which contain volatile substances like d-allethrin and d-trans allethrin which possessed good knock-down and adequate killing activity against mosquitoes. In the present study, the synthetic insecticide chlorpyriphos contain 0, 0-diethyl 0-(3, 5, 6-trichloro-2 pyridyl) Phoshorothioate which possess good knock-down and adequate killing activity against the dengue vector, Aedes aegypti. Hence, present study also the sub-lethal dose of chlorpyriphos might have interacted with plant toxins and ultimate death of larvae.

H. azteca exposed to chlorpyrifos and methyl mercury eliminated methyl mercury at a significantly reduced rate compared to methyl mercury-mercury-chlorpyrifos elimination curve is positive indicating the inability of the organisms to eliminate methyl mercury. The chronic sensitivity of M. beryllina, the least sensitive of the three species tested with chlorpyriphos, is similar to that of the freshwater fathead minnow (Pimephales promelas), which has lower and upper chronic values of 1.6 and 3.2µg technical chlorpyrifos/L (Jarvinen and Tanner, 1982). In the present study, the lethal concentration of chlorpyriphos against the larvae of Aedes aegypti was ranged from 0.88 to 1.52%. When compared the other products, it was very lesser value.

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 Ludlum et al. (1991) have reported that aromatic compounds and plant allelochemicals increase Bti activity. The result of this study indicate that Plectranthus amboinicus and Canarium strictum extracts enhance the
Bacillus thuringiensis var. israelensis activity, it may be an effective alternative to conventional synthetic insecticides for the control of A. aegypti the use of plant extracts as an additive to the Bt may play a more prominent role in Integrated Pest Management Programme in future.

Addition of Bti at concentrations ranging from 20 to 100 ppm to plant extracts had adverse effect upon the larval mortality. When combined with plant extracts, the Bti increased the percentage of larval mortality and decreased the time 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 (Gould et al., 1991). This may be due to antifeedant potential of plant products and particularly from neem principles.

In the present study the combined effect of plant extracts and Bti produced higher toxicity, in terms of mortality of larvae and pupae of Aedes aegypti, similar study had been conducted by using resucethrin, piperonyl butoxide mixtures toxicity co efficient Coomber et al. (1977). This suggests that plant (neem) principles have array chemicals that may be on the gut system and caused toxicity and led to death.

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 of plant extract with Bt toxin we can improve the efficacy of Bt toxin (Murugan et al., 2003).

In the present study, the extract of Plectranthus amboinicus and Canarium strictum with Bacillus thuringiensis var. israelensis showed considerable effect of larvicidal and pupicidal properties against the dengue vector Aedes aegypti. When PAME and CSME combined with Bacillus thuringiensis var. israelensis had significant larval and pupal toxicity of Aedes aegypti. It reveals that the cry toxin from Bt crystal affected gut lining and brought out mortality.
In the present study the results showed higher mortality in the combined treatment of Bti and PAME. 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 and growth and increase mortality (Lindroth et al., 1988). In the 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. Integrated pest management (IPM) aimed at optimization would ultimately replace the key-test oriented control. It should be recognized, however, that when successful control of mosquitoes like Aedes aegypti is achieved through the use of control tactics other than synthetic insecticides, it will contribute to a great reduction in the application of synthetic insecticides, which in turn increase the opportunity for natural control of various mosquitoes by microbial and botanical pesticides. Since, neem limonoids and other plant tannin have proved on gut binds properties (Isman, 1993; Murugan et al., 1996).

The seaweeds Caulerpa scalpelliformis and Dictyota dichotoma and mangrove Rhizophora apiculata were extracted in acetone, combined with synthetic insecticides (DDT, BHC, HCH and malathion) and evaluated for activity against fourth instar larvae of Aedes aegypti. The extract showed synergism with the insecticide. The highest synergistic activity with all three insecticides especially HCH (Subramonia Thangam et al., 1991).

Effect of an insecticide and acetone leaf extracts of Withania somnifera and Argemone mexicana against Anopheles stephensi, Culex quinquefasciatus and Aedes aegypti was studied. The insecticide effectively checked all mosquitoes population. Both W.somifera and A.mexicana were found to be more effective in controlling the mosquito’s population. The early larval instars were highly susceptible to plant extracts than later stages. When the effects of two extracts were compared, it was discernible that the leaf extract of W. somifera was more effective in controlling the population of A. aegypti that the leaf extract of A. mexicana (Uma et al., 2003). In the present study, Plectranthus amboinicus extract was more effective in controlling the population of Aedes aegypti. Hence, this plant posses number of active alleloids that may be responsible for the larvicidal activity.
The 22 plant samples were extracted in acetone and petroleum ether separately. The extracts were tested for their activity against larvae of *Culex quinquefasciatus*. The petroleum ether extract was the most effective. The extract was studied further with pyrethrum for its synergistic larvicidal activity. The extract exhibited synergism. The synergistic factor was 0.81 at 5 mg/l. (Thangam and Kathiresan, 1991). Petroleum ether extracts of ten indigenous plants were studied for the larvicidal activity against the *Culex quinquefasciatus, Anopheles stephensi* and *Aedes aegypti*. Plant extracts were also studied for the synergistic effect with the synthetic larvicide phenthoate and fenthion *Croton sparsiflorus*. A common weed was found to be effective at 1 ppm level while extracts from the other plants were found to be effective at higher concentration (Kalyanasundaram and Das, 1985). The extracts for the synergistic activity were benzene extract of the leaf and seeds of *Acacia nilotica* as the LC$_{50}$ value of the leaf extract was low 19.90 and the mortality was observed even at 31.25 ppm. The synergistic factor value of benzene extract of leaves was 1.73 and in benzene extract of seed the synergistic factor value was 2.18 and this showed synergism (Chokalingam *et al.*, 1989). In the present study the combined effect of PAME and CSME with chlorpyriphos showed higher synergistic activity against *Aedes aegypti*.

Neem products can be mixed with other bioproducts (for instance, insecticides based on *Bacillus thuringiensis*) or with synergists to increase their efficacy, if necessary. However, for crops with high quality demands, neem pesticides are less suitable or not suitable at all, because they are less efficient in quickly killing target insects than are synthetic products. By neem treatment due to lack of feeding, the insect starved by the way as well as the gut (Vahitha, 2002).

Harve and Kamath (2004) carried out the synergistic study by using 0.05 ppm of Temphos and Fenthion with 25 ppm of *M. koenigii, F. asafetida, T. foenum garceum* and 100 ppm of *C. sativum*. All the plants showed potential synergistic activity although showed comparatively poor larvicidal activity when tested individually. In the present study, the synergistic activity of methanolic extracts of PAME and CSME with chlorpyriphos showed nil-synergistic activity, but comparatively high larvicidal activity when tested individually.
Earlier work on the effect of IGR on insects, the petroleum ether extract of *Acorus calamus*, *Ageratum conyzoides*, *Annona squamosa*, *Bambusa arundanasia*, *Madhuca longifolia* and *Citrus medica* were tested for larvicidal and juvenile hormone activity against different mosquito species viz *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi*. *Acorus calamus* extract was found to be effective with LC$_{50}$ values of 33.7, 39.4 and 48.8 mg/l against *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi*, respectively. *Bambusa arundanasia*, *Madhuca longifolia* and *Citrus medica* extracts were found to be effective at concentrations higher than 50 mg/l. The juvenile hormone activity of these extracts showed that only *Acorus calamus*, *Madhuca longifolia* and *Ageratum conyzoides* were found to produce significant inhibition in adult emergence at the test concentration of 5 and 10 mg/l (Sujatha *et al.*, 1988). Similar to above studies in the present study also the suppression of growth of various larval instars was evident after the treatment of PAME, CSME, Econeem, Bti and chlorpyriphos. Growth suppression effect of plant extracts may be due to the slow toxicity on physiological process of insect.

Acetone extracts of neem seed coat was used against *Aedes aegypti* and *Culex quinquefasciatus* to assess its toxicity growth regulating capacity and its impact on hatchability. When *Aedes aegypti* larvae in their first instar exposed to low concentration, no mortality was observed. At higher concentrations the larval mortality occurred in dose dependent manner. A concentration of 40 ppm caused 100% mortality of the first instar larvae while in *Culex quinquefasciatus*, mortality of same magnitude was caused by a dose of 20 ppm. Even at lower concentrations, pupal mortality was observed in *Culex quinquefasciatus* but no pupal casualty occurred in *Aedes aegypti*. Sagar and Sehgal (1996) reported that the insect growth effect of acetone extract of neem seed coat were assessed in the pronounced eggs of both the species treated with acetone extract of neem seed coat had no adverse effect on hatchability.

In the present study Econeem treated larvae did not grow well and malformation of larvae and pupae were evident. The growth regulatory effects of neem related products are of considerable theoretical and practical interest. Treatment of insects by injection, oral ingestion or topical application of azadirachtin caused larval growth, inhibition
malformation and mortality. These are effects that are similar to those observed in treating insects with insect growth regulators (IGRs). This activity has been proven in Orthoptera, Hemiptera, Lepidoptera and Diptera (Schmutterer, 1988, 1990; Ascher, 1993; Mordue and Blackwell, 1993). The hatchability of treated egg rafts was also very poor after the Econeem treatment.

In the present study Econeem treatment greatly affected the growth and development of *Aedes aegypti* and proved to be a good larvicide, pupicide as well as growth regulatory effects against *Aedes aegypti*. The interruption of insect reproduction is also an important feature of the azadirachtin compounds. Because ecdysteriod is one of the hormones regulating vitellogenesis and azadirachtin can modify haemolymph ecdysteroid by inhibiting the release of PTTH and allatotropins from the brain-corpus cardiacum complex of adverse effects on ovarian development, fecundity and fertility (egg viability) occur in Orthoptera, Hemiptera, Heteroptera, Coleoptera, Lepidoptera, Diptera and Hymenoptera Schmutterer, 1988,1990; Ascher, 1993; Mordue and Blackwell, 1993).

Zebitz (1986) observed that mosquito larvae treated with neem seed kernel extracts produced deformities including larval - pupal intermediates. Bioactive compounds to that of azadirachtin, the principal active compound in the neem seeds was reported by Mwangi and Rembold (1988) and Mwangi and Mukiama (1988). In the present study PAME and CSME extracts produced various larval, pupal and adult deformities.

The failure in morphogenesis in Azadirachtin treated animal was due to the low body weight, reduction of pupal weight, delay in pupation and inhibition of adult emergence. Adult emerging from azadirachtin treated larvae, was smaller and showed various malformations (Bidmon *et al.*, 1987). The antipupational effect of neem oil and neem seed kernel extract prepared against *Anopheles stephensi*. The percentage of pupation and adult emergence were markedly reduced by neem oil and NSKE treatments, suggesting growth regulatory effects and post-ingestive toxicity of neem extract in mosquito control (Murugan *et al.*, 1996).
In the present study, after the treatment of PAME, CSME, Econeem, Bti and chlorpyriphos, higher effect on the delay in pupation and growth inhibition as well as significant anti-emergence properties was noted. Azadirachtin and other related neem products act as insect growth regulator to inhibit the development of the immature stage of Culex quinquefasciatus even at very low concentrations. The effects on pupae and adults were dose dependent (Gaaboub and Hayer, 1984).

In the present study methanolic extract of Plectranthus amboinicus and Canarium strictum to inhibit the development of the immature stage of Aedes aegypti. Extracts from dry Melia volkensii fruits contain certain growth-inhibitory activity against the larvae of the mosquito Aedes aegypti. The active compound of this fraction is not identical with azadirachtin. It may be more potent as an insect growth inhibitor and more lethal to Aedes larvae than azadirachtin (Mwangi and Rembold, 1988).

In plants producing bioactive materials, the neem tree Azadirachta indica A. Juss, and closely related species A. excelsa Jack, A. siamens valeton, Melia azadirach L., and M. volkensii in the family Meliaceae have been well investigated and proven to possess considerable insecticidal properties. Atleast 35 components exhibiting some insecticidal properties have been identified from tree (Rao and Parmer, 1984), which includes triterpenoid and azadirachtin. The azadirachtin moiety has structural similarity to the moulting hormone of insects. It is quite unique that azadirachtin can act and affect multiple systems in insects and exhibit various mode of action. The petroleum ether fraction of Solanum trilobatum and the ethyl acetate fraction of Leucas aspera were less toxic to the larvae. The active fractions of the plant extracts disrupted moulting and metamorphosis induced malformation, extended the larval duration and inhibited adult emergence (Muthukrishnan et al., 1997). In contrast to the earliest work, in the present study the methanolic extract of PAME, CSME showed significant toxicity and growth regulatory effects on Aedes aegypti.

The larvicidal effect of acetone extracts from Melia volkensii and Melia azadirach seeds were compared with the pure natural growth inhibitor Azadirachtin in their morphogenetic effects against Culex pipiens (Sharook et al., 1991) Similar to Melia
volkensii and Melia azadirach, in this study PAME and CSME showed considerable toxicity and inhibition of growth regulation on Aedes aegypti. In the present study, the Econeem treated insects deposited lesser number of eggs and it may be due to anti-reproductive effect of neem on mosquito (Babu et al., 1997).

Some way the fires are for cooking. A possible new, low technology method to release plant volatiles is thermal expulsion where the plant material is placed on a metal plate over a fire (Seyoum et al., 2002a). It appears to provide greater expellency than direct burning of plant material, possibly since it release different repellent compounds and may carry lower risk of causing respiratory disease since less smoke is produced this way.

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 material such as saw dusts are also cheap and often an effective method of repelling mosquitoes. They are probably derived from the incense use in religious ceremonies by Hindus, Buddhist and the followers of confucious. In today, the same in cense used in ceremonies to honour ancestors is also used on a daily basis to repel mosquitoes (Sangat –Roemantyo, 1990).

In the present study also smoke emerged from Plectranthus amboinicus leaf and Canarium strictum seed powder have considerably affected by adult mosquitoes and brought out considerable mortality and also treated individual layed minimum number of eggs. The mortality of mosquitoes may be due to toxic volatile ingredients of thymol, carvacrol, 1, 8-cineole, p-cymene, spathulenol and terpinnen-4-ol from Plectranthus amboinicus leaf and Oleo-gum-resins from Canarium strictum seed used into mosquito coil.

Traditionally smoke from burning dried plant leaves such as Vitex negundo, neem, pongamia seed kernel powder and Acorus calamus rhizome powder have been used for domestic protection. In Malaysia, (Janten et al., 1999) have prepared nineteen mosquito coil formulations, each containing a different plant and investigated for their knockdown and 24 hours mortality values against Aedes aegypti. Mosquito coil of the
leaves of *Cymbopogan nardus* and *Aloe vera* and seed kernel of *Azadirachta indica* incorporation with D-trans-allethrin significantly increased their efficiency against mosquitoes in terms of knocking down and killing effects. Therefore, use of these plant materials as organic filters in mosquito coil formulation may allow a reduction in the active concentration of pyrethroid and reduced health hazards. In view of growing concern about safety of chemical based repellents, interest in reviewed in oils extracted from plants as repellent for mosquitoes. Traditional repellents not only provide protection against mosquito bites but also curtail malarial transmission. In the present study the smoke from the *Plectranthus amboinicus* leaf and *Canarium strictum* seed powder also had considerable smoke repellency. Hence, these plant parts can be preferably employed for the development of mosquito coil in future. Since *Plectranthus amboinicus* and *Canarium strictum* showed higher smoke repellent properties against dengue vector *A. aegypti*. Since, plant compounds contain toxic substances to target pests and gather to the human being.

Ten mosquito coil formulations were prepared using ten mangrove plants samples separately. The smokes from the coils were tested against biting of female mosquitoes of *Aedes aegypti*. Among the samples tested, the leaf of *Acanthus ilicifolius* was found most effective against the biting activity and also reduced the mosquito population in F1 generation (Subramonia Thangam and Kathiresan, 1992). In the present study, similar to above studies the mosquito coil were prepared using *Plectranthus amboinicus* leaf and *Canarium strictum* seed samples separately. The smoke from the coils was tested against the biting action of *Aedes aegypti*. Among the samples tested were found most effective against biting activity and also reduced the mosquito population in F1 generation.

The smoke of the two plants *Vitex negundo* and *Leucas aspera* showed their repellent activity in moderate degrees against *Culex quinquefasciatus* (Kalyanasundaram and Babu, 1982). In the present study *Plectranthus amboinicus* leaves and *Canarium strictum* seeds were made from coils. The smoke from coils against the repellent on *Aedes aegypti*.

Subramonia Thangam and Kathiresan (1992) stated that the smoke from marine plant samples especially the leaf of *E. agallocha* provided protection against *Cx. quinquefasciatus*, reduced the number of egg rafts laid and the ensured mosquito
population. This reduction in the number of egg-rafts may be due to the complete eggs reduction in the fed mosquitoes and to the non-fed mosquitoes of blood meal taken among the response of smoke treatment and reduction in the egg raft as well as correspondingly. In the present study also smoke emerged from *Plectranthus amboinicus* and *Canarium strictum* plant parts have considerably affected the adult mosquitoes and brought out considerable mortality and also treated individual layed minimum number of eggs. Smoke emerged from plant materials not only act as ovicidal and also toxic effect on eggs.

The mosquito mats contain an insecticide, which is slowly vapourised by heating. The vapour in the air affects the central nervous system of mosquitoes (Mohan, 1990). The mosquito mats showed toxic effects on *Culex quinquefasciatus* after exposing the mosquitoes to the vapour of mosquito mats. Some are known to contain toxic principle can play a useful role in the control of vectors (Sujatha et al., 1988). The smoke of *Vitex negundo* and *Leucas aspera* exhibited similar toxic effects like that of synthetic mosquito mats but the offset of mortality for the herbal smoke was earlier than the mosquito mats and so extracts of these plants can be mixed with a minimum amount of synthetic chemicals to minimize the health hazards (Selvaraj Pandian et al., 1995).

In the laboratory, powdered preparation of the leaves of *Adhatoda vasica*, *Azadirachta indica* and *Ocimum sanctum* were combined with burning charcoal to produced smoke which repelled *Armigeres subalbatus* and *Culex quinquefasciatus*. All the plants smoke was more toxic to *Culex quinquefasciatus* (Selvaraj Pandian et al., 1995). In this present study, *Plectranthus amboinicus* and *Canarium strictum* exhibited similar toxic effect against *Aedes aegypti*.

Plants used as repellents are particularly beneficial for rural areas where access to commercial preparations is minimal and expense is a limiting factor. The only plant based repellent that has been reported to be as effective and long-lasting as deet is PMD (Quwenling). Several different types of repellent may be used at once for instance a fumigant concurrent with a skin repellent to provide extra protection although the use of a partially effective method is better than no protection at all. The use of smoke as protection from malaria this practice may provide (Vander Hoek et al., 1998).
In the present study, smoke from *Plectranthus amboinicus* and *Canarium strictum* had much repellent properties against *Aedes aegypti*. Among the two plants, *Plectranthus amboinicus* showed higher repellency than the *Canarium strictum*. This is because of the presence of volatile compounds like thymol, carvacrol, p-cymene and spathulenol present in the *Plectranthus amboinicus* leaf.

Further more, burning neem oil in kerosene may provide personal protection from mosquitoes (Sharma and Ansari, 1994) kerosene lamps containing neem oil were burned in a living room, and mosquitoes resting on the walls (or) attached to human bait were collected inside rooms from 1800 to 0600 hrs. Neem oil (0.01 – 1%), mixed in kerosene reduced biting of human volunteers and catches of mosquitoes resting on the walls in the rooms. However, in cage tests with female *Aedes aegypti*, neem oil showed no repellent potency (Zebitz and Schmutterer, 1987). Azadirachtin fed in blood meals to adult female *Aedes aegypti* through an artificial membrane did not cause feeding inhibition over a wide dose range (0-200 mg/female) (Ludlum and Sieber, 1988). In the present study also, the smoke emerged from the *Plectranthus amboinicus* and *Canarium strictum* plant parts were target specific on mosquito and it was observed that higher smoke repellency was found on leaves of *Plectranthus amboinicus*.

Field trial have been conducted to test on the efficacy of methanolic extracts of *Plectranthus amboinicus* leaf and *Canarium strictum* seed with Econeem, Bt toxins and chlorpyriphos at different breeding habitats of *Aedes aegypti* in and around of Vadavalli Panchayat, Coimbatore. For the field trial the quantity of plant residues and *Bti* required (Based on the laboratory of LC50 values) for each treatment was determined by calculating the total surface area of the each habitat. Dipper sampling and counting of larvae has been monitored on the larval density before 24 hours, 48 hours and 72 hours after treatment. Among the plant extract treatment, PAME showed better than the CSME. Combined treatment of PAME and *Bti* brought out higher mortality with in shorter span of time suggest the interactive effect of plant extract with Bt toxins. The percentage reduction of larval mortality also showed the variations among the different breeding habitats. This may due to the impact of geographical effect and environmental pollutions on *Aedes aegypti*. 

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Earlier studies demonstrated that the leaf of extract of *Polyalthia longifolia* evaluated against larvae and pupae of *Culex quinquefasciatus*. Three habits 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 (Suryanarayana murty *et al.*, 1997). In the present study, the field trails were conducted by different breeding habits of *Aedes aegypti* by using methanolic extract of *Plectranthus amboinicus* leaf and *Canarium strictum* seed. The methanolic extracts of *Plectranthus amboinicus* leaf and *Canarium strictum* seed and *Bti* were applied on breeding place spread on the water surface and from a thin layer which cuts of oxygen supply to mosquito come up to the water surface to breath, the oil substance penetrates the larva through the respiratory tube and kills if either by suffocation or by poisoning. Methanolic extract of *Plectranthus amboinicus* leaf had promising larvicidal effect on *Aedes aegypti* and this was done by Amutha devi *et al.* 1999 by using pongamia oil against *Culex quinquefasciatus*.

The application of neem oil-based formulations were also effective in reducing the pupal density of *Anopheles subpictus* Grassi and *Anopheles vagus* Donitz when combined with intermittent irrigation (Rao, 1977). In the present study, the combined and individual of Econeem with *Plectranthus amboinicus* and *Canarium strictum* were used for the field trails on different breeding sites of *Aedes aegypti*. The combined treatment reduced all the stages of larval density of *Aedes aegypti*. The percentage reduction was considerably increased in the combined treatment than the individual treatment. Hence, *Plectranthus amboinicus* and *Canarium strictum* plants can also employ for the mosquito control programmes. Higher efficacy by combined treatment may be due to the interactive effect plant compound on mosquito larvae.

Application of mineral oils like diesel oil, fuel oil, kerosene, white petrol etc. is widely practiced for the control of mosquito larvae oils with a mixture of spreading agent mosquito larvicidal oil and Malariol are available and preferred for this purpose when applied to a breeding place, oil spreads on water surface and forms a thin layer which

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cuts off the oxygen supply to mosquito larvae and pupae, when larvae come up to the water surface to breathe, the oil penetrates the larva through the respiratory tubes and kills it either by and kills it either by suffocation or by poisoning (Amuthadevi et al., 1999).

In the present study, the field trials were conducted by different breeding habitats of Aedes aegypti. The extracts of PAME, CSME with Econeem, Bti and chlorpyriphos were applied on breeding place spread on the water surface and form a thin layer which cuts off oxygen supply to mosquito larvae come up to the water surface to breathe, the oil penetrates the larva through the respiratory tube and kills it either by suffocation or by poisoning. All the extract showed higher field trial activity against Aedes aegypti dissolves into the water, methanolic extract and combined also killed the mosquito population.

Garcia and Rochers (1979) reported appreciable mortality only with high concentrations (1 x 10^7 cells/ml) of Bacillus thuringiensis var. israelensis. Recent trials of Bti in replicated experimental ponds by Ali (1981) have shown that the biocide at 1 to 10 kg/ha (0.25 to 2.5 ppm) caused 18 to 88% mortality of midges during 4 week evaluation period. Younger instars are more susceptible than older ones as shown by Culex quinquefasciatus. Consequently lower rates would be required for their control, and overall 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 Bacillus thuringiensis var. israelensis against chironomids is the length of exposure. In the field trials (Ali, 1981), midges were exposed to the pathogen for longer periods of time than the 48 hrs laboratory exposures. Exposure periods longer than 48 hrs in the laboratory may produce better activity results of the Bacillus thuringiensis var. israelensis formulations against the midges species. In the present study after the treatment of Bti at different concentration of various larval instars and pupae of Aedes aegypti showed higher mortality. The effect was dose also dependent.

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. Moreover, they are selective to insects and are consequently considered soft to non-target fauna commercial products. Based on Bacillus thuringiensis subsp. israelensis (Bti) is currently available (Thiery et al., 1996). After ingestion of the spore crystal complex by mosquito larvae, the protein-crystal matrix quickly dissolves in the lumen of the anterior stomach through the combined action of midgut proteases and the high pH.

Botanical pesticides like many other natural products show a limited persistence under field conditions. Temperature, ultraviolet light, pH on treated plant parts, rainfall, and other environmental factor may exert a more or less negative influence on the active principle. Therefore, the residual effect of neem based products is, in general, restricted to a few days, mostly around five to seven days. In case of systemic effects, after application of high concentrations, it lasts somewhat longer. In the present study, Econeem treatment not only affected the growth of the mosquito the treated individual and prolonged larval and pupal duration, and also affected egg laying pattern of mosquito. These bioassay effects are mainly due to the physiological as well as hormonal interference of neem products on insects.

The type of formulation used and several mosquito habitat factors such as pollution, water depth, turbidity, temperature, associated microflora, ionic composition of the water, presence of larval food, and canopy can markedly affect activity. Biological factors that effect efficiency include species of mosquito, age, larval density and feeding strategy. Younger larvae are considerably more susceptible than old instar and culicine larvae are usually more susceptible than anophelines (Lacey and Singer, 1982).

Application of Azadirachtin rich and neem oil-based formulations either alone (or) coated over urea at the time of transplantation in rice paddies was shown to significantly reduce the density of the Culex vishnui sub group including Culex tritaeniorhynchus Giles. In the present study, also the combination and individual of Bti with PAME and CSME used for field trials on different breeding sites of Aedes aegypti. The combined treatment reduced the all the stages of larval density of Aedes aegypti.
The percentage reduction was considerably increased in combined treatment than the individual treatment. Hence, *Plectranthus amboinicus* and *Canarium strictum* plants are also employed for the mosquito control programmes since lower dose with combined plant products may facilitate the slow release active compound.