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Adult emergence and prolong larval duration effects of *Spathodea campanulata* aqueous leaf extract against the dengue vector *Aedes aegypti*

Mani Saranya[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

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ABSTRACT

With the development of resistance to conventionally used synthetic insecticides, vector management has become very difficult. Hence, scientists have interest on botanicals. It is in this regard, present study was aimed to evaluate the efficacy of aqueous extract of Spathodea campanulata leaves against the dengue vector Aedes aegypti. EI_{50} and EI_{90} were shown at 0.79% and 0.88% of the leaf extract respectively. The aqueous leaf extract at 0.1, 0.2 and 0.3% tested against the Ae. aegypti was found to prolong larval and pupal period. In the control it took 10 days for all the larvae to become pupae, whereas the aqueous extract at 0.1% and 0.2% took 19 days and 24 days respectively. In 0.3% the larvae required 26 days to become pupae. The data highlights the importance of Spathodea campanulata as a promising local plant with mosquitocidial activities.

Key words: *S. campanulata* leaves, *Ae. aegypti,* Adult emergence and prolong larval duration effects

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INTRODUCTION

Mosquitoes are major public health pests throughout the world. Among the 3492 species of mosquitoes recorded worldwide, more than a hundred species are capable of transmitting various diseases to humans [1]. Mosquito menace is particularly high in South East Asian countries [2] and in recent years global warming has lead to the spread of mosquitoes into temperate countries and higher altitude regions and the people in these regions are severely affected [3].

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 [4]. Compared to any other species of *Aedes*, *Ae.aegypti* shows more dependency on human blood [5]. *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 [6].

Ae. aegypti is generally known as a vector for an arbovirus responsible for dengue fever, which is endemic to Southeast Asia, the Pacific island area, Africa and the Americas. This mosquito is also the vector of yellow fever in Central and South America and West Africa. Dengue fever has become an important public health problem as the number of reported cases continues to increase, especially with more severe forms of the disease, dengue hemorrhagic fever, and dengue shock syndrome, or with unusual manifestations such as central nervous system involvement [7]. About two-thirds of the world's population lives in tropical and subtropical areas infested with dengue vectors, mainly *Aedes aegypti* [8].

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 [9]. 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 [10]. In 2010, there were a total of 28, 292 cases and 110 deaths [11]. In 2012 a total of 9,000 cases and 50 deaths were reported in Madurai, Tirunelveli and Kanyakumari districts (Tamil Nadu) [12]. According to the Central Health Ministry of India in 2013, 17,000 people affected by this disease, in Tamilnadu alone 4,000 affected by dengue [13].

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 [6]. 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 [10]. In 2013, a total of 500 cases were reported in Thirunelvali district (Uthakulam village) Tamilnadu, India [14]. These diseases devastate Indian economy every year [15].

At present, no effective vaccine is available for dengue; therefore, the only way of reducing the incidence of this disease is mosquito control [16]. The control methods should aim at the weakest link of the life cycle of the mosquito, which is the larval stage. Larviciding is a successful way of reducing mosquito densities in their breeding places before they emerge into adults. During the immature stage, mosquitoes are relatively immobile; remaining more concentrated than they are in the adult stage [17].

Many control strategies for mosquitoes have been suggested since the ancient times. Among the various control measures, viz., mechanical control by source of reduction [18] ; biological control, using endopathogenic bacteria [19,20]; larivorous fish [21] as well as predatory arthropods [22] and chemical control [23].

Mosquito control has been becoming increasingly difficult because of the indiscriminate uses of synthetic chemical insecticides which have an adverse impact on the environment and disturb ecological balance. Majority of the chemical pesticides are harmful to man and animals, some of which are not easily degradable and spreading toxic effects. The increased use of these insecticides may enter into the food chain. They even result in mutation of genes and these changes become prominent only after a few generations [24].

These problems have warranted the need for developing alternative strategies using eco-friendly products.

During the last decade, various studies on natural plant products against mosquito vectors indicate them as possible alternatives to synthetic chemical insecticides [25,26]. In this regard, India has a rich flora that is widely distributed throughout the country. More than 2000 plants species have been known to produce chemical factors and metabolites of value in the pest control programmes [27] and among these plants, products of some 344 species have been reported to have a variety of activities against mosquitoes [28].Phytochemicals are advantageous due to their eco-safty, target- specificity, non development of resistance, reduced number of applications, higher acceptability and suitability for rural areas. Plants being rich source of bioactive chemicals [29] and so far there is no report of resistance to plant extracts [30].

Botanical insecticides also have potential uses such as larvicidal, ovicidal, oviposition deterrence, growth and reproduction inhibitors, repellents, growth regulation, fecundity suppression, male sterility [31,32]. Some of the plant leaf extract tested for their diverse insecticidal properties on the medically important mosquitoes are: methanolic extracts of *Derris elliptica* leaves [33]; aqueous extract of *Solanum nigrum* leaves [34];acetone extract of *Solanum trilobatum* leaves [35]; methanol, benzene and acetone leaf extract of *Cassia fistula* [36]; petroleum

ether extract of *Azadirachta indica, Ocimum gratissimum* and *Hyptis suaveolens* leaves [37];aqueous and chloroform extracts of *Leucas aspera* leaf [38]; methanol, petroleum ether, hexane extract of *Pseudocalymma alliaceum* leaves[39]; aqueous, methanolic extract of *Spathodea campanulata* leaves [40,41, 42]; methanol extract of *Agerantina adenophora* leaves [43] ; methanol extract of *Ipomoea pes-caprae* leaves [44]; methanol, hexane, benzene ,chloroform, ethyl acetate extract of *Pithecellobium dulce* leaf [45]; petroleum ether extract of *Pongamia pinnata* leaves [46]; acetone extract of *Vernonia cinerea, Propis juliflora, Cassia tora* leaves [47].

As far as our literature survey could ascertain no information was available on the adult emergence and prolong larval duration 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* aqueous 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*,(plate 1) 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[48,49,50].

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 (Plate 3) 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 [51] *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 [52,53].

Both females and males were provided with 10% glucose solution as described by [54]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 (Plate 2) 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.

Adult emergence inhibition (EI) bioassay

A series of sub lethal concentrations (0.2, 0.4, 0.6, 0.8, 1, 1.2 and 1.4) were prepared. The 3rd instar larvae were introduced into each of the concentration cups. The larvae were fed by fish food *ad libitum* till they become pupae. The pupae were kept separately in the net cage to count the adults emerged. Mortality of the larvae, pupae and the adults was recorded at 24 hrs intervals. At the end of observation period, the impact is expressed as EI % based on the number of larvae that do not develop successfully into viable adults. In recording EI % for each concentration, moribund and dead larvae and pupae, as well as adult mosquitoes not completely separated from the pupal case, were considered as dead. The experiments were stopped when all larvae (or) pupae in the controls died or emerged as adults. Results of each concentration obtained on each occasion were subjected to computer log probit analysis, EI_{50} and EI_{90} values (in percent) were estimated by linear regression analysis [32].

Laboratory assay for larval duration

To determine the effect of aqueous leaf extract of *S. campanulata* on the length (duration) of the larval stage (egg-pupation) of different concentrations (0.1 to 0.3) test solutions were prepared. 50 freshly laid eggs were placed in the treated water and allowed to hatch and develop further. The medium was watched every 24 hours. The total larval duration in days was recorded from hatching to pupation. In parallel, the duration of larval stage was calculated for the larvae reared in untreated water for comparison [55].

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

RESULTS AND DISCUSSION

Effect of aqueous leaf extract of *S. campanulata* on adult emergence inhibition (EI)

The statistical data of adult emergence inhibition (EI) activity of aqueous leaf extract of *S. campanulata* against *Ae. aegypti* presented in (Fig. 1). EI_{50} and EI_{90} were shown at 0.79% and 0.88% of the leaf extract respectively. This reflects the possible insect growth regulatory activity of the extract against *Ae. aegypti*.

Effect of aqueous leaf extract of *S. campanulata* on total larval duration of *Ae. aegypti*

The aqueous leaf extract at 0.1, 0.2 and 0.3% tested against the *Ae. aegypti* was found to prolong larval and pupal period. In the control it took 10 days for all the larvae to become pupae, whereas the aqueous extract at 0.1% and 0.2% took 19 days and 24 days respectively. In 0.3% the larvae required 26 days to become pupae (Fig. 2).

The statistical data of adult emergence inhibition (EI) activity of aqueous leaf extract of *S. campanulata* against *Ae. aegypti* was found to be 0.79% and 0.88% as EI_{50} and EI_{90} respectively. The obtained results agree with some of the previous studies.

Effective EI was found in leaf acetone extract of the *Aegle marmelos*, ethyl acetate extract of *Andrographis lineate*, methanol leaf etracts of *Cocculus hirsutus*, and *Tagetes erecta* against *An. subpictus* (EI_{50} = 128.14, 79.39, 143.97 and 92.82 ppm; EI_{90}=713.53, 293.70, 682.72 and 582.59 ppm)[56]; adult emergence inhibition (EI) activity of *Calotropis procera* leaves against *An. arabiensis* and *Cx. quinquefasciatus*, EI_{50} – EI_{90} was shown at 277.90 –

677.64 ppm and 183.65 – 453.94 ppm respectively[31]; 50% of adult emergence inhibition (EI_{50}) were 374.97 and 1180.32 ppm of aqueous leaf extract of *Ricinus communis* against 3rd instar larvae of *An. arabiensis* and *Cx. quinquefasciatus[32]* ; emergence inhibition (EI_{50}) of 150$_{\mu}$ particle sized leaf powder of *Vinca rosea* was found to be most effective on *Ae. aegypti* with the (EI_{50}) of 24.81 mg/100 ml of water [57].

This reflects the activity of the plant extracts as possible insect growth regulator against *Ae. aegypti.* The failure in adult emergence could be due to insufficient availability of chitin during metamorphosis resulting in death of larvae and pupae entangled in the weak integument.

The aqueous leaf extract of *S. campanulata* tested against the *Ae. aegypti* at 0.1, 0.2 and 0.3% were found to be showing prolonged larval and pupal period. The results of present study are comparable with earlier reports. Results of hexane, diethyl ether, dichloromethane and ethyl acetate extracts of *Murraya koenigii* leaf revealed that treated individuals took prolonged larval and pupal period when compared to control in all species of *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*, tested and the larval and pupal period lasted for 9 and 3days in *Ae. aegypti*; 11 and 4 in *An. stephensi*; 10 and 4 days *Cx. quinquefasciatus*, and in the case of control it was 8 and 2 days. Larval duration significantly increased in treated individuals and total development period in *Ae. aegypti, An. stephensi* and *Cx.quinquefasciatus* was 12,(1000 ppm) 15(1000 ppm) and 14 (1000 ppm) days respectively and 10 days in control[58]; [59]reported the prevention of pupation upto day 7 at dosage of 1000 ppm in the immature of *Ae. aegypti* in ethanolic extract of *Cassia holosericea; Leucas aspera* leaf (500, 1000 ppm) showed prolonged larval and pupal periods among *Ae. aegypti, Cx. quinquefasciatus* and *An. stephensi*. It took 8 days for all the extracts to become pupae; whereas in the aqueous extract it took 11 days for *Ae. aegypti* and 9 days in the case of *An. stephensi* and *Cx. quinquefasciatus*,[60]; the hexane, dimethyl ether, dichloromethane and ethyl acetate extract of *Abutilon indicum* leaf against *Ae. aegypti, Cx. quinquefasciatus. An. stephensi* showed that larval and pupal periods were prolonged with an overall increases in the developmental period[61]; There was a delay in the development of *Cx. pipines* larvae to pupal stage (21days) when the second instar larvae were exposed to concentrations 40 and 20 mg/ml of methanolic leaf extract of *Azadirachta excela*[62]; exposure of *An. stephensi* larvae to sub–lethal doses of neem leaves in the laboratory prolonged larval development [63].

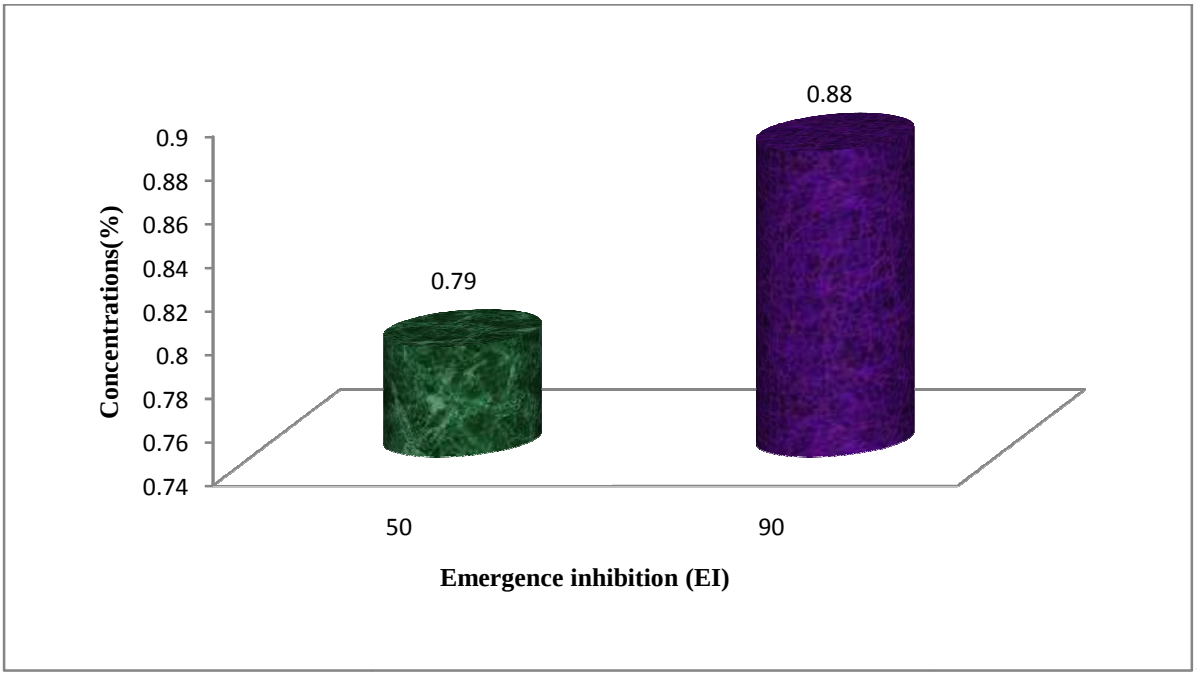

Fig. 1. EI_{50}, EI_{90} values (%) of aqueous leaf extract of *Spathodea campanulata* against *Aedes aegypti*

In the present study, lengthening of larval and pupal periods indicates the interference of the bio–active compounds of leaf extract of *S. campanulata* with the normal hormonal activity coordination of the metabolic process of the

developing stages. Prolongation of development period of mosquito larvae treated with plant extracts were generally attributed to interference of the active ingredients of the extracts with the endocrine system [64].

The screening of locally available medicinal plants for mosquito control would generate local employment, reduce dependence on expensive imported products and stimulate local efforts to enhance public health. It is in this regard, the study adds to the knowledge on the efficacy of the local plant *S. campanulata.*

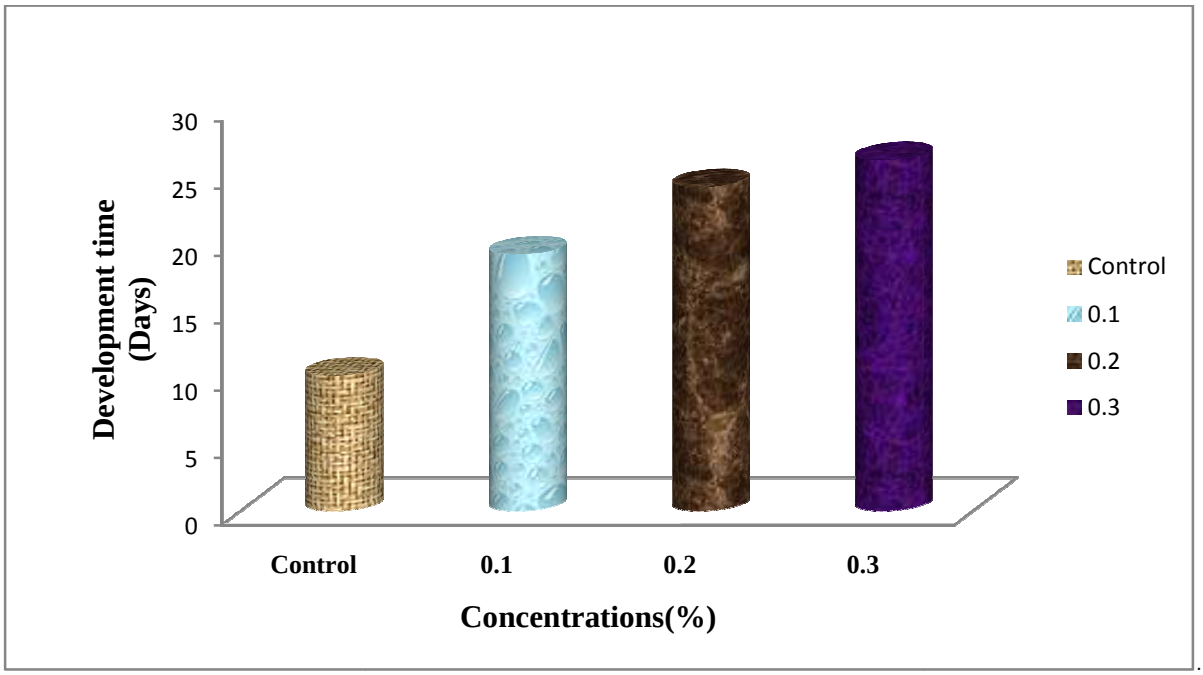

Fig.2. Change in development time (days) of *Aedes aegypti* larva reared in different concentrations of the aqueous leaf extract of *Spathodea campanulata* and control

Plate 1 : Shows the tree *Spathodea campanulata* from which leaves collected for this study

Plate 2 : Shows the leaves of *Spathodea campanulata* with which the extract was prepared

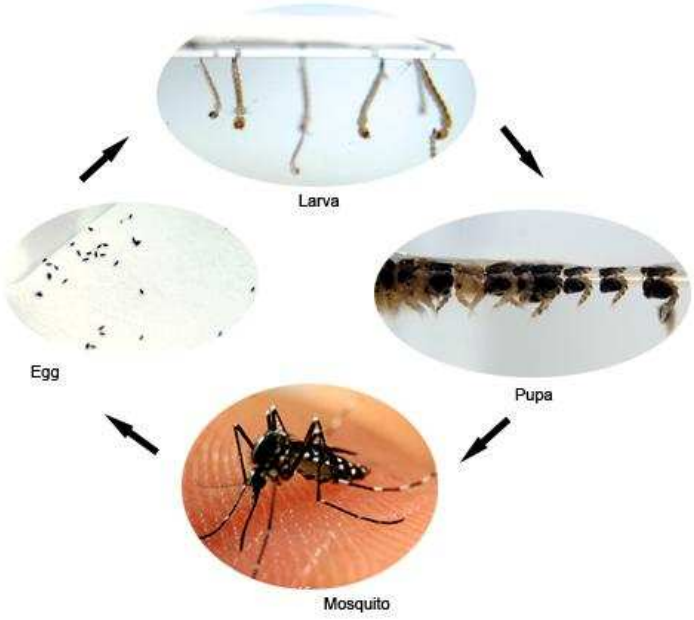

Plate 3: Shows the life cycle of *Aedes aegypti*

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