



REGULAR ARTICLE

LARVICIDAL AND SMOKE REPELLENT ACTIVITIES OF *SPATHODEA CAMPANULATA* P.BEAUUV. AGAINST THE MALARIAL VECTOR *ANOPHELES STEPHENSI* LIS (DIPTERA: CULICIDAE)

N. Aarthi* and K. Murugan

Department of Zoology, Division of Entomology, School of Life Sciences, Bharathiar University, Coimbatore,
Tamil Nadu, India

SUMMARY

As we talk about diseases certainly we have to say about the vectors and the major disease causing vectors is mosquito. The natural products have been a rich source of medicines since they provide a host of many bioactive compounds with a wide range of applications. *Anopheles stephensi* which transmits plasmodium responsible for malarial fever. Vector control can be achieved by using insecticidal larvicidal organophosphorates or synthetic compounds. The toxicity of the chemical has side effects. More over the constant use of these chemicals will lead to gain the resistance to these mosquitoes. As a result Investigations were made to evaluate the larvicidal activity and smoke repellent potential of *Spathodea campanulata* P.Beauv. (Family: Bignoniaceae) to identify suitable bio active compound in selected medicinal plants. The present paper deals with larvicidal and mosquito repellency activity of *Spathodea campanulata*. The extracts of *Spathodea campanulata* were found most effective with LC50 value of 1.343, 1.607, 1.981, 2.165, 2.432 of I, II, III, IV and pupa respectively. The smoke toxicity was more effective against the *Anopheles stephensi*. Smoke exposed gravid females oviposited fewer eggs when compared to those that were not exposed.

Key words: *Spathodea campanulata*, Larvicidal, *A. stephensi*, Malarial vector

N.Aarthi and K.Murugan. Larvicidal and Smoke Repellent Activities of *Spathodea campanulata* P.Beauv. against the Malarial Vector *Anopheles stephensi* Lis (Diptera: Culicidae). J Phytol 2/8 (2010) 61-69.

*Corresponding Author, Email: goyahred@gmail.com

1. Introduction

Mosquito-transmitted disease continues to be a major source of illness and death. In India, malaria is one of the most important causes of direct or indirect infant, child, and adult mortality. About 2 million confirmed malaria cases and 1,000 deaths are reported annually, although 15 million cases and 20,000 deaths are estimated by WHO South East Asia Regional Office. India contributes 77% of the total malaria in Southeast Asia (Kumar et al., 2007). Mosquitoes are also becoming increasingly resistant to traditional chemical pesticides and there is growing concern about the potential health and environmental risks surrounding these products. Environmental protection agencies have banned or placed severe restrictions on

the use of many pesticides which were formerly used in mosquito control programmes and there are now fewer adulticides available than there have been for the last 20 years (Carvalho et al. 2008). More over the constant use of these chemicals will lead to gain the resistance to these mosquitoes, undesirable effects on non-target organisms and fostered environmental and human health concern (Hayes and Laws 1991), which initiated a search for alternative control measures. Plants are considered as a rich source of bioactive chemicals (Wink 1993) and they may be an alternative source of mosquito control agents. Natural products of plant origin with insecticidal properties have been tried in the recent past for control of

variety of insect pests and vectors. Phytochemicals derived from plant sources can act as larvicide, insect growth regulators, and repellent and ovipositor attractant and have different activities observed by many researchers (Babu and Murugan 1998, Venketachalam and Jebasan 2001, and Venketachalam and Jebasan 2001). However, insecticides of plant origin have been extensively used on agricultural pests and to a very limited extent, against insect vectors of public health importance.

S.campanulata P. Beauv is extensively used in Indian traditional and folklore medicines to cure various human ailments. The Siddha/Tamil name of this species is Patadi and in folk it is popularly called as Ruugatuuraa. It is very commonly found and planted in the gardens, South Tamilnadu. In English the species is called as Syringe tree, Fountain tree, African tulip tree, Flame-of-the-forest or Nandi Flame. It is a medium-size tree (15-25 m high), characterized by red garish flowers. It is often employed in gardening in tropical and subtropical areas including South America (Joly, 1985). The stem bark preparations are employed against enemas, fungus skin diseases, herpes, stomachaches, diarrhea (Jardim *et al.*, 2003). Several phytochemicals studies were performed with different parts of *S.campanulata*, including stem barks, leaves, flowers and fruits (Ngouela *et al.*, 1990; Amusan *et al.*, 1996). The leaves have furnished spathodol, caffeic acid, other phenolic acids and flavonoids (Ngouela *et al.*, 1991; El-Hela, 2001a; El-Hela, 2001b). Banerjee and DE (2001) showed the presence of anthocyanins in flowers of *S. campanulata*. In India, Ayurvedic system of medicine has existed for over four thousand years. From ancient literature it is evidence that the various parts of the plants were used in Siddha, Ayurvedha and Unani medicine for the treatment of disease of human beings and animals (Palaniswamy *et al.*, 2008). There is no literature that the plant is used as mosquitocidal or used for the control of vectors and hence the present study was an attempt to find new larvicidal and repellent products from the extracts of plants to control the malarial vector *A. stephensi*.

2. Materials and Methods

Colonization of *Anopheles stephensi*

Collection of eggs

The eggs of *A.stephensi* were collected from National Institute for Communicable Diseases (NICD), Mettupalayam, Coimbatore, Tamil Nadu, India without expose to any insecticide and in and around Coimbatore, India at different breeding habitats with the help of a 'O' type brush. The eggs were then brought to the laboratory and transferred to 18 x 13 x 4 cm size enamel trays containing 500 ml water and kept for larval hatching. They were hatched and reared have been still maintained from many generations in the laboratory. The eggs and larvae obtained from this stock were used for different experiments.

Maintenance of larvae

The freshly hatched larvae were fed with dog biscuits and yeast at 3:1 ratio. The feeding was continued till the larvae transformed into the pupae stage. The larvae reared in plastic cups. 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 500 ml of water with help of a sucker. The pupae containing glass beaker were kept in 90 x 90 x 90 cm size mosquito cage for adult emergence. The cage was made up of wooden frames and covered with polythene sheets on four sides (two laterals, one back and other one upper) and the front part was covered with a muslin cloth. The bottom of the cage was fitted with strong cardboard. The freshly emerged adults were maintained 27.2°C, 75 - 85% RH, under 14L: 10D photoperiod cycles. The adults were fed with 10% sugar solution for a period of three days before they were provided an animal for blood feeding.

Blood feeding of adult *Anopheles stephensi* and egg laying

The females were fed by hand every alternate day at 6.00 p.m. feeding mosquitoes on human arm for experimental purposes was suggested by Judson (1967) and Briegel (1990). Both females and males were provided with 10% glucose solution as described by Villani *et al.* (1983) on cotton wicks. The cotton was always kept moist with the solution and changed every day. Theoder and Parsons (1945) noticed that glucose as well as ordinary sugar appeared equally attractive to the mosquitoes. An egg trap (cup) lined with filter paper containing pure water was always placed at a corner of the cage. This arranged made collection of eggs easier.

Collection of plant materials

S.campanulata P.Beauv. (Family: Bignoniaceae) was collected from Siruvani hills, Western Ghats, Southern India, Coimbatore. The plants were authenticated at BSI (Botanical Survey of India) and the specimens were deposited at Zoology Department, Bharathiar University, and Coimbatore-641 046, India.

Preparation of plant extracts

S. campanulata leaves were washed with tap water and shade dried at room temperature. The dried plant materials were powdered by an electrical blender. From the powder 200g of the plant material were extracted with 2.5 liters of organic solvents (Methanol) for 8 hrs in a soxhlet apparatus (Vogel, 1978). The crude plant extracts were evaporated to dryness in rotary vacuum evaporator.

Preparation of required plant extracts concentration

One gram of the plant residue was dissolved in 100 ml of acetone (stock solution) considered as 1% stock solution. From this stock solution concentrations were prepared ranging from 2% to 10%.

Determination of median lethal concentration

LC50 (lethal concentration 50%) is the concentration of any toxic substance reducing by mortality the number of tested individuals to 50% in a prefixed time (Ravera, 1986). According to Rand and petrocelli (1985) the LC50 (median lethal concentration) is estimated to produce mortality in 50% of a test solution over a specific period of time. Preliminary toxicity tests were carried out to find the median lethal tolerance limit of *A. stephensi* larvae to *S.campanulata* for 24h. Determining LC50 concentration separate glass beakers of 500 ml of water capacity were taken. Then, different concentrations of *S.campanulata* were added to different glass beakers. Then, 25 *A. stephensi* larvae were introduced into each glass beaker. A control beaker with 500 ml of water and 25 larvae were also maintained. The mortality/survival of larvae in the treatment beakers was recorded after every 24 h. the concentration at which 50% mortality of larvae occurred after 24h was taken as the medium lethal concentration (LC50) for 24 h. The LC50 concentration for 24 h. was calculated by the probit analysis method of Finney (1971).

Test for larvicidal and pupicidal activity (WHO, 1996)

A laboratory colony of *A.stephensi* larvae were used for the larvicidal activity. Hundred numbers of first, second, third and fourth instars larvae were kept in 500 ml glass beaker containing 249 ml of dechlorinated water and 1ml of desired concentration of plant extracts were added. Larval food was given for the test larvae. In each concentration five replicates were tested. The control was set up by mixing 1ml of acetone with 249 ml of dechlorinated water. The control mortalities were corrected by using Abbott's formula (Abbott's, 1925).

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatme nt} - \text{Observed mortality in control}}{100 - \text{Control mortality}} \times 100$$

$$\text{Percentage mortality} = \frac{\text{Number of dead pupae}}{\text{Number of pupae introduced}} \times 100$$

The values of LC50, LC90 and their 95% confidence limit of upper confidence limit(UCL) and lower confidence limit(LCL) , regression and chi-square values were calculated by using probit analysis (finney,1971).the levels of significance by Duncan's Multiple Range Test (Duncan,1963).

Pupal toxicity test

A laboratory colony of *A.stephensi* pupae were used for pupicidal activity. Twenty five numbers of freshly emerged pupae were kept in 500 ml glass beaker containing 249 ml of dechlorinated water and 1 ml of desired concentrations of plant extract was added. Five replicates were set up for each concentration and control was setup by mixing 1ml of acetone with 249 ml of dechlorinated water. The control mortality was corrected by Abbott's formula (Abbott's, 1925).

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{Control mortality}} \times 100$$

$$\text{Percentage mortality} = \frac{\text{Number of dead pupae}}{\text{Number of pupae introduced}} \times 100$$

The values of LC50, LC90 and their 95% confidence limit of upper confidence limit(UCL) and lower confidence limit(LCL), regression and chi-square values were calculated by using probit analysis (finney,1971).the levels of significance by Duncan's Multiple Range Test (Duncan,1963).

Smoke toxicity test

S. campanulata leaves were used for smoke toxicity assay. The mosquito coils were prepared by following the method of Saini *et al.* (1986) with minor modifications by using 2.5 gram of plant ingredients, 4 grams of coconut shell and charcoal powder as burning material. These ingredients were thoroughly mixed with distilled water to form a semisolid paste. A Mosquito coil (0.6 cm thickness) was prepared manually and shade dried. The control coils will be prepared by without the plant ingredient.

The experiments were conducted in glass chamber measuring 140 X 120 X 60 cm. A window measuring 60 X 30 cm was situated at mid bottom of one side of the chamber.

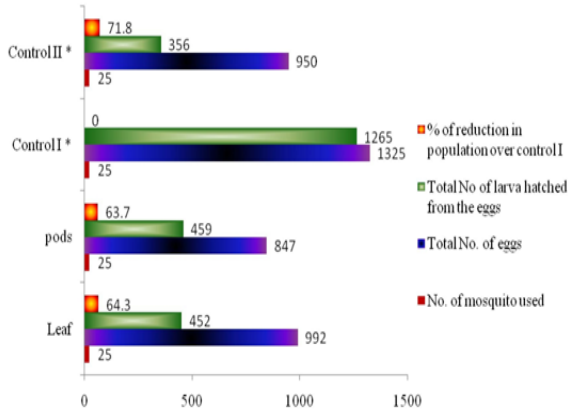
One hundred three or four day's old blood-starved adult female mosquitoes were released into the chamber and were provided with 10% sucrose solution. A belly shaven pigeon was kept tied inside the cage in immobilized condition. The experimental chamber was tightly closed. The experiment was repeated five times on separate days including control groups using mosquitoes of same age. The data were pooled and average values were subsequently used for calculations. Control was maintained in two sets. One set was run with coil lacking the active ingredient of plant powder (control 1) another one was a commercial coil (control 2), which was used for positive control to compare the effectiveness of plant coils. After the experiment was over, the fed, unfed (active and dead) mosquitoes were counted. The protection given by the smoke from plant samples against the biting of *A. stephensi* was calculated in terms of percentage of unfed mosquitoes due to treatment. Data were analyzed using analysis of variance (ANOVA) and means separated by Duncan's multiple range tests.

$$= \frac{\text{Number of unfed mosquitoes in treatment} - \text{Number of unfed mosquito in control 1}}{\text{Number of mosquitoes treated}} \times 100$$

The live blood fed mosquitoes were reared in a mosquito cage, measuring 30 x 30 x 15 cm. The top and bottom of the cage were fit with glass and all other sides were covered with muslin cloth. Water soaked raisins and a 5% sucrose solution soaked in cotton balls were provided as a food source. Water containing powdered yeast and dog biscuits were also kept inside the cage in a glass bowl for oviposition. The eggs from the cage were collected daily till all the mosquitoes died. A total 50- 100 eggs were allowed to hatch in plastic trays measuring 30 x 25 x 6 cm, containing about 2.5 liters of unchlorinated tap water. Hatched larvae's were fed with a mixture of dog biscuits and yeast powder in the ratio of 2:1 and water in the tray was changed daily. Survival and dead instars were counted and reduction in the population from the smoke treated mosquitoes was calculated using the formula.

$$\text{Population reduction (\%)} = \frac{\text{Number of larvae hatched in control 1} - \text{Number of larvae hatched in treated}}{\text{Number of larvae hatched in control 1}} \times 100$$

Fig. 1: Smoke toxicity effect of leaves of *S. campanulata* on reproduction and survival of *Anopheles stephensi*



Statistical analysis

All data were subjected to analysis of variance (ANOVA), Completely Randomised Design (CRD) and the means were separated

using Duncan’s multiple range test (DMRT) (Alder and Rossler, 1977).

3. Results

Larvicidal and pupicidal activity of ethanol extract of *S.campanulata* leaf extract (SCLE) at various concentrations against malarial vector, *A.stephensi* is given in the Table 1. Considerable mortality was evident after the treatment of SCLE for all larval instars and pupae. Mortality was increased as the concentration increased, for example, 37% mortality was noted at I instar larvae by the treatment of SCLE at 0.5% whereas it has been increased to 73% at 2.5% of SCLE treatment. Similar trend has been noted for all the larval instars and pupae of *A.stephensi* at different concentrations of SCLE treatment. The LC50 and LC90 values were represented as follows: LC50 value of I instar was 1.343%, II instar was 1.607%, III instar was 1.981%, IV instar was 2.165 %, and pupa was 2.432%, respectively. LC90 value of I instar was 4.026%, II instar was 4.207%, III instar was 4.699%, IV instar was 4.852. % and pupa was 4.816%,respectively.

Table 1: Insecticidal activity of *S. campanulata* extract against different instars and pupae of malarial vector *Anopheles stephensi*

Larval instar And pupa	Larval mortality (%)					LC 50 & (LC90)	Regression Equation	95% Confidence limit		Chi-Square value
	Concentration of S.C. (%)							LCL	UCL	
	0.5	1	1.5	2	2.5			LC50(LC90)	LC50(LC90)	
I	37 ^d	42 ^e	50 ^e	62 ^e	73 ^d	1.343(4.026)	Y=- 0.641+0.478X	1.070(3.353)	1.580(5.352)	0.962
II	31 ^c	36 ^d	48 ^{de}	57 ^{de}	68 ^{cd}	1.607(4.207)	Y= - 0.792+0.493X	1.375(3.513)	1.863(5.549)	0.422
III	25 ^b	31 ^c	40 ^c	51 ^c	60 ^b	1.981(4.699)	Y= - 0.945+0.477X	1.735(3.848)	2.344(6.312)	0.154
IV	22 ^b	28 ^{bc}	37 ^{bc}	48 ^c	56 ^a	2.165(4.852)	Y= - 1.033+0.477X	1.898(3.983)	2.596(6.604)	0.136
pupa	16 ^a	21 ^a	30 ^a	41 ^a	52 ^a	2.432(4.816)	Y= - 1.308+0.538X	2.152(4.018)	2.900(6.332)	0.198

Chi square value Significant at P <0.05 level

Within a column means followed by the same letter (s) are not significantly different at 5% level by DMRT

Table 2: Smoke toxicity effect of leaves of *S. campanulata* against biting activity of *Anopheles stephensi*

<i>S.campanulata</i> parts used in grams	No. of mosquito tested	Fed mosquito	Unfed mosquito		Total	% unfed over control I
			Alive	Dead		
Leaf 2 G	100	20 ^{bc}	36 ^c	44 ^b	80 ^b	60 ^a
Pods 2 G	100	25 ^d	40 ^{cd}	35 ^d	75 ^c	55 ^c
Control I *	100	80 ^a	20 ^e	0 ^e	20 ^d	0 ^e
Control II *	100	10 ^e	43 ^d	47 ^a	90 ^a	70 ^a

Within a column means followed by the same letter (s) are not significantly different at 5% level by DMRT

Control I *: Negative control - blank without plant material

Control II *: Positive control - mortein coil

Table 3: Smoke toxicity effect of leaves of *S. campanulata* on reproduction and survival of *Anopheles stephensi*

<i>S.campanulata</i> parts used	No. of mosquito used	Total No. of eggs	Total No of larva hatched from the eggs	% of reduction in population over control I
Leaf	25	992 ^e	452 ^e	64.3 ^b
Pods	25	847 ^c	459 ^c	63.7 ^a
Control I *	25	1325 ^a	1265 ^a	-
Control II *	25	950 ^d	356 ^c	71.8 ^c

Within a column means followed by the same letter (s) are not significantly different at 5% level by DMRT

Control I *: Negative control - blank without plant material

Control II *: Positive control - mortein coil

Table 2 provides the results of smoke toxicity effect of *S. campanulata* leaf on *A. stephensi*. Two gram of plant ingredients from the plant leaf and pods was used for the smoke toxicity. The control was maintained without plant ingredients. It acts as negative control. The commercially available (Morstein) mosquito coil used as positive control. One hundred 3-4 days starved *A. stephensi* larvae were used. After the individual treatment of each plant, the fed and unfed mosquitoes were counted. There were 20 fed and 80 unfed mosquitoes counted after the treatment of *S. campanulata* leaf was counted. The comparisons of positive control with the plant product showed very high efficacy, but the plant products alone showed good smoke toxicity effect on *A. stephensi*.

Table 3 and fig 1 shows the result of smoke toxicity effect of different parts of (leaves and pods) *S. campanulata* ensured

population of *A. stephensi*. The numbers of eggs laid by the alive, fed females were shown. Number of eggs laid and the hatchability were greatly reduced or affected by the exposure of smoke from *S. campanulata*. The percentage reduction of hatchability by the smoke from leaves showed 64.3 % and from the pods showed 63.7%. The leaves showed a significant effect on the fecundity and hatchability.

4. Discussion

Today, the environmental safety 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) but it should prevent the breeding. Phytochemicals may serve as suitable alternatives to synthetic insecticides in future as they are relatively safe, inexpensive, and

are readily available throughout of the world. Protection against mosquito bites can be achieved by avoiding infested habitats, by wearing protective clothing, and by applying repellent (Fradin 2001). At present, the main threat to effective mosquito control is resistance to insecticide in the mosquito (Chandra et al. 1998). Botanical insecticides provide an alternative to synthetic insecticides because they are generally considered safe, are biodegradable, and can often be obtained from local sources (Prabhakar and Jabanesan 2004). In addition, the use of medicinal plants for mosquito control is likely to generate local employment, reduce dependence on expensive imported products, and stimulate efforts to enhance public health (Bowers et al. 1995).

In the present study, we sought to determine whether an ethanol extract from *S. campanulata* could be used for mosquito control. We observed a functional response by all immature life stages of *A. stephensi* to the ethanolic extract of leaves of this plant species. This biological activity is attributed to the compounds in the leaves, including flavonoids, phenols, and steroids that together or independently produce morbidity and mortality effects in *A. stephensi*. In the previous study Babu and Murugan, (2000) investigated that the larvicidal effect of resinous exudates from the tender leaves of *Azadirachta indica*. Crude extract of leaves of *solanum nigrum* in water showed larvicidal activity against *A. culicifacies*, *C. quinquefasciatus* and *A. aegypti* at a dose equivalent to LC₉₀ ranging between 0.18 and 0.21 % (Singh et al., 2002). Sujatha et al., 1988 observed differential susceptibilities of larva of three mosquito species to petroleum ether extract of *Acorus calamus* L. *citrus medica*. In the present study also the *S. campanulata* ethanol extract showed larvicidal activity against the malarial vector *A. stephensi* at a dose equivalent to LC₅₀ ranging between 1.343, 1.607, 1.981, 2.165 and 2.432 % for I, II, III, IV instar larvae and pupa respectively and previous literature of smoke toxicity Smoke emerged from *Albizia amara* and *Ocimum basilicum* considerably affect the mosquito survival and pronounced

high repellent potential (Murugan et al. 2007) and in the study of (Aarthi and Murugan, 2010) also the smoke emitted from the *Vetiveria zizanioides* also showed a good knock down effect. In the present smoke repellent test or study also the plant showed a good smoke repellency activity. The smoke exposed females also laid only limited number of eggs, in that also only a limited hatched out. This reduces the mosquito population also. The result of this study indicate that *S.campanulata* leaf extracts enhance the larvicidal and pupicidal activity, the leaves and pods enhances in the smoke repellency test it may be an effective alternative to conventional synthetic insecticides for the control of *A. stephensi*.

5. Conclusion

These approaches are providing important evidence for the potentiality of botanicals in public health integrated management and hence the plant can be used in the vector control for reducing many vector borne diseases such as in the malaria transmission.

Acknowledgement

The authors are thankful to Dr. K. Sasikala, Professor and Head, Department of Zoology, Bharathiar University, Coimbatore, India for providing the laboratory facilities. The financial help given by University Grants Commission, New Delhi is also duly acknowledged.

References

- Aarthi, N., Murugan, K. 2010. Larvicidal and repellent activity of *Vetiveria zizanioides* L, *Ocimum basilicum* Linn and the microbial pesticide spinosad against malarial vector, *Anopheles stephensi* Liston (Insecta: Diptera: Culicidae). *J of Biopes.*, 3: 199-204.
- Abbott W.S. 1925. A method of computing the effectiveness of insecticides. *J. Econ. Entomol.* **18**: 265 - 267.
- Alder, H.L., Rossler, E.B. 1977. Introduction to probability and Statistics (Sixth edition), W.H. Freeman Company, San Francisco, pp. 246.
- Amusan, O. O. G., Adesogan, E. K., Makinde, J. M. Antimalarial active

- principles of *Spathodea campanulata* stem bark. *Phy Research*, London.
- Babu R, Murugan K. Interactive effect of neem seed kerna and neem gum extract on the control of *Culex quinquefasciatus* Say. *Neem Newsletter* 1998; 15(2): 9-11.
- Babu, R., Murugan, K., 2000. Larvicidal effect of resinous exudate from the tender leaves of *Azadirachta indica*. *Neem Newsletter*, 17 (1).
- Banerjee, A., DE, B., 2001. Anthocyanins in some flowers of West Bengal. *J Med Aro Plant Sc*, Lucknow, v.23, p.600-604.
- Bowers, WS, Sener, B, Evans, PH, Bingol, F, Endoganl, GH. Activity of Turkish medicinal plants against mosquitoes *Aedes aegypti* and *Anopheles gambiae*. *Insect Sci Appl* 1995; 16:339- 342.
- Briegel, H., 1985. Mosquito reproduction: incomplete utilization of the blood meal protein of oogenesis. *J. Insect physiol.*, **31**: 15 - 21.
- Carvalho, A.F.U., Melo, V.M.M., Craveiro, A.A., Machado, M.I.L., Bantim, M.B., E. F. Rabelo., 2008. Larvicidal activity of the essential oil from *Lippia sidoides* Cham. against *Aedes aegypti* L. *Mem. Inst. Oswaldo Cruz* 98: 569-571. (s)
- Chandra, F, Darriet, F, Darder, M, Cuany, A, et al. Pyrethroid resistance in *Culex quinquefasciatus* from West Africa. *Med Vet Entomol* 1998; 12:359-366.
- Duncan, J., 1963. Post-treatment effects of sublethal doses of dieldrin on the mosquito *Aedes aegypti*. *Ann. App. Biol.*, **52**: 1-6.
- El-Hela, A. A., 2001a. Phenolics from *Spathodea campanulata* Beauv. Leaves. *Al-Azhar J Pharm Sc*, Cairo, v.27, p.152-162.
- El-Hela, A. A., 2001b. A new iridoid glucoside from *Spathodea campanulata* Beauv. leaves. *Al-Azhar J Pharm Sc*, Cairo, v.27, p.115-120.
- Finney, D.J., 1971. Probit analysis. Cambridge University, London. pp. 68 - 78.
- Fradin, MS. Protection from blood-feeding arthropods. In: Auerbach, P, ed. *Wilderness Medicine*. St. Louis: Mosby, 2001:754-768.
- Hayes, J. B., Jr, Laws, E.R, Jr., 1991. *Handbook of pesticide toxicology*, Vol 1. San Diego, CA: Academic Press.
- Jardim, M. A., Mendonça, L. F. R., Ferreira, M.M.D., 2003. *Os produtos naturais para o desenvolvimento sustentável biotecnológico*. Disponível em: <http://www.mdic.gov.br/tecnologia/revistas/artigos/200112pa/mariojardim.pdf>. Acesso em: 20 dez.
- Joly, A. B., 1985. *Introdução à taxonomia vegetal*. 7. ed. São Paulo: Ed. Nacional, 503p.
- Judson, C.L., 1967. Alternation of feeding behavior ad fertility in *Aedes aegypti* by the chemosterilant apholate. *Entomol. Exp. Appl.*, **10**: 387 - 394.
- Kabaru, J.M., Gichia, L., 2001. Insecticidal activity of extracts derived from different parts of the mangrove tree *Rhizophora mucronata* (Rhizophoraceae) Lam. against three arthropods. *African J Sc Tech*, 2(2): 44-49.
- Kumar, A., Valecha, N., Jain, T., Aditya ,P. Dash., 2007. Burden of Malaria in India: Retrospective and Prospective View. *Am. J. Trop. Med. Hyg.*, 77(6): 69-78.
- Murugan, K., Murugan, P., Noortheen, A., 2007. Larvicidal and Repellent Potential of *Alhizzia amara* Boivin and *Ocimum basilicum* Linn against dengue vector, *Ades aegypti* (Insecta:Diptera:Culicidae). *Biores technol.* 98:198-201.
- Ngouela, S., Nyasse, B., Tsamo, E., Sondengam, B. L., Connolly, J. D., 1990. Spathodic acid: a triterpene acid from the stem bark of *Spathodea campanulata*. *Phytochemistry*, Oxford, v.29, n.12, p.3959-3961.
- Ngouela, S., Tsamo, E., Sondengam. B. L., Connolly, J. D., 1991. Spathodol, a new polyhydroxysterol from the leaves of *Spathodea campanulata*. *J Nat Prod*, Washington, v.54, n.3, p.873-876.
- Palaniswamy, M., Dhanabalan, R., Doss, A., Jagadeeswari, M., Karthic, R., Angayarkanni, R., 2008. Preliminary Phytochemical Screening and Antimalarial Studies of *Spathodea campanulatum* P. Beauv Leaf Extracts. *Ethno Leaflets* 12: 811-19.
- Prabhakar, K, Jabanesan A. Mosquitocidal effect of bitter gourd leaf extracts. *Bioresour Technol* 2004; 95:113-114.

- Rand, G.M. and Petrocelli, S.R. (1985). In: 'Fundamentals of aquatic toxicology. Methods and application (Eds.) Hemisphere publishing corporation, Washington, USA, pp.28.
- Ravera, O., 1986. Cadmium in freshwater ecosystems. In: "Cadmium in the Environment". Vol.50. Mislin, H. and Ravera, O. (Eds.). Birkhauser Verlag, Basel Switzerland. Pp.75-87.
- Saini, H.K., Sharma, R.M., Bami, H.L., Sidhu, K.S., 1986. Preliminary study on constituents of mosquito coil smoke. *Pesticides*. 20, 15-18.
- Singh, S.P., Ragavendhira, K., Singh, R.K., Subbarao, S.K., 2002. Studies on larvicidal prosperities of leaf extract of *solanum nigrum* Linn (Family: Solanaceae). *Curr Sci*, **81**:1529.
- Sujatha, C.H., Vasuki, V., Mariappan, T., Kalyanasundaram., Das, P.K., 1988. Evaluation of plant extracts for biological activity against mosquitoes. *Inyl. Pest Control*. 20: 122-124.
- Theoder, M.O.H., Parsons, B.T., 1945. Insectory rearing of *Anopheles pharoensis*. *Bull. Ent. Res.*, 36: 79 - 83.
- Venketachalam MR, Jebasan A. Repellent activity of *Ferronia elephantum* Corr. (Rutaceae) leaf extract against *Aedes aegypti*. *Biores Technol* 2001; 76(3): 287-8.
- Venketachalam MR, Jebasan A. Larvicidal activity of *Hydrocotyl javanica* Thunb (Apiaceae) extract against *Cx. quinquefasciatus*. *J Exptl Zool India* 2001; 4(1): 99-101.
- Villani, F., White, G.B., Curtis, C.F., Miles, S.J., 1983. Inheritance and activity of some estrases associated with organophosphate resistance in mosquitoes of the complex of *Culex pipiens* L. (Diptera: Culicidae). *Bull. Ent. Res.*, 73: 153 - 170.
- Vogel., 1978. Textbook of Practical Organic Chemistry. The English Language BookSociety and Longman. London, pp.1368.
- Wink M. Production and application of phytochemicals from an agricultural perspective. In: Van Beek TA, Breteler H, editors. *Phytochemistry and agriculture*. Oxford, UK: Clarendon Press 1993; p. 171-213.