Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Asian Journal of Plant Science and Research, 2013, 3(4):138-149

ISSN : 2249-7412
CODEN (USA): AJPSKY

Mosquitocidial activities of *Spathodea campanulata* methanolic leaf extract against the dengue vector *Aedes aegypti*

Kandasamy Karthika Devi[1], Ramanathapuram Sundaram Mohanraj*[1] and Balasubramaniam Dhanakkodi[2]

[1]*Department of Zoology, Government Arts College (Autonomous), Coimbatore, Tamilnadu, India*
[2]*Department of Zoology, Kongunadu Arts and Science College, Coimbatore, Tamilnadu, India*

ABSTRACT

Mosquitoes are important vectors of etiological agents of diseases to humans and domestic animals. The present study aimed to investigate larvicidal, pupicidal, ovicidal and ovipositional deterrent activity of methanol leaves extract of Spathodea campanulata against Aedes aegypti. The methanolic leaf extract of S. campanulata were found most effective with LC_{50} and LC_{90} values. The decrease in hatchability was found to be dose dependent. The mosquitoes were subjected to choice- oviposition test and no- choice oviposition test. The extract showed oviposition deterrence and effective repellence against Aedes aegypti at different concentrations, with the observation on that maximal eggs were laid in low concentration of the extract and control. These results suggest that the methanolic leaf extract of S. campanulata have the potential to be used as an ideal ecofriendly approach for the control of mosquitoes.

Key words: *S. campanulata* leaves, *Ae. aegypti* ,larvicidal, pupicidal, ovicidal, ovipositional deterrent activity

---

INTRODUCTION

Mosquitoes are insects that have been around for more than 30 million years. And it seems that, during those million years, mosquitoes have been honing their skills so that they are now experts at finding people to bite. Mosquitoes are common flying insects in the family that are found around the world. There are about 3500 species.

Among the thirteen genera of the family Culicidae, besides *Anopheles* and *Culex*, individuals of genus *Aedes* are considered dangerous because they cause significant public health threat all over the world. One of the dominant species of *Aedes* showing wide geographic distribution and spanning both temperate and tropical climate zones is *Aedes aegypti*. *Ae. aegypti* is a medium- sized blackish mosquito easily recognized by a silvery- white Iyre- shaped pattern of scales on its scutum. The colouration of both males and females is similar. It is breeds in many types of household containers, such as water storage jars, drums, tanks and plant or flower containers [1]. Compared to any other species of *Aedes,Ae.aegypti* shows more dependency on human blood [2]. *Ae.aegypti* breeds throughout the year. The eggs laid singly on the side of containers at or above the water line and also on the water surface. Hatching can take place in 2 or 3 days. These mosquitoes go through distinct stages of development: egg, larva, pupa and adult. The life cycle can be completed in about 10 days. The adult life-span of a mosquito is 50-55 days or approximately two months [3].

Ae.aegypti is the only known potential vector of dengue and urban yellow fever [4,5]. This species of mosquito was shown to be a competent laboratory vector of Chikungunya (CHIK) virus [6]. *Ae.aegypti* has also been noted to transmit filariasis and encephalitis [7].

Dengue or 'break bone' fever had been known in our country for every long time. Epidemic outbreaks of dengue fever have also been reported in India. For instance, in 1980 a total of 4,601 cases were recorded [3]. In October

2001, an outbreak of dengue resulting in 16 deaths was reported in Chennai (Tamil Nadu) India [8]. In October, 2006, a total of 5,710 cases were recorded in India. Delhi had the highest (1,637) patients. Tamilnadu, India had 307 patients; 103 deaths were also reported [9]. In 2010, there were a total of 28, 292 cases and 110 deaths [10]. In 2012 a total of 9,000 cases and 50 deaths were reported in Madurai, Tirunelveli and Kanyakumari districts (Tamil Nadu) [11].

Chikungunya, a febrile disease is caused by Chikungunya virus which is transmitted by *Ae.aegypti*. There was an outbreak of this disease in Calcutta in 1963-1964 and another in Madras (Chennai) in 1965 which gave rise to 3,00,000 cases in Madras city alone [3]. According to Central Health Secretary of India, in 2006, 13 lakh people affected by this disease. In Tamil Nadu alone 63,000 persons were affected by this disease [9]. These diseases devastate Indian economy every year [12].

At present, no effective vaccine is available for dengue; therefore, the only way of reducing the incidence of this disease is mosquito control [13]. The control methods should aim at the weakest link of the life cycle of the mosquito, which is the larval stage. During the immature stage, mosquitoes are relatively immobile, remaining more concentrated than they are in the adult stage [14].

Many control strategies for mosquitoes have been suggested since the ancient times. Among the various control measures, viz., mechanical control by source of reduction [5] ; biological control, using endopathogenic bacteria [15,16]; larivorous fish [17] as well as predatory arthropods [18] and chemical control [19].

Over and injudicious use of synthetic insecticides in vector control has resulted in environment hazards through persistence and accumulation of non–biodegradable toxic components in the ecosystem, development of insecticide resistance among mosquito species, biological magnification in the food chain and toxic effects on human health and non–target organisms [20,21].

These inevitable dilemmas have promoted renewed interest in the search and development of better or alternate vector control strategies that destroy the insects over a wide range, with minimal effect to non-target organisms and the environment.

During the last decade, various studies on natural plant products against mosquito vectors indicate them as possible alternatives to synthetic chemical insecticides [22,23]. More than 2000 plants species have been known to produce chemical factors and metabolites of value in the pest control programmes [24] and among these plants, products of some 344 species have been reported to have a variety of activities against mosquitoes [25].

Botanical insecticides also have potential uses such as larvicidal, ovicidal, oviposition deterrence, growth and reproduction inhibitors, repellents, growth regulation, fecundity suppression, male sterility [26,27]. Some of the plant leaf extract tested for their diverse insecticidal properties on the medically important mosquitoes are: methanolic extracts of *Derris elliptica* leaves [28]; aqueous extract of *Solanum nigrum* leaves [29];acetone extract of *Solanum trilobatum* leaves [30]; methanol, benzene and acetone leaf extract of *Cassia fistula* [31]; petroleum ether extract of *Azadirachta indica*, *Ocimum gratissimum* and *Hyptis suaveolens* leaves [32];aqueous and chloroform extracts of *Leucas aspera* leaf [33];hexane extracts of leaves of *Citrus sinensis* [34]; aqueous extract of *Spathodea campanulata* leaves [35,36]; methanolic extract of *Trichodesma africanum* leaves and *Cleome rupicola* [37]; acetone, chloroform , methanol extracts of *Baraleria prionitis* leaves [38]; methanolic extract of *Artemisia vulgaris* leaves [39]; methanolic extracts of *Nepeta cataria* and *Azadirachta indica* leaves [40]; ethyl acetate , aqueous, ethanol extract of *Nerium olender* leaves [41]; methanol, ethanol, chloroform, acetone and dichloromethane extracts of *Biophytum sensitivum* leaf [42]; ethanolic leaf extract of *Leucas aspera* [43].

As far as our literature survey could ascertain no information was available on the larvicidal, pupicidal, ovicidal and ovipositional detterence effects of the experimental plant species given here against *Ae. aegypti*.

The present study was therefore carried out to evaluate mosquitocidal properties of *S. campanulata* methanolic leaf extract against the vector mosquito, *Ae. aegypti*.

Spathodea is a monotypic genus in the flowering plant family Bignoniaceae. It contains the single species, *Spathodea campanulata*, which is commonly known as the African Tulip Tree, Flame-of –the forest in English, Rugtoora in Hindi, Patadi in Tamil. It is a tree that grows between 7-25 m (23-82ft) tall and native to tropical Africa. This tree is planted as ornamental tree throughout the tropics and much appreciated for its very showy reddish orange colour, campanulated flowers. It is commonly planted as a street tree in south Tamil Nadu. The tree is considered evergreen but it sheds leaves in dry summers and hence it is a dry season deciduous tree. *S.*

campanulata commonly employed to control epilepsy. This species has many uses in folk medicine. The flowers are employed as diuretic and anti–inflammatory while the leaves are used against kidney diseases, urethra inflammation and as a antidote against animal poisons. The leaves have furnished Spathodol, caffeic acid and other phenolic acids and flavonoids. The plant leaf is used for anti-plasmodial activity, anti-microbial activity and anti - larvicidal activity [44,45,46]. The aim of the present study is therefore to find out the larvicidal, pupicidal, ovicidal and ovipositional detterence effects of the methanolic leaf extract of the *S. campanulata* aganist *Aedes aegypti.*

MATERIALS AND METHODS

Colonization of *Aedes aegypti*

Collection of eggs

The eggs of *Aedes aegypti* were collected from National Institute for Communicable Disease (NICD), Mettupalayam, Coimbatore, Tamil Nadu, India without exposure to any insecticide. The eggs were then brought to the laboratory and transferred to enamel trays containing water and kept for larval hatching. They were hatched and reared and have been still maintained for many generations in the laboratory. The eggs and larvae obtained from this stock were used for different experiments.

Maintenance of larvae

The larvae were reared in plastic cups. They were daily provided with commercial fish food [47] *ad libitum.* Water was changed alternate days. The breeding medium was regularly checked and dead larvae were removed at sight. The normal cultures as well as breeding cups used for any experimental purpose during the present study were kept closed with muslin cloth for preventing contamination through foreign mosquitoes.

Maintenance of pupae and adult

The pupae were collected from culture trays and were transferred to glass beakers containing water with help of a sucker. The pupae containing glass beaker were kept in side mosquito cage for adult emergence. The cage was made up of steel frame wrapped with mosquito netting. The cage had a provision (a hole) for handling of materials and animals placed inside. The hole was guarded with a sleeve which was useful to close suddenly after being used.

Blood feeding of adult *Ae.aegypti* and egg laying

The females were fed by hand every alternate day. Feeding mosquitoes on human arm for experimental purposes was suggested by [48,49].

Both females and males were provided with 10% glucose solution as described by [50] on cotton wicks. The cotton was always kept moist with the solution and changed every day.

An egg trap (cup) lined with filter paper containing pure water was always placed at a corner of the cage. This arrangement made the collection of eggs easier.

Collection of plant materials

S. campanulata P. Beauv. (Family :Bignoniaceae) leaves were collected from Government Arts college campus, Coimbatore, Southern India. The identification of the plants was authentified at BSI (Botanical Survey of India), Coimbatore.

Preparation of plant extract

The fresh leaves of the plant *S. campanulata* were collected in our college campus area. Then the leaves brought to the laboratory. The plant leaves were observed carefully for anykind of diseases or infection and if found any, those parts were separated and not used for the experiment. The selected leaves washed with distilled water in order to clean dust or any particle stuck to them. Then the leaves kept for drying under shade at room temperature (27± 2^{o}C) for about 2 weeks till they dried completely. The leaves were finely powdered using electric blender. 250g of leaf powder was dissolved in 200ml of methanol (as a solvent) and extracted in the Soxhlet apparatus for 8 h over a mantle heater at 55 ◦C. The methanol extract was concentrated using a vacuum evaporator at 45◦ C under low pressure. After complete evaporation of the solvent , the concentrated extract was collected and stored in a refrigeratore for later use.

Preparation of stock solution and different concentrations of leaf extract

1 g of the concentrated extract of leaves of *S. campanulata* was dissolved in 100ml of methanol and kept as stock solution. This stock solution was used to prepare the desired concentrations of the extract for exposure of the mosquito larvae.

Bioassay test

Bioassay tests were carried out for testing the efficacy of methanolic leaf extracts of *S. campanulata* on *Ae.aegypti* at different stages of development viz I, II, III and IV instars and pupae. Instructions of WHO (1960) as detailed by [51] for conducting bioassay experiment with mosquito larvae were carefully followed.

The values of LC_{10}, LC_{30}, LC_{50} and LC_{90} and their 95% confidence limit of upper confidence limit (UCL) and lower confidence limit (LCL), regression and chi- square values were calculated using probit analysis[52] . The SPSS 17.0 (Statistical Package of Social Sciences) used for statistical analysis.

Ovicidal assay

Effect of methanolic leaf extract of *S. campanulata* on the hatchability of *Ae.aegypti* eggs were determined adopting the following procedure.

Twenty freshly laid eggs were exposed to a particular concentration of a test compound. The hatchability was recorded after 96 hours from the initial time of the experiment. The time was fixed because it was demonstrated that the completion of embryogeny occurs within 4 days [53].

Hatching rate was calculated on the basis of non-hatchability of eggs according to [54]. To ensure non-hatchability, the eggs from any test container were collected after 96 hours. Unhatched and decapped eggs were separated and counted using dissection microscope. Five replications were conducted at each concentration of test compound.

The data were statistically examined using Student's *t*- test.

Oviposition bioassay

Fifteen pairs of mosquitoes were kept in a cage and maintained. They were blood fed every alternate day.

The effect of methanolic leaf extract of *S. campanulata* on oviposition of *Ae. aegypti* was determined under two set of conditions as suggested by [55,56,57].

Choice oviposition test

Four egg traps containing any one of the concentrations (0.1, 0.2 and 0.3%) of the test compound and control (unchlorinated water) were placed inside the cage with 15 pairs of blood fed mosquitoes. After 24 hours, the traps were taken out and the eggs present in each were separately counted. The test was replicated 10 times (10 days) for each concentration of test compound.

No-choice oviposition test

Egg trap containing any one of the test concentrations of the test compound was placed at the time in the cage with 15 pairs of mosquitoes, along with a control trap. After 24 hours, the containers were removed and the number of eggs were counted. The trial was repeated 3 times for each concentration.

Oviposition Active Index (OAI) was calculated as detailed by [58] using the formula,

$$OAI = \frac{Nt - NS}{Nt + NS}$$

Where

Nt is the total number of eggs in test solutions and
NS is the total number of eggs in control

This would indicate wheather the effect of the compound on oviposition is positive or negative.

Further, the percentage of oviposition deterrence (oviposition deterrent index of Lundgren, 1975) was determined according to the formula given by [59].

$$\text{Oviposition Deterrent Index (ODI)} = \frac{B-A}{A+B} \times 100$$

Where
A – is the number of eggs laid on treated
B – is the number of eggs laid on control.

The data were statistically examined using Student's *t*- test.

RESULTS AND DISCUSSION

Toxicity of methanolic leaf extract of *S. campanulata* to the developmental stages of *Ae.aegypti*

Bioassay tests were conducted to find out the toxicity of methanolic extract to I, II, III, IV instars and pupae of the mosquitoes of *Ae. aegypti*. The data were subjected to Finney's method of probit analysis. The results expressed in terms of LC_{10}, LC_{30}, LC_{50} and LC_{90} / 24 hours.

LC_{10}, LC_{30}, LC_{50} and LC_{90} / 24 hours values of methanolic leaf extract of *S. campanulata* to I instar larvae was 0.084, 0.125, 0.242 and 0.398 % (24hrs), and this was found to gradually increase with the age of larvae. Pupae showed the highest resistance to the methanolic leaf extract of *S. campanulata* as evident from the relatively higher LC_{10},LC_{30}, LC_{50} and LC_{90}/ 24 hour values 0.240 , 0.533, 0.561 and 0.713 % (Fig.1).

Effect of methanolic leaf extract of *S. campanulata* on hatching of *Ae. aegypti* eggs

Freshly laid eggs obtained from the general stock of mosquitoes were tested for their hatching ability in relation to the different concentrations of methanolic leaf extract of *S. campanulata* . Percent hatch of eggs placed in control medium was 85 % where as in 0.20, 0.30, 0.40 and 0.50 % concentrations it was 55, 35, and 20 %. 0.50 % dose completely arrested hatching eggs (Fig.2). The decrease in hatchability was found to be dose dependent.

Effect of methanolic leaf extract of *S. campanulata* on oviposition of *Ae.aegypti*

For determining the influence of methanolic leaf extract of *Spathodea campanulata* on the ovipositional pattern of *Ae.aegypti*, the mosquitoes were subjected to choice – oviposition test and no – choice oviposition test. The data were substituted under appropriate formulae to calculate oviposition active index (OAI) and oviposition deterrent index (ODI). The results are furnished figures 3 & 4.

Choice oviposition test

Mosquitoes showed more preference towards control ovitrap for oviposition, though, media of different concentrations of methanolic leaf extract of *S. campanulata* were available along with control (Choice oviposition test). The total number of eggs laid in ovitraps containing any concentration of the methanoilc leaf extract of *S. campanulata* was always less than that in the control. Among the total number of eggs laid, 60.54 % was present in control medium when placed along with ovitraps with 0.01, 0.10 and 0.20 % methanolic leaf extract of *S. campanulata* in which appeared 22.40, 11.42 and 5.617 % of eggs respectively. This was also indicated by ODI values (45.97, 68.25 and 83.01). Rate of oviposition in ovitraps with any concentration of test compounds was significantly ($P<0.001$) less than in control.

No - choice oviposition test

The ovipositional deterrence of methanolic leaf extract of *S.campanulata* against *Ae. aegypti* was also confirmed by the results of 'no – choice test' where ovitrap with any one of the concentrations accompanied the control. Percent oviposition in 0.01, 0.10 and 0.20 % of methanolic leaf extract of *S. campanulata* was 21.07, 19.61 and 10.52 % which were significantly ($P<0.001$) less compared to their control counterparts 78.92, 80.38 and 89.47 %, respectively. The data of no – choice oviposition test clearly exhibited interference of methanolic leaf extract of *S.campanulata* on the oviposition preference of mosquitoes.

The results showed that the methanolic leaf extract of *S. campanulata* possesses significant larvicidal properties against *Ae. aegypti*. The findings agree with some of the previous reports.

The leaf extract of *Acalypha indica* with different solvents viz, benzene, chloroform, ethyl acetate and methanol was tested for larvicidal activity against *An.stephensi* and the LC_{50} values/24hrs were observed to be 19.25, 27.76, 23.26 and 15.03ppm respectively [60]; the leaf extract of *Cassia fistula* with different solvents viz, methanol, benzene, acetone was tested for the larvicidal activity against *Ae. aegypti* and the 24hrs LC_{50} of the extract against *Ae. aegypti* were 10.69, 18.27 and 23.95 mg/l respectively [31]; larvicidal efficacy of leaf extract of *Pavonia zeylanica* and *Acacia ferruginea* (Malvaceae)were tested against the late third – instar larvae of *Cx.quinquefaciatus*, and their LC_{50} values were 2214.7 and 5362.6 ppm respectively [61]; 24 hrs exposure to early fourth instar of *Ae. aegypti* with hexane extract of the leaves of *Citrus sinensis* resulted in 50% mortality at 446.84 ppm [34]; [62] reported that at 1 mg/ml the ethanol extract of the leaves of *Lantana camara* caused 84% larval mortality while the methanol extract showed 48% mortality in the fourth instar larvae of *Ae. aegypti* [63]; found that the hexane extract of *Abutlion indicum* leaves caused 100% mortality at 1000 ppm with LC_{50} value of 261.31 ppm against the larvae of *Ae. aegypti* at 24hrs; the LC_{50} values of methanol, benzene, acetone leaf extracts of *Pemphis acidula* against *Cx.quinquefasciatus* and *Ae. aegypti* were 10.81 ppm, 41.07 ppm, 53.22 ppm and 22.10 ppm, 43.99 ppm, 57.66 ppm

respectively [64]; the larvicidal efficacy was determined of benzene, hexane, ethyl acetate, methanol and chloroform leaf extract of *Cardiospermum halicacabum* against *Cx. quinquefasciatus* and *Ae. aegypti*, the LC_{50} values were 174.24, 193.31, 183.36, 150.44, 154.95 ppm and 182.51, 200.02, 192.31, 156.80, 164.54 ppm respectively [65]; the larvicidal activity of hexane, acetone and methanol extracts of the leaves of *Toddalia asiatica* against *Ae. aegypti* and *Cx. quinquefasciatus* was investigated and the LC_{50} values were 133.80, 177.20 and 79.48 and 164.53, 175.28 and 87.87 ppm[66]; acetone leaf extract of *Biophytum sensitivum* displayed the highest larvicidal and pupicidal with LC_{50} values of 21.79 and 13.05 mg/ ml against *Ae. aegypti* [67]; methanolic leaf extract of *Spathodea campanulata* were found most effective with LC_{50} (LC_{90}) values of 1.343(4.026), 1.607(4.207), 1.981(4.699), 2.165(4.852) and 2.432(4.861) I, II, III, IV and pupa of *An. stephensi* respectively [68]; the LC_{50}/LC_{90} values of ethanolic leaf extract of *Ocimum sanctum* against *Anopheles stephensi* larvae ranged from 1.52 ppm to 6.44 ppm and 7.38 ppm to 15.23 ppm respectively [69]; the LC_{50} and LC_{90}/24 h values of methanolic leaf extract of *Delonix elata* against early third instar of *Culex quniquefasciatus* were 124.84 mg /L and 213.88 mg/L respectively [70]; the LC_{50} and LC_{90} values of diethyl ether extracts of *Phyllanthus emblica* leaves against 4 th instar larvae of *Aedes aegypti* and *Culex quinquefasciatus* were 114.77 ppm, 333.50 ppm and 82.65 ppm , 206.65 ppm [71]; methanolic leaf extract of *Areca catechu* exhibited highest larvicidal activity followed by *Nicotana tabacum* and *Piper betle* with LC_{50} and LC_{90} values of 124.28 and 95.75; 236.73 and 98.45; 313.58 and 122.99 ppm against 3 rd instar larvae of *Aedes aegypti* [72]; the LC_{50} values of 34.756 ug / ml ,31.351 ug/ml and 28.577ug/ml were calculated of *Barleria prionitis* leaves against 4 th instar larvae of *Culex tritaeniorhynchus*[38]; the LC_{50} /24 hrs values of methanolic leaf extract of *Artemisia vulgaris* against *Culex quinquefasciatus* was 803.2ppm [39]; the *Nepeta cataria* shows highest mortality ratio at different concentration of methanolic leaf extract with LC_{50} of 0.98 mg/L against 4 th instar larvae of *Anopheles gambiae* [40]; LC_{50} and LC_{90} values of ethanolic leaf extract of *Leucas aspera* aganist 1st , 2 ndinstar larvae and pupa of *Anopheles stephensi* was 4.31, 4.46 and 8.94% and 10.80,11.00 and 17.24% [43]; the LC_{50} and LC_{90} values of methanol leaf extract of *Calotropis procera* aganist *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* third instar larvae in 24 h were 63.24, 81.99, 94.08, and 237.07, 249.43, 251.58 ppm respectively [73]; the LC_{50} values of methanol leaf extract of *Euphorbia hirta* aganist the first to fourth instars larvae and pupae of *Anopheles stephensi* were 137.40, 172.65, 217.81, 269.37 and 332.39 ppm [74]; the petroleum ether crude extract of *Centratherum anthelminticum* leaves exhibited significant larvicidal activity aganist third instar larvae of *Anopheles stephensi* with LC_{50} values of 522.94, 154.21 and 70.51 ppm, respectively after 24, 48 and 72 h [75]; the LC_{50}(LC_{90}) 24 h values of ethanolic leaf extract of *Delonix elata* to 1,2, 3, 4 instars larva of *Aedes aegypti*, were 4.91(8.13),5.16(8.44), 5.95(7.76) and 6.87(11.23)% [76]; the LC_{50} (LC_{90}) 24 h values of methanolic leaf extract of *Alocasia macrorrhiza* from first instar to pupae of *Anopheles stephensi* were 126.55(278.81), 143.19(327.47), 165.10(380.01), 186.13(421.04) and 205.68(456.92) ppm [77]; the LC_{50}/LC_{90} 24 h values of petroleum ether leaf extract of *Aloe vera* against the first to fourth instar larvae of *Aedes aegypti* were 162.74, 201.43, 253.30 and 300.05 ppm and 442.98, 518.86, 563.18 and 612.96 ppm respectively [78]; the LC_{50} 24 h values of acetone leaf extract of *Tagates erecta* against the first instar to pupa of *Aedes aegypti* were 4.15, 4.93, 10.21, 23.22 and 48.17 ppm respectively [79]; the LC_{50}(LC_{90}) 24 h values of methanolic leaf extract of *Artemisia nilagirica* against the first to pupa of *Anopheles stephensi* and *Aedes aegypti* were 272.50(590.07), 311.40(688.81), 361.51(789.34), 442.51(901.59) and 477.23(959.30) ppm and 300.84(646.67), 338.79(726.07), 394.69(805.49), 470.74(892.01) and 542.11(991.29) ppm respectively [80]; the $LC_{50/}$ 24 h values of hexane, chloroform, ethyl acetate, acetone and methanol leaf extract of *Orthosiphon thymiflorus* against third instar larvae of *Anopheles stephensi* were 201.39,178.76, 158.06, 139.22 and 118.74 ppm; *Culex quinquefasciatus* were 228.13, 209.72, 183.35, 163.55 and 149.96 ppm and *Aedes aegypti* were 215.65, 197.91, 175.05, 154.80 and 137.26 ppm respectively [81]; at 24 hrs exposure the *Ocimum tenuiflorum* and *Datura alba* ethyl acetate leaf extract against 4 th instar *Anopheles* larvae the LC_{50} value were 44 mg/L and 46.00 mg/L respectively, similary at 48 hrs LC_{50} values were 33.6 mg/L and 30.25 mg/L [82]; ethanolic extract of *Cadaba indica* leaf had higher mortality with the valeus of LC_{50} 115.70, 96.09, 144.50, and 143.75 ppm and LC_{90} 215.46, 204.98, 233.82 and 260.86 ppm was observed after 24 h exposure against *Ae. aegypti* [83].

S. campanulata leaves have furnished Spathodol, caffeic acid, phenolic acids and flavonoids [84, 46 ,85]. These compounds may jointly (or) independently contribute to larvicidal activity against *Ae. aegypti*. The phytochemicals interfered with proper functioning of mitochondria more specifically at the porton transforming sites [86] and phytochemicals primarily effect the midgut epithelium and secondarily affect the gastric caeca and the malpighian tubules in mosquito larvae [87,88]. The death of treated larvae may be due to the inability of the moulting bodies to swallow sufficient volume of air to split the old cuticle and expand the new one during ecdysis or to a metamorphosis inhibiting effect of the plant extract which is possibly based on the disturbance of the hormonal regulation [89].

The crude methanol and benzene leaf extract of *Cardiospermum halicacabum* exerted 100% reduction of egg hatching at 300 ppm against *Cx.quinquefasciatus* and in *Ae. aegypti* 100% reduction of egg hatching at 400 ppm [65]; methanol and ethyl actate leaf extract of *Andrographis paniculata* exerted 100% reduction of egg hatching at

200 ppm against *Cx.quinquefasciatus* and at 250 ppm against *Ae. aegypti* [90]; aqueous leaf extract of *Calotropis procera* treatment at 1000 ppm *Cx. tritaeniorhynchus* and *Cx. gelidus* eggs resulted in to 100% ovicidal activity [91]; methanol, benzene leaf extract of *Cassia fistula* was tested for ovicidal activity against *Ae. aegypti* and in 120 mg/l of methanol leaf extract 100% reduction was observed where as in benzene leaf extract 100% reduction was observed in 140 mg/l [31]; an acetone extract of *Solanum trilobatum* leaves was evaluated for its ovicidal activity on the *Cx.quinquefasciatus* and *Cx. tritaeniorhynchus*, by exposing eggs ranging concentrations of 50 – 200 ppm of the extract and a 100 ppm of the extract killed all the eggs from both the species [30]; in the laboratory, eggs of *Cx.quinquefasciatus* and *Ae. aegypti* were tested at 1000 ppm concentration ethyl acetate extract of *Swertia chirata* leaves shows 23% egg hatchability [92]; mortality (no egg hatchability) was observed 100 percent with ethyl acetate and methanol extracts of *Andrographis paniculata, Eclipta prostrata and Tagetes erecta* leaves at 998.85 mg/l against *An. subpictus* [93]; at a dose of 82.5 mg/ml the ethanolic leaf extract of *Hyptis suaveolens* completely inhibited *An.gambiae* hatching whereas the aqueous extract could inhibit only 70.42% egg hatching at the same dose [94]; the crude methanol leaf extract of *Ervatamia coronaria* exerted zero hatchability (100% mortality) at 250 ppm, 200 and 150 ppm , for *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* respectively and the curde methanol leaf extract of *Caesalpinia pulcherrima* exerted zero hatchability (100% martality) at 375, 300 and 225 ppm for *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* respectively [95]; hundred percent ovicidal activities were observed at 350 ppm and 450 ppm of methanol, benzene, acetone extract of *Pemphis acidula* leaves [64]; aqueous extract of *Leucas aspera* was found to be ovicidal against *Ae. aegypti*, *An.stephensi* and *Cx.quinquefasciatus* with hatchability values of 39.4 and 21.2; 42.4 and 27.8; 50.6 and 30.2 percent at 500 and 1000 ppm respectively [96]; ovicidal activity with ethyl acetate , aqueous solution, ethanol leaf extract of *Nerium oleander* against *Anopheles stephensi* at 100,150,200,250,and 300 ppm were calculated. With each extract at a concentration of 100 ppm, the percentage of hatchability was very high and nil hatchability was recorded when the concentration of extract was increased to 300 ppm in the case of aqueous and ethanol extract [41]; at 300 ppm of ethanolic leaf extract of *Celosia argentea*, *Anthocephalus cadamba, Gnetum ula, Solena amplexicaulis and Srermacoce hispida* showed 100% ovicidal activity aganist *Anopheles stephensi, Aedes aegypti and Culex tritaeniorhynchus* [97]; percent hatch of eggs placed in control medium was 80% where as in 0.1%, 0.2%,0.4% and 0.6% concentrations of aqueous leaf extract of *Spathodea campanulata* against *Aedes aegypti* was 65, 46, 40 and 2%. 0.8% dose completely arrested hatching eggs [36]. In the case of ovicidal activity, exposure of freshly laid eggs was more effective than that of the older eggs [98].

The methanolic extract treated eggs exhibited an allayed hatchability and this may be due to the action of phytochemicals present in the extract. The extract may inhibit the hatchability of the eggs by interfering with their chorion [99]. Eggs and egg shells treated with plant extracts become damaged probably due to endosmosis. After the initial phase of swelling, eggs become desicated, followed by shrinkage and death of larvae trapped within [100]. It is also evident from the present study on exposure of *Ae. aegypti* eggs to the methanolic leaf extract of *S. campanulata*. The treated eggs contained developed embryos the eclosion of the egg was incomplete [98].

The findings of the present investigation were comparable with other ovicidal studies and revealed that the methanolic *S. campanulata* leaf extracts possesses ovicidal activity against the eggs of *Ae. aegypti.*

Oviposition active index for both ethanolic leaf extract of *Ocimum kilimandscharicum* (OK) and O*cimum suave* (OS) experiments egg lay in a negative side ranged from -1% to -0.19%. It was showed that OS and OK deter ovipoistion in *An. gambiae* [101]; ethanolic extract of *Pongamia pinnata*, *Coleus forskohlii* and *Datura stramonium* leaves reduced egg laying by 97.62%, 77.3%, 100% against *Ae. aegypti* and 59.10%, 39.22%, 82% against *Cx.quinquefasciatus* at higher concentration (0.1%) [102]; the ethanolic leaf extract of *Sloanum trilobatum* was tested under laboratory conditions for oviposition deterrent activities against the adult *An.stephensi* and the concentrations of 0.01, 0.025, 0.05, 0.075 and 0.1% reduced egg laying by females from 18 to 99% [103]; 100% oviposition deterrency was obtained with *Melia azedarach* leaf extract at lg/L against *Ae. aegypti* [104]; the oviposition active index (OAI) value of acetone, ethyl acetate, and methanol extracts of *Aegle marmelos*, *Andrographis lineate* and *Cocculus hirsutus* leaf at 500 ppm against *An. subpictus* were -0.86, -0.87, -0.90, -0.78 and -0.87, -0.86, -0.91, -0.94 and -0.86 respectively and the OAI values revealed that the solvent plant extracts have deterrent effect, and they caused a remarkable negative response resulting an oviposition of very few eggs [105]; ethanolic leaf extract of *Andrographis paniculata* observed against *An. stephensi* and OAI values for the species were -0.28, -0.45, -0.49 and -0.59 for extract concentrations of 29, 35, 41 and 46 ppm respectively [106]; in oviposition deterrent activity, the highest concentration of (0.1%) ethanolic leaf extract of *Vitex negundo* produce 94.2% in *Ae.aegypti*, 96.4% in *An.stephensi* and 99.8% in *Cx. quinquefasciatus* [107]; the highest concentration of (0.1%) acetone, chloroform, hexane, petroleum ether and ethanol extracts of *Annona squamosa* leaves produce oviposition deterrent activity 99.6% against *An. stephensi*, 92.4% against *Cx. quinquefasciatus* and 92.4% against *Ae. aegypti* respectively [108].

The oviposition is one of the most important events in the life cycle of mosquitoes[109]. If oviposition is prevented the mosquito life cycle is disrupted and population growth is reduced. The present oviposition study shows that the methanolic leaf extract of *S. campanulata* act as oviposition deterrent, this indicates that *Ae. aegypti* mosquitoes were acutely sensitive to phytochemical stimuli and respond to the odour of the leaf extract. The strong odour produced by higher concentration of methanolic leaf extract produce maximum effective repellence against oviposition. The mosquitoes are known to select or reject their specific oviposition sites by sensing chemical signals that are detected by sensory receptors on the antenna [110].

The findings of the present investigation revealed that methanolic leaf extract of *S. campanulata* possess remarkable larvicidal, pupicidal, ovicidal and ovipositional activity against the *Aedes aegypti*. The present finding is encouraging as the plant extract seems to be target specific, effective at low dose and easily available and the study is of great importance in formulation of an effective vector control strategy based on environmental friendly alternative(plant origin) insecticides.

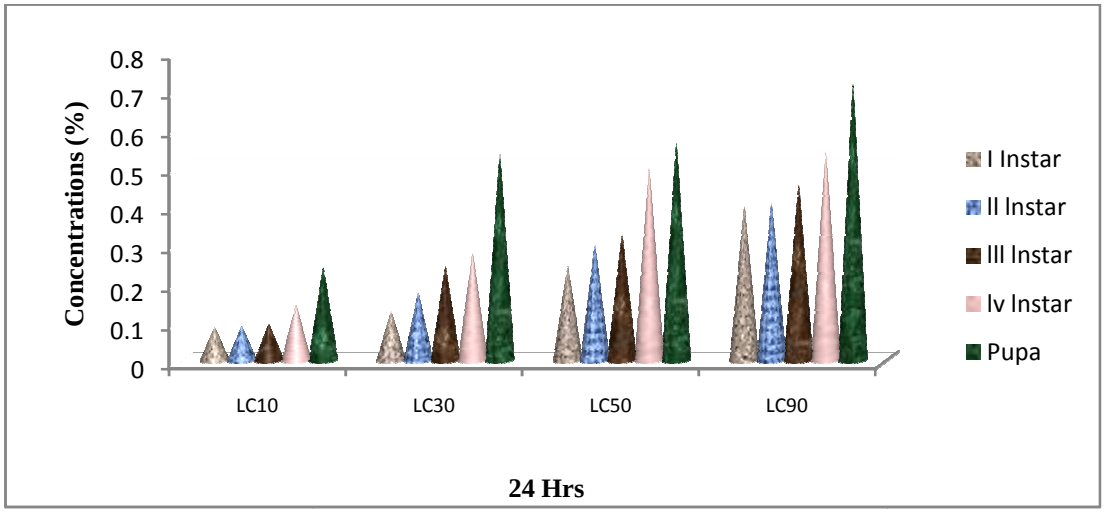

Fig.1. LC_{10}, LC_{30}, LC_{50} and LC_{90} /24 hour values (%) of methanolic leaf extract of *Spathodea campanulata* to different pre adult stages of *Aedes aegypti*

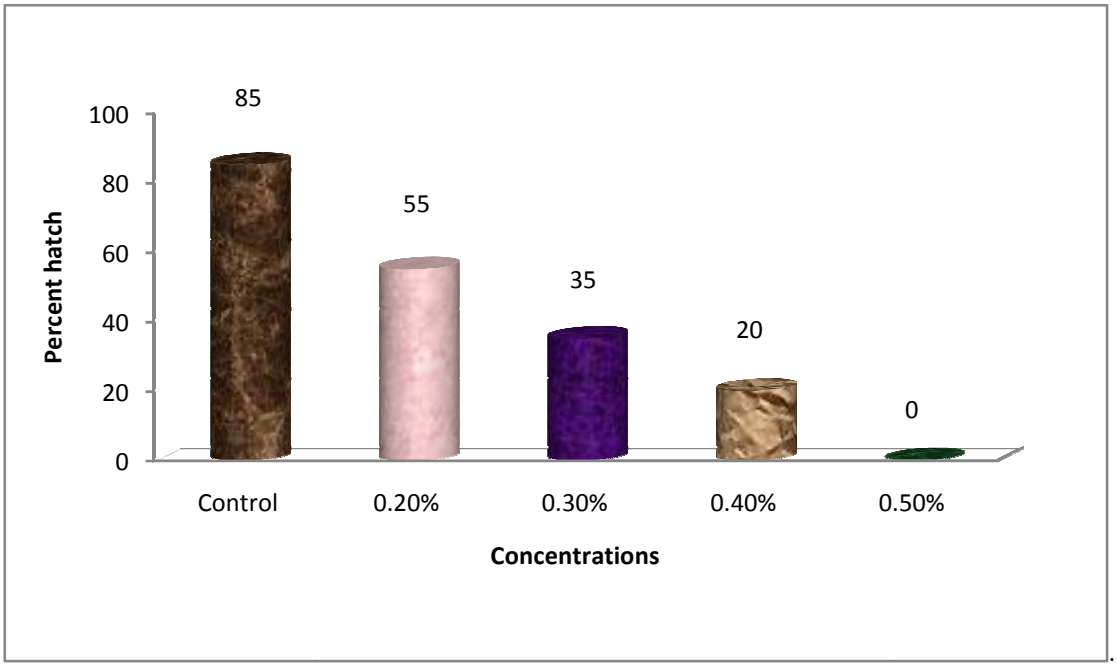

Fig.2. Changes in the hatchability (percent hatch) of *Aedes aegypti* eggs exposed to different concentrations of the methanolic leaf extract of *Spathodea campanulata* and control

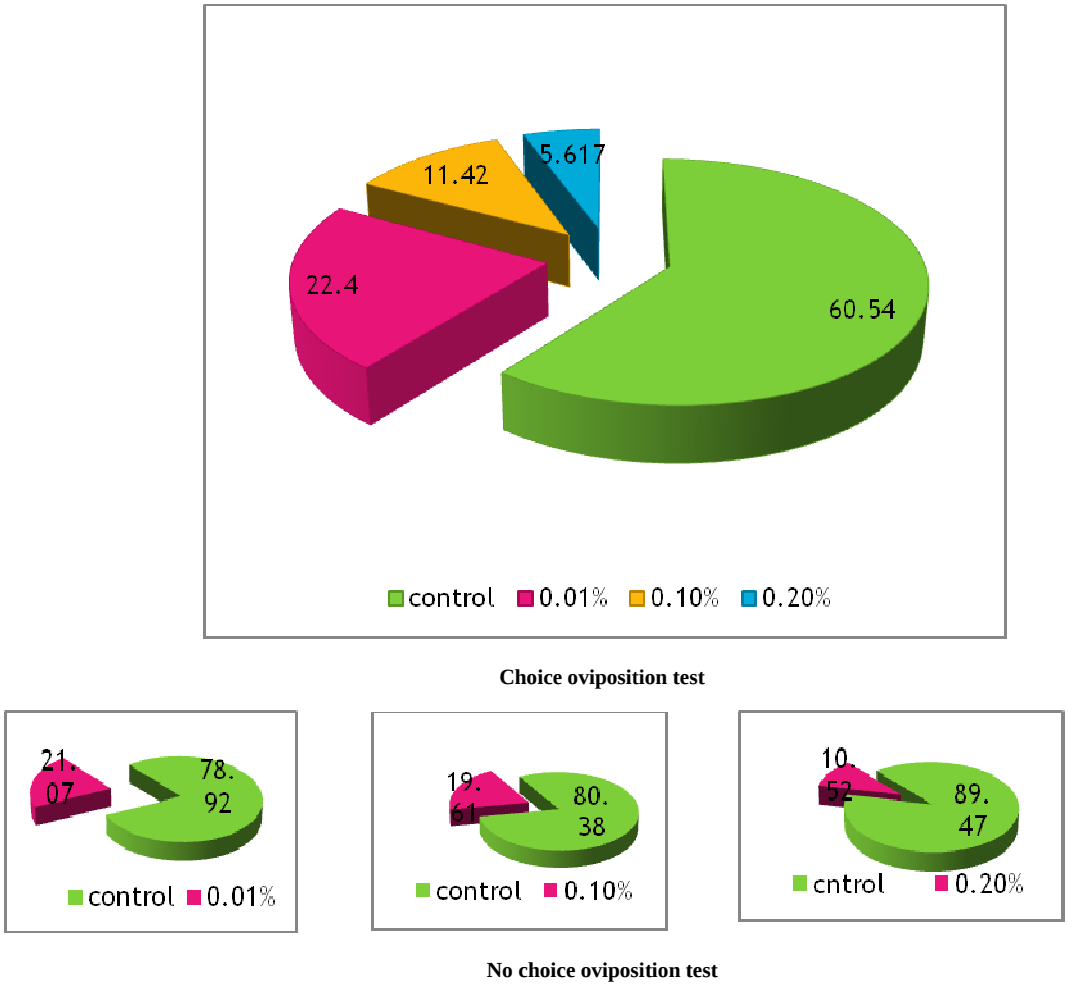

Fig.3. Percentage of eggs oviposited by *Aedes aegypti* in ovitraps contained different concentrations of the methanolic leaf extract of *Spathodea campanulata* under the choice and no – choice oviposition tests

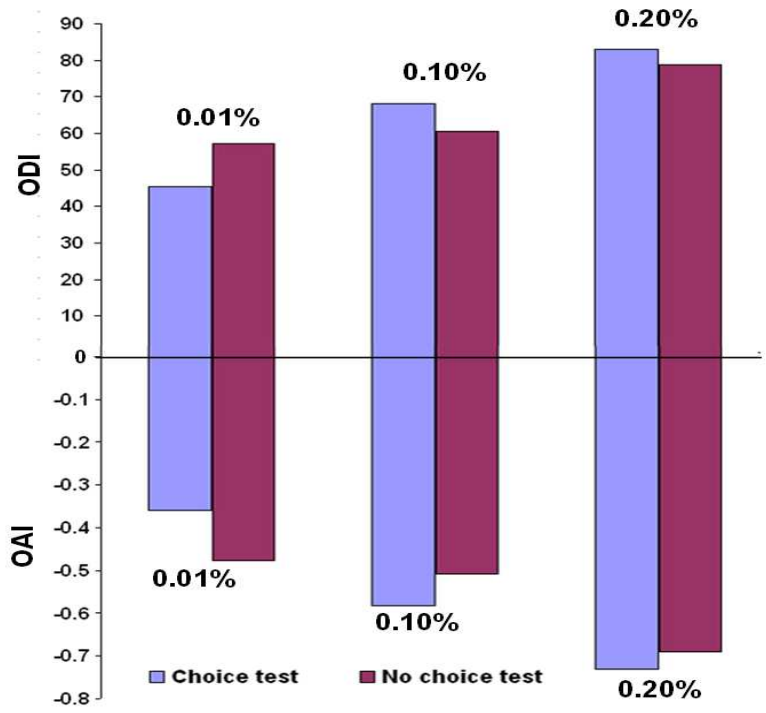

Fig.4. Oviposition deterrent index (ODI) and oviposition active index (OAI) at different concentration (0.01%, 0.10%, 0.20%) of methanolic leaf extract of *Spathodea campanulata* against *Aedes aegypti* under choice and no-choice oviposition tests

REFERENCES

[1]Harrington. LC, Scott. TW, Lerdthusnee. K, Coleman. RC, Coctero.A. Clark. GG, Jones.JJ, Kitthawee. S, Kittqyapong. P, Sithiprasasna. R, Edman. JD, *Am.J. Trop. Med. Hyg.*, **2005** 72: 209-220.
[2]Scott .TW, Chow. E, Strickman. A, Kittqyapong. P, Writz. RA, Lorenz. H, Edman. JD, *J. Med. Ent.*, **1993** 30: 922 – 927.
[3]Park. JE, Park. K, Text book of preventive and social medicine. XI ed. M/s Banarsides Bhanot, India. **1987.** pp:217-221.
[4]Mekuria. Y, Gwinn. TA, Williams. DC, Tidwell. MA, *J. Am. Mosq. Control Assoc.*, **1991** 7:69-72
[5]Mazzarri. MB, Georghiou. P, *J. Am. Mosq. Control Assoc.*, **1995** 11:315-322.
[6]Jupp. PG, Mclntosh. BM, *J.Am.Mosq. Control Assoc.*, **1990** 12:415-419.
[7]Dorland's illustrated Medical Dictionary, 26th Edition W.B. Saunder Company.Igaku-Shoin/Saunders. London. **1982.** p.35.
[8]The Hindu, The Hindu daily. **2001**. 18th October.
[9]The Hindu, The Hindu daily. **2006**.18th October.
[10]National Vector Borne Disease Control Programme (NVBDCP),**2011.** Dengue cases and Deaths in the country since 2007. Available at : http:// nvbdcp.gov.in/ den . cd. html.
[11]The Hindu, The Hindu daily. **2012**. 15th July.
[12]Jaswanth. A, Ramanathan. P, Rukmani. K, *Indian J. Exp. Biol.*, **2002**. 40:363-365.
[13]Sarita. K, Naim.W, Monika. M, Radhika. W, *Front. Physiolo.*, **2012** 3 : 1 -6.
[14]Rutledge. CR, Clarke. F, Curtis. A, Sackett. S, *Tech. Bull. Florida Mosq. Control Assoc.*, **2003** 4 :16-19.
[15]Seleena. P, Lee. HL, Lecadet. MM *J.Am. Mosq. Control Assoc.*, **1995** 13: 471-473.
[16]Mulla. MS, Su. TY, Thavara. U, Tawatsin. A, Nagamsuk .W, Pan Ural. P, *J. Vec. Eco.*, **1999** 24:99-110.
[17]Gluckman. TL, Hartney. KB, *Car. J. Sci.*, **2000** 36:104-111.
[18]Bohidar. K, Mohapatra. S, *Insect Environment*, **2000** 5:173-174.
[19]Laird. M ,Miles. JW, Integrated mosquito control methodologies. Academic Press Inc. (London) Ltd., **1983** London.
[20]Bansal. SK, Singh. KV, Sharma. S, Sherwani. MRK, *J. Environ. Biol.*, **2011** 32 : 71-75.
[21]Devine. GJ, Furlong. MJ, *Agr. Human Values.*, **2007** 24:281-306.
[22]Mittal. PK, Subbarao. SK, *I.C.M.R. Bull.*, **2003** 33:1-12.
[23]Promsiri. S, Naksathit. A, Kruatrachue. M, Thavara. U, *Insect Science.* **2006** 13: 179 - 188.
[24]Ahmed. S. Graivge. M, Hylin. JM, Mitchell. MC, Listinger. JA**,** Some promising plant species for use as pest control agents under traditional farming system. In: Schmutterer. and Ascher K.R.S., (eds) Proc. 2nd International neem conference, Rauischolzhausen, Germany, **1984**. 1983 pp. 565-580 GTZ, Eschborn, Germany.
[25]Sukumar. K, Perich. MJ, Boobar. LR, *J. Am. Mosq. Control. Assoc.*, **1991** 7: 210-237.
[26]Elimam. AM, Elmalik. KH, Ali. FS, *Saudi J. Biol. Sci.*, **2009a** 16 : 95-100.
[27]Elimam.AM, Elmalik. KH, Ali. FS, *Trop. Biomed.*, **2009b**. 26 : 130-139.
[28]Prempree. P, Sukhapanth .N, *J. Sci. Soc. Thailand*, **1990.** 16 : 133 -139.
[29]Singh .SP, Raghavendra. K, Singh. PK, Subbarao. SK, *Current Science*, **2001**. 81 : 1529-1530.
[30]Rajkumar. S, Jebanesan. A, *Int. J. Trop. Ins. Sci.*, **2004** 24: 340-342.
[31]Govindarajan. M, *Eur. Rev. Med. Pharmacol. Sci.*, **2009** 13:99-103.
[32]Okigbo. RN, Okeke. JJ, Madu. NC, *J. Agri. Tech.*, **2010** 6 : 703-719.
[33]Ramanibai. R, Mahavarani. A, Boomilingam. M, *J. Pharma. Res.*, **2011** 4 : 4424 – 4425.
[34]Radhika. W, Ankita. R, Jasdeep. KS, Roopa. S, Naim. W, Sarita. K**,** *Asian Pac. J.Trop.Biomed.*, **2012.** 152 - 155.
[35]Saranya. M, Mohanraj .R S, Dhanakkodi. B, *Int. J. Rec. Sci. Res.*, **2013 a** 4: 28-34.
[36]Saranya. M, Mohanraj .R S, Dhanakkodi. B, . *Euro.J. Exp.Bio.*, **2013b** 3: 203-213.
[37]Al-Mekhlafi. FA, Taha. NA, Mashaily. AMA, Wadaan. MA, *Pakistan J. Zool.*, **2013** 45: 241-246.
[38]Jeyasankar. A, Premalatha. S, Krishnappa. K, Elumalai. K, *Int. J. Interdis. Res. Revi.*, **2013** 01 :116-120.
[39]Ikram Ilahi, Farman Ullah, *Int. J. Innov. Appl. Stu.*, **2013** 2 :189-195.
[40]Adewole. A, Oderinde. A, Bankole. O, Faparusi. F, Oyede. RT, *Acad. J. Med. Plants* **2013** 1:037-040.
[41]Roni . M, Murugan. K, Christina Mary, Sivapriyajothi .S, Sugana. S, Dinesh. NA, Vivek. S, *Int.J. Innovat. Res.*, **2013** 1:12-18.
[42]Shivakumar. MS, Natarajan. D, Srinivasan .R, *Res. J. Biotech.*, **2013** 8:67-70.
[43]Sivapriyajothi .S, Murugan. K, ChristinaMary. S, Roni. M, Suganya. NA, *Int. J. Curr. Trop. Med. Heal. Res.*, **2013** 1: 019-025.
[44]Kowti .R, Harsha. R, Ahmed. MG, Hareesh. AR, Gowda. TSS, Dinesha. R, *Res. J. pharm . Biol & Chem. Sci.*, **2010** 3 : 691 – 698.
[45]Kowti. R, Joshi. V, Dabadi. P, Gowda. TSS, Sathish. BP, Dinesha. R, *Int. J. Curr. Pharm. Res.*, **2011** 3 : 87 - 89.

[46]EI – Hela. AA, *Al – Azar J. pharm. Res.,* **2001**. 27 : 152 – 162.
[47]Lymio. EO, Takken.W, Koella. JC, *Entomol. Exp. Appl.,* **1992** 63: 265-271.
[48]Judson. CL, *Entomol. Exp. Appl.,* **1967** 10: 387-394
[49]Briegel. A, *J. Insect physiol.,* **1990** 36 : 165 - 172.
[50]Villani. F, White. GP, Curtis.CF, Miles. SJ, *Bull. Ent. Res.,* **1983** 73: 153 – 170.
[51]Pampana. E,A text book of malaria eradication. Oxford University Press, London . **1963**. pp:459-462.
[52]Finney. DJ, Probit Analysis. Cambridge university, London. **1971** 68 – 78pp
[53]Judson. CL, Gojrati. HAN, *Entomol. Exp. Appl.,* **1967** 10:181-188.
[54]Sahgal. A, Pillai. MKK, *Entomon,* **1993** 17:149-154.
[55]Beehler. JW, Mulla. MS, *J. Am. Mosq. Control Assoc.,* **1993** 9: 13 -16.
[56]Chen. CC, Dong. YJ, Cheng. LL, Hou. RF, *J. Econ. Entomol.,* **1996** 89 : 462 – 466.
[57]Dhanakkodi. B, Saraswathi. S, Thangavel. K, Mohanraj. RS, Influence of neem based formulations on ovipositional preference of *Aedes aegypti*. In : Biopesticides in insect pest management. S. Ignacimuthu and Aloksen. (Eds). Phoenix Publishing House Pvt. Ltd., New Delhi. **1999**. pp. 68 – 70.
[58]Vasuki .V, *Entomon,* **1991**. 16 : 175 -178.
[59] Dimetry. NZ, Barakat. AA, Abdulla. EF, EI – Metwally. HE, Abd EI – Salam. AME, *Anz., Schadlingskde., Pflanzenschutz, Umweltschut,* **1995** 68 : 39 – 41.
[60]Govindarajan. M, Jebanesan. A, Pushpanathan. T, Samidurai. K, *Parasitol Res.,* **2008** 103 : 691-695.
[61]Vahitha. R, Venkatachalam. MR, Murugan. K, Jebanesan. A, *Bioresour. Technol.,* **2002** 82 : 203-204.
[62]Kumar. MS, Maneemegalai. S, *Adv. Biol. Res.,* **2008** 2:39-48.
[63]Arivoli. S, Samuel. T, *J. biopesticides* . **2011 c** 4 : 27-35.
[64]Samidurai. K, Jebanesan. A, Saravanakumar. A, Govindarajan. M, Puspanathan. T, *Academic J. Entomol.,* **2009** 2 : 63 – 66.
[65]Govindarajan. M, *Eur. Rev. Med. Pharmacol Sci.,* **2011b** 15 : 787-794.
[66]Borah. R, Kalita. MC, Kar. A, Talukdar, AK, *African J. Biotech.,* **2010** 9 : 2527 – 2530.
[67]Shivakumar. MS, Srinivasan. R, Natarajan. D, *Res. J. Pharma. Bio. Che. Sci.,* **2012** 3 : 885-892.
[68]Aarthi. N, Murugan. K, *Journal of phyto.,* **2010** 2 : 61 – 69.
[69]Murugan. K, Madhiyazhagan. P, Nareshkumar. A, Nataraj. T, Dinesh. D, Hwang. JS, Nicoletti. M, *J. Ent.and Acaro. Res.,* **2012** 44: 90-97.
[70]Govindarajan. M, Rajeswary. M, Sivakumar. R, *Asian.Pac. J. Tro.Dis.,* **2012a** s571- s573.
[71]Jeyasankar. A, Premalatha. S, Elumalai. K, *Asian.Pac. J. Tro.Dis.,* **2012** s399-S 403.
[72]Samule. T, Arivoli. S, Raveen. R, Bobby. M, Dhinamala. K, *Int.J. Res. Biolo. Sci.,* **2012b** 2: 157- 160.
[73]Gokulakrishnan. J, Selvakumar. B, Elumalai. K, Krishnappa. K, *Int. J. Ren. Env. Sci.,* **2013** 2: 17-21.
[74]Pannerselvam. C, Murugan. K, Kovendan. K, Kumar. PM, Subramaniam. J, *Asian Pac. J. Trop. Med.,* **2013** 6: 102-109.
[75]Srivastava. A, Bartarya. R, Tonk. S, Srivastava. SS, Maharaj kumara. K, *J. Env. Bio.,* **2008** 29: 669-672.
[76]Vasugi. C, Kamalakannan. S, Murugan. K, , *Asian Pac. J. Trop. Dis.,* **2013** 3: 119-126.
[77]Dura Devi. G, Murugan. K, *Int.J. Innovat. Res.,* **2013** 1: 24-30.
[78]Jayapal. S, Kovendan. K, Mahesh kumar. P, Murugan. K, Walton. W, *Saudi J. Bio. Sci.,* **2012** 19: 503-509.
[79]Rajmohan. D, Logankumar. L, *Asian Pac. J. Trop. Biomed.,* **2012** 1-4.
[80]Pannerselvam. C, Murugan. K, Kovendan.K,Mahes kumar. P, *Parasitol. Res.,* **2012** 111: 2241-2251.
[81]Kovendan. K, Murugan. K, Vincent. S, Barnard.DR, *Asian Pac. J. Trop. Med.* **2012** 5: 299-305.
[82]Mehdi. SH, Qamar. A, Iram khan, Priyanka ., *Euro.J. Exp.Bio.,* **2012** 2: 1370-1375.
[83]Kalimuthu. K, Murugan. K, Pannerselvam. C,Hwang. JS, *Asian J. Plant Sci. Res.,* **2012** 2: 633-637.
[84]Ngouela .S, Tsamo. E, Sondengam. BL, Connolly. JD, *J. Nat. Prod. Washington.,* **1991** 54 : 873-876.
[85]EI – Hela. AA, *Al – Azar J. Pharm. Sc. Cario.,* **2001b** 27 : 115 -120.
[86]Usta. J, Kieydiyyeh. S, Bakajian.K, Nakksah – Chmaisse .H, *Food Cem. Toxicol.* **2002**, 40:935-.
[87]Rey. D, Pautou. MP, Meyran. JC, *J. Invertebrate Pathol.,* **1999** 73 : 173-181.
[88] David. JP, Rey. D, Pautou. MP, Meyran. JC, *J. Invertebrate Pathol.,* **2000** 75 : 9-18.
[89]Al – Sharook. Z, Balan. K, Jiang. J, Rembold. H, *J. Appl. Ent.,* **1991** 111:425-530.
[90]Govindarajan. M, *Asian Pac.J. Trop. Med.,* **2011a** 4 : 176-181.
[91]Kumar. G, Karthik. L, Venkata. BRK, Kirthi. AV, Jayaseelam. G, Rahuman A, *Bangladesh J. Pharma.,* **2012** 7 : 1-5.
[92]Balaraju. K, Maheswaran. R, Agastian. P, Ignacimuthu. S, *Indian J. Sci. and Tech.,* **2009** 2 : 46-49.
[93]Elango. G, Rahuman. AA, Kamaraj. C, Bagavan. A, Zahir. AA, *Parasitology Res.,* **2011b** 108 : 1437-1445
[94]Ivoke. N, Okafor, Chukwuemenam .F, Laura Onyi. O, *Animal Res. Int.,* **2009** 6 : 1072 – 1076.
[95]Govindarajan. M, Mathivanan. T, Elumalai .K, Krishnappa. K, Anandan. A, *Asian. Pac. J. Trop. Biomed.,* **2011d** 43 - 48.
[96]Arivoli. S, Samuel. T, *World Appl. Sci. Journal.,* **2011a** 14 : 565 -568.
[97]Dhanasekaran. S, Krishnappa. K, Anandan. A, Elumalai. K, *J.Agri.Tech.,* **2013** 9 : 29-47.

[98]Miura. T, Schaefer. CH, Takahashi. RM, Mulligan. FS, *J. Econ. Entomol.,* **1976** 69: 655-658.
[99]Rajkumar. S, Jebanesan. A, Nagarajan. R, *Asian Pac. J. Trop. Med .,* **2011** 4 : 948-951.
[100]Arivoli. S, Samuel. T, *Asian J. Exp. Biol. Sci.,* **2011d** 1 : 721 - 730.
[101]Kweka. EJ, Lyatuu. EE, Mboya. MA, Beda. JM, Mahande. AM, *J. Glob. Infect . Dis.,* **2010** 2 : 242-245.
[102]Swathi. S, Muruganathan. G, Ghosh. SK, *Pharmacogn. Mag.,* **2010** 6 : 320 – 324.
[103]Rajkumar. S, Jebanesan. A, *J. Insect. Sci.,* **2005 a**. 5:3.
[104]Coria. C, Almiron. W, Valladares .G, Carpinella. C, Ludena. F, Defago. M, *Bioresour. Technol.,* **2008.** 99:3066-3070.
[105]Elango. G, Bagavan. A, Kamaraj .C, Abdul Zahir. A, Abdul Rahuman. A, *Parasitol Res.,* **2009** 105 : 1567-1576.
[106]Kuppusamy. C, Murugan. K, *Ent. Res.,* **2008** 38 : 119-125.
[107]Vijaya kumar. S, Arunkumar. R, Mani. P, John Bastin. TMM, Ravikumar. G, *J. Pharma. Res.,* **2011a** 4 : 2060-2063.
[108]Vijaya kumar. S, Mani. P, John Bastin. TMM, Arunkumar. R, Ravikumar. G, *Int. J. Pharma. Tech.,* **2011b** 3 : 3143 -3155.
[109]Xue. RD, Barnard. R , Ali. A, *Med. Vetri. Entomol.,* **2001** 15: 126-131.
[110]Davis. EE, Bowen. FM, *J. Am. Mosq. Control Assoc.,* **1994** 10 : 316-325.