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European Journal of Experimental Biology, 2013, 3(2):205-213

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Larvicidal, pupicidal activities and morphological deformities of *Spathodea campanulata* aqueous leaf extract against the dengue vector *Aedes aegypti*

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ABSTRACT

In the persent study , aqueous leaf extract of Spathodea campanulata were investigated for larvicidal, pupicidal activities and morphological deformities against Aedes aegypti . LC_{10}, LC_{50} and LC_{90}/24 ,48, 72, 96 hours values of aqueous leaf extract of S. campanulata to 1 instar larvae was 1.42, 4.0 and 5.40 %(24 hours), 0.47, 0.96 and 2.12 % (48 hours) , 0.28, 1.14 and 1.84 % (72 hours) and 0.14, 0.59 and 1.12 % (96 hours) and this was found to gradully increase with the age of larvae. Pupae showed the highest resistance to the aqueous leaf extract of S. campanulata. Moreover, wide range of morphological deformities was observed and recorded in different categories.The study is of great importance in formulation of an effective vector control strategy based on enviromental friendly alternative (plant origin) insecticides.

Key words: *S. campanulata* , *Ae. aegypti* ,larvicidal, pupicidal, morphological deformities

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INTRODUCTION

Among the various groups of invertebrate animals, insects have a very close relationship with life and existence of mankind[1]. In the insect group, many insects of the order Diptera act as vectors and play a role in spreading disease among man.

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 the only known potential vector of dengue and urban yellow fever [2,3]. This species of mosquito was shown to be a competent laboratory vector of Chikungunya (CHIK) virus [4]. *Ae.aegypti* has also been noted to transmit filariasis and encephalitis [5].

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 [6]. In October 2001, an outbreak of dengue resulting in 16 deaths was reported in Chennai (Tamil Nadu) India [7]. 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 [8]. In 2010, there were a total of 28, 292 cases and 110 deaths [9]. In 2012 a total of 9,000 cases and 50 deaths were reported in Madurai, Tirunelveli and Kanyakumari districts (Tamil Nadu) [10].

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 [5]. 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 [8]. These diseases devastate Indian economy every year [11].

At present, no effective vaccine is available for dengue; therefore, the only way of reducing the incidence of this disease is mosquito control [12]. 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 [13].

Many control strategies for mosquitoes have been suggested since the ancient times.

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 [14,15].

This has necessitated the need for the search and development of environmentally safer, low cost, indigenous methods for vector control.

During the last decade, various studies on natural plant products against mosquito vectors indicate them as possible alternatives to synthetic chemical insecticides [16,17]. More then 2000 plants species have been known to produce chemical factors and metabolites of value in the pest control programes [18] and among these plants, products of some 344 species have been reported to have a variety of activities against mosquitoes [19].

Botanical insecticides also have potential uses such as larvicidal, ovicidal, oviposition deterrence, growth and reproduction inhibitors, repellents, growth regulation, fecundity suppression, male sterility [20,21]. Some of the plant leaf extract tested for their diverse insecticidal properties on the medically important mosquitoes are: aqueous extracts of *Senna didymobotrya* leaves [22]; ethanolic extract of *Centella asiatica* leaves [23] ; aqueous extracts of *Gymnema sylvestre* and *Eclipta prostrata* leaves [24]; methanolic extracts of *Azadirachta excelsa*, *Cleome glaucescens* and *Ricinus communis* leaves [25]; aqueous leaves extract of *Calotropis procera* [20]; aqueous extract of *Gymnema sylvestre*, *Nerium indicum* and *Datura metal* leaves [26]; methanol leaf extract of *Ervatamia coronaria* and *Caesalpinia pulcherrima* [27]; methanolic extracts of *Acalypha alnifolia* leaves [28]; aqueous leaf extract of *Lantana camara* [29].

It could be as certained from the literature survey that there was no information available on the larvicidal, pupicidal, deformities effects of the aqueous leaf extract of the *S. campanulata.*

The present study was therefore carried out to evaluate mosquitocidal properties of *S. campanulata* aqueous leaf extracts 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 Fountain Tree, African Tulip Tree, Flame-of –the forest, Rudra palash, Pichkari or Nandi Flame in different parts of the world. It is a tree that grows between 7-25 m (23-82ft) tall and native to tropical Africa. 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. The leaves have furnished Spathodol, caffeic acid and other phenolic acids and flavonoids.

MATERIALS AND METHODS

Colonization of *Aedes aegypti*

Collection of eggs

The eggs of *Ae. 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 *ad libitum* [30]. 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 [31,32].

Both females and males were provided with 10% glucose solution on cotton wicks [33]. 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 any kind 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. Different concentrations of the leaf extract was prepared taking a particular amount of leaf powder in glass beaker containing a known quantity of unchlorinated filtered tap water. The solution was allowed to stand for 72 hrs and the suspension was filtered through Whatman No.1 filter paper. For instance, 2g powder mixed in 200 ml of water for getting experimental medium of 1%. This solution was used for experiments.

Bioassay test
Bioassay tests were carried out for testing the efficacy of aqueous 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 [34] for conducting bioassay experiment with mosquito larvae were carefully followed.

Different concentrations of the test compound were prepared using unchlorinated filtered tap water as described earlier. Clean plastic cubs of 500 ml capacity were used as test containers. Batches of 20 larvae were exposed to 200 ml of particular concentration of test solution. The larvae of either I, II, III, IV instar stage and pupae were collected with an eye dropper placed onto filter paper strips and immediately transferred to test cup containing test solution according to [35].

Mortality rates of larvae were recorded after 24, 48, 72 and 96 hours. Five or more concentrations of a test compound giving between 0 and 100% mortality for larvae at different instar stages were tested. Parallel controls were maintained. Two replicates were done at each concentration. In recording the percentage moralities for each concentration, the moribund and dead larvae in both replicates were combined.

It was described that dead larvae are those that cannot be induced to move when they are probed with a needle in the siphon or the cervical region, moribund larvae are those incapable of rising to the surface [34].

The values of LC_{10}, LC_{50} and LC_{90} and their 95% confidence limit of upper confidence limit (UCL) and lower confidence limit (LCL), regression were calculated using probit analysis [36]. The SPSS 17.0 (Statistical Package of Social Sciences) used for statistical analysis.

Record of deformities
During the course of lethal experiments, the morphological features of larvae at different stages, pupa and adults from treated and control media were compared. Any notable difference in appearance between treated and control was recorded as deformity. The deformities were designated according to their similarity to those previously exhibited by [37- 42].

RESULTS AND DISCUSSION

Toxicity of aqueous leaf extract of *S. campanulata* to the developmental stages of *Ae. aegypti*
Bioassay tests were conducted to find out the toxicity of aqueous 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_{50} and LC_{90} / 24, 48, 72, 96 hours.

LC_{10}, LC_{50} and LC_{90} / 24, 48, 72, 96 hours values of aqueous leaf extract of *Spathodea campanulata* to I instar larvae was 1.42, 4.0 and 5.40% (24 hrs), 0.47, 0.96, and 2.12% (48 hrs), 0.28, 1.14 and 1.84% (72 hrs) and 0.14, 0.59 and 1.12% (96 hrs) and this was found to gradually increase with the age of larvae. Pupae showed the highest resistance to the aqueous leaf extract of *Spathodea campanulata* as evident from the relatively higher LC_{10}, LC_{50} and LC_{90}/ 24, 48, 72, and 96 hours values 19.72, 21.0 and 23.81% (24 hrs) , 16.35, 18.85 and 21.0% (48 hrs), 13.64, 16.10 and 17.22% (72 hrs) and 11.67, 12.93 and 14.58% (96 hrs) (Tables 1- 3).

Deformities
Visible morphological deformities occurred among the larvae exposed to lethal concentrations of aqueous leaf extract of *Spathodea campanulata.* Larvae and pupae survived through treatment frequently showed a variety of changes viz., dechitinized larva with damaged digestive tract (Plate 1a); exuvia of the proceding instar attached to the dead larvae (Plate 1b); death of larval stage with no intiation of pupation (Plate 1c), malformations like demelanized pupa with straight abdomen (Plate 2a); dwarf pupa with retarded abdomen (Plate 2b); Pupa with some melanization (brown pupa) (Plate 2c); and partly emerged adult with attached pupal case (Plate3).

The findings agree with some of the previous reports.

The leaf extract of *Ocimum canum* against *Ae. aegypti* showed LC_{50} values for 2^{nd} , 3^{rd} and 4^{th} instar larvae at 77.82, 229.08 and 331.13 ppm respectively [43]; 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 [44]; the leaf extract of *Cassia fistula* with different solvents viz, methanol, benzene, acetone was 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 [45]; 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 [46]; ethyl acetate, petroleum ether and methanol leaf extract of *Citrullus colocynthis* and *Cucurbita maxima* showed LC_{50} values of 47.58, 66.92 and 118.74 ppm and 75.91,117.73 and 171.64 ppm respectively against *Cx, quinquefasciatus* larvae [47]; the petroleum ether extract of the leaves of *Vitex negundo* were evaluated for larvicidal activity against larval stage of *Cx. tritaeniorhynchus* in the laboratory with LC_{50} and LC_{90} values of 2.4883 and 5.1883 mg/l, respectively [48]; 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 [49]; 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* [50]; methanol extract of the leaves of *Achyranthes aspera* caused 50% mortality of *Ae. aegypti* larvae at 409 ppm [51]; 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 24 hrs [52]; 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 [53]; the LC_{50} values of *Ficus benghalensis* leaf extract against early second, third and fourth larvae of *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi* were 41.43, 58.21 and 74.32 ppm,56.54, 70.29 and 80.85 ppm and 60.44, 76.41 and 89.55 ppm respectively [54]; the LC_{50} and LC_{90} values of crude methanol extract of leaves of *Ervatamia coronaria* on *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi* larvae in 24hrs and the results were 72.41 and 65.67 mg/l, 62.08 and 136.55 mg/l, 127.24 and 120.86 mg/l respectively [55]; 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 [56]; 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 [57]; 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* [58]; methanolic leaf extract of *Spathodea campanulata* were found most effective with LC_{50} and LC_{90} value of 4.026, 4.207, 4.699, 4.852 and 4.861 if I, II, III, IV and pupa of *An. stephensi* respectively [59].

The *S. campanulata* leaves have furnished Spathodol, caffeic acid, phenolic acids and flavonoids [60- 62]. 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 [63] and phytochemicals primarily effect the midgut epithelium and secondarily affect the gastric caeca and the malpighian tubules in mosquito larvae [64,65]. 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 [66].

In the present study, the *Ae. aegypti* larvae reared in lethal dose of aqueous leaf extracts of *S. campanulata*, in addition to changes in the indices of development, also exhibited, in common, a variety of metamorphic aberrations. Some of the visible malformations were dechitinized larva with damaged digestive tract, exuvia of the proceding instar attached the dead larvae, death occurred during the larval stage with no initiation of pupation, demelanized pupa with straight abdomen, dwarf pupa with retarded abdomen, pupa with some melanization (brown pupa) and partly emerged adult with attached pupal case. Similar deformities were found to occur during the development of *Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi* in media treated with hexane, diethyl ether, dichloromethane and ethyl acetate extracts of *Murraya koenigii* leaf [42]; early fourth instar larvae of *Ae. aegypti* exposed to acetone leaf extracts of *Saraca indica* and *Cassia fistula* showed, a variety of morhogenetic effects[67]; ethanolic, acetone and petroleum ether extracts of leaves from *Cupressus semprevirens* were tested against 3rd instar larvae of *Cx. pipiens*, various degrees of morphogenic abnormalities were observed in immature and adult stages [68]; the hexane, diethyl ether, dichloromethane and ethyl acetate extracts of *Abutilon indicum* leaf were evaluated for their pupal deformities against *Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi* [52]; Phytoextracts affect larval morphology, resulting in pigmentation and alternations in head and abdomen shape [69].

These morphogenetic abnormalities are commonly caused by botanical extracts and are atributed to result from disturbance to growth regulating hormones [70].

The study concludes that this leaf extract has potent larvicidal, pupicidal property along with morphogenetic effects. So this extract can be used as a solution for mosquito problem in the developing countries without damaging the environment.

Table.1 Log probit analysis and regression analysis of *Spathodea campanulata* aqueous leaf extract against different larval instars and pupae of *Aedes aegypti*.

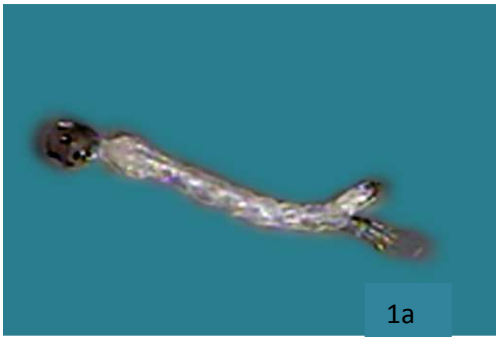
Larval instars	Period of bioassay(h)	LC_{10} (%)	Confidence interval(95%)		Regression equation	Regression value
			LL	UL		
First	24	1.42	1.06	1.87	y=43x-54	0.533
	48	0.47	0.28	0.52	y=230x-81	0.445
	72	0.28	0.12	0.33	y=215x-32.5	0.650
	96	0.14	0.093	0.197	y=289x-29.06	0.768
Second	24	3.97	3.14	4.83	y=20.5x-68.5	0.511
	48	0.94	0.45	1.21	y=205x-173	0.259
	72	0.38	0.26	0.43	y=205x-68.5	0.511
	96	0.23	0.21	0.34	y=200x-47	0.598
Third	24	6.65	5.78	7.32	y=22.5x-124.5	0.324
	48	4.67	3.96	5.15	y=21.5x75.5	0.446
	72	3.37	3.24	3.45	y=22x-56	0.509
	96	2.27	2.01	2.95	y=22.5-34.5	0.638
Fourth	24	12.45	11.67	13.28	y=21x-242	0.180
	48	9.25	8.58	10.62	y=23.5x-199	0.255
	72	7.76	6.82	8.85	y=22x-143	0.298
	96	4.37	3.69	5.13	y=22x-76	0.480
Pupae	24	19.72	19.11	20.56	y=21.5x-399	0.110
	48	16.35	15.84	17.47	y=23x-357	0.141
	72	13.64	12.45	4.37	y=21.5x-270	0.157
	96	11.67	10.86	12.42	y=22x-231	0.203

Table.2 Log probit analysis and regression analysis of *Spathodea campanulata* aqueous leaf extract against different larval instars and pupae of *Aedes aegypti.*

Larval instars	Period of bioassay(h)	LC_{50} (%)	Confidence interval(95%)		Regression equation	Regression value
			LL	UL		
First	24	4.0	3.25	4.72	y=22.5x-40	0.810
	48	1.43	0.92	1.96	y=45x-15.5	0.944
	72	1.14	0.71	1.85	y=230x-223	0.346
	96	0.59	0.50	0.67	y=225x-81	0.654
Second	24	7.17	6.45	7.84	y=23.5x-116.5	0.090
	48	3.18	2.56	3.73	y=20x-119	0.906
	72	2.15	1.58	2.93	y=50x-50	0.747
	96	1.05	0.48	1.53	y=250x-200	0.358
Third	24	9.13	8.09	10.15	y=24.5x-171.5	0.398
	48	6.85	6.24	7.35	y=22x-101	0.564
	72	5.90	4.03	6.86	y=22.5x-81	0.627
	96	3.84	3.12	4.68	y=22x-35	0.826
Fourth	24	16.12	15.63	16.95	y=22.5x-306	0.269
	48	11.95	10.55	12.94	y=23x-223	0.346
	72	9.97	8.76	10.98	y=24x-187	0.383
	96	6.96	6.14	7.85	y=23.5x-112.5	0.530
Pupae	24	21.0	19.31	21.85	y=22x-500	0.170
	48	18.85	17.49	19.92	y=23.5x-394.5	0.221
	72	16.10	15.35	17.21	y=21.5x-291	0.283
	96	12.93	12.22	13.72	y=20.5x-214.5	0.349

Table.3 Log probit analysis and regression analysis of *Spathodea campanulata* aqueous leaf extract against different larval instars and pupae of *Aedes aegypti.*

Larval instars	Period of bioassay(h)	LC_{90} (%)	Confidence interval(95%)		Regression equation	Regression value
			LL	UL		
First	24	5.40	4.79	6.13	y=19.5x-27.5	0.774
	48	2.12	1.67	1.87	y=19.5x-10.01	0.809
	72	1.84	0.97	2.66	y=37x-16.5	0.750
	96	1.12	0.86	1.68	y=190x-119	0.508
Second	24	7.74	6.91	8.52	y=19.5x-86	0.589
	48	4.36	3.89	4.83	y=20.5x-13	0.810
	72	3.44	2.57	3.38	y=19.5x-11.5	0.809
	96	4.63	3.84	5.36	y=20x-10	0.805
Third	24	10.64	9.83	11.25	y=21x-141	0.449
	48	7.42	7.32	7.42	y=18.5x-76	0.602
	72	7.36	6.45	8.37	y=21x-79	0.603
	96	5.73	5.14	6.58	y=21x-37	0.739
Fourth	24	17.50	16.46	18.25	y=21x-289	0.291
	48	13.15	12.21	13.28	y=18x-161	0.417
	72	11.63	10.58	12.33	y=21x-163	0.426
	96	8.45	7.80	9.26	y=22x-109	0.521
Pupae	24	23.81	23.10	24.73	y=21.5x-426.5	0.212
	48	21.0	18.41	20.56	y=20.5x-341	0.258
	72	17.22	16.46	18.12	y=19.5x-261.5	0.319
	96	14.58	13.79	15.43	y=19x-196	0.386



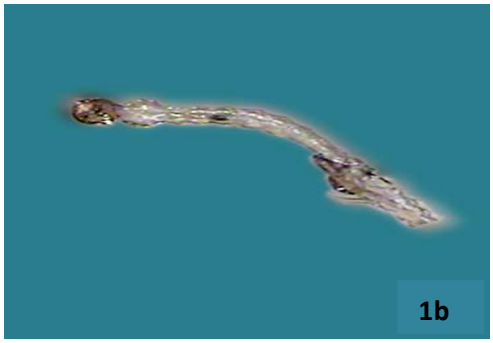


Plate 1a. Shows dechitinized larvae with damaged digestive tract of *Aedes aegypti* resulting from aqueous leaf extract of *Spathodea campanulata*
Plate 1b. Shows exuvia of the proceding instar attached to the dead larvae
Plate 1c. Shows death occurred during the larval stage with no initiation of pupation due to aqueous leaf extract of *Spathodea campanulata*
Plate 1d. Shows normal larvae of *Aedes aegypti*

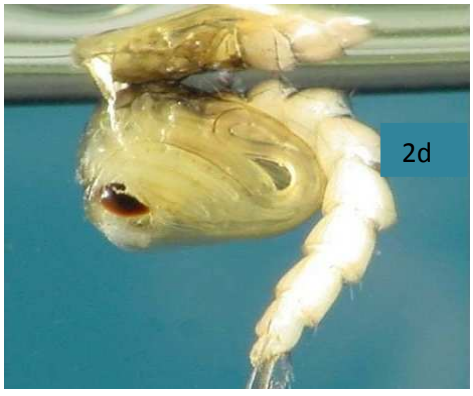


Plate 2a. Shows demelanized pupa except eye pigment of *Aedes aegypti* with straight abdomen
Plate 2b. Exhibits dwarf pupa of *Aedes aegypti* with retared abdomen
Plate 2c. Shows brown pupa with some melanization
Plate 2d. Shows normal pupae

Plate 3: Shows partly emerged adult of *Aedes aegypti* with attached pupal case

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