

Original Research Article

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***Tecoma stans* (L.) Juss.ex Kunth (Bignoniaceae) a Prospective Mosquitocide in the Management of Zika Virus Vector Mosquito *Aedes aegypti* (Diptera:Culicidae)**

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A B S T R A C T

Mosquitoes are found throughout the world excluding the place which are completely frozen. Three fourth (3/4) of the existing mosquito species were native to tropical and subtropical region. Mosquitoes are likely to transmit disease to more than two fifth(2/5) of the world population. The major dreadful disease such as Malaria, Chikungunya, Dengue, Filariasis and Japanese encephalitis were transmitted by mosquito bites. In order to reduce the awful diseases, extensive research has been done on mosquitoes for few decades. Vector control techniques face serious threats because of the emergence of resistance to synthetic insecticides. Plant are rich sources of bioactive compounds and synthesize a number of secondary metabolites to serve as defensive chemicals for control the insect pests. Plants offer an advantage over than synthetic pesticide, which used as an alternative to chemical insecticides. The present investigations were carried out to assess the ovicidal activity and ovipositional deterrence efficiency of aqueous leaf extracts of *Tecoma stans* against *Aedes aegypti*. Hatching rate was calculated on the basis of non-hatchability of eggs. The effect of aqueous leaf extract of *T.stans* on oviposition of *Ae. aegypti* was determined under two set of conditions (Choice oviposition test, No-choice oviposition test). Oviposition Active Index (OAI) and Oviposition Deterrent Index (ODI) was calculated. The hatchability of *Ae. aegypti* eggs was decreased when placed in media of aqueous leaf extract. The reduction in percent hatch was inversely proportional to the concentration of aqueous leaf extract used. The aqueous leaf extract of *T.stans* were found to deter mosquitoes from oviposition. Oviposition Active Index and Oviposition Deterrent Index indicated a low preference of *Ae. aegypti* to leaf extract treated medium for egg laying. This study suggest that, the aqueous leaf extract of *Tecoma stans* should be considered as promising anti- mosquito agent.

Keywords

Aedes aegypti,
Tecoma stans,
Leaf Extract,
Ovicidal Activity
and Ovipositional
Deterrent Activity.

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Introduction

A variety of organism like insects and arthropods affects the human health by causing various fatal disease. The insects related to epidemiology include mosquitoes, houseflies, sandflies, rat flea, tse-tse fly etc., and among these insects, mosquitoes play an important role. They are the causative agent of various fatal disease like malaria, filariasis, encephalitis, yellow fever and dengue (Gulber, 1998.).

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 (Reuda, 2008). Mosquito menace is particularly high in South East Asian countries (Rao *et al.*, 2008) and in recent years global warming has led to the spread of mosquitoes into temperate countries and higher altitude regions and the people in these regions are severely affected (Nerio *et al.*, 2010).

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 breeds in many types of household containers, such as water storage jars, drums, tanks and plant or flower containers (Harrington *et al.*, 2005). Compared to any other species of *Aedes*, *Ae. aegypti* shows more dependency on human blood (Scott *et al.*, 1993). *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 (Park and Park, 1987).

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 (Pancharoen *et al.*, 2002). About two-thirds of the world's population lives in tropical and subtropical areas infested with dengue vectors, mainly *Aedes aegypti* (Hahn *et al.*, 2001).

In 1906, dengue fever seemed to be a viral illness. Dengue fever first recognized documented signs were cited in Chinese Encyclopedia in the Chin Dynasty (AD 265-420). This disease was connected by means of airborne arthropods close to H₂O and called as 'the water poison' (Khanani *et al.*, 2011). The word 'dengue' is derivative from the Swahili saying Ka-dinga pepo, standing for "cramp-like seizure". The first medically documented dengue outbreaks happened in the 1780s, concurrently in Asia, Africa and North America. The first clinical case report dating from 1779 to 1780 epidemic in Philadelphia was by Gupta *et al.*, 2012 who invented the word "break bone fever" due to indications of arthralgia and myalgia (Gupta *et al.*, 2012).

Dengue or 'break bone' fever had been known in our country for every long time. Dengue fever virus (DENV) belongs to RNA virus of the Flaviviridae family and it was first isolated from Japan in 1942 by Hotta (Izabela *et al.*, 2010). The viruses of dengue are round shape particles and 50 nm in diameter. It holds a single strand of RNA bounded with bi layer lipid. Full-grown viruses are made up of 9% carbohydrate, 17% lipid and 6 % RNA. The genomic RNA is approximately 11 kb in length. Dengue virus is composed of three structural protein genes (Ma *et al.*, 2004; Modis *et al.*, 2003). First protein gene is Enveloped (E) protein found on viral surface, second protein is membrane (M) protein which is very important for formation of viral particles. Third protein gene is non structural (NS) proteins which has seven subtypes. These NS proteins are believed to be involved in replication of viral RNA. It is estimated that 34% of the global cases are from India (Bhatt *et al.*, 2013) and the country is known to be endemic, with all four serotypes (DENV-1, 2, 3 &4) circulating throughout the year in different parts (Gupta *et al.*, 2012).

Chikungunya is an infection caused by the chikungunya virus. Chikungunya virus, also referred to as CHIKV, is a member of the alphavirus genus, and Togaviridae family. It is an RNA virus with a positive-sense single-stranded genome of about 11.6kb (Weaver *et al.*, 2012). It is a member of the Semliki Forest virus complex and is closely related to Ross River virus, O'nyong'nyong virus, and Semliki Forest virus (Powers *et al.*, 2001). Because it is transmitted by arthropods, namely mosquitoes, it can also be referred to as an arbovirus (arthropod-borne virus). The virus consists of four non-structural proteins and three structural proteins (Weaver *et al.*, 2015). The structural proteins are the capsid and two envelope glycoproteins: E1 and E2, which

from heterodimeric spikes on the viron surface.

Zika virus (ZIKV) is a member of the virus family Flaviviridae and the genus Flavivirus, transmitted by day time-active *Aedes* mosquitos, such as *Ae. aegypti*. Its name comes from the Zika forest of Uganda, where the virus was isolated in 1947. It was later identified in humans in 1968 for the first time in Nigeria. The first major outbreak, with 185 confirmed cases, was reported in 2007 in Yap Islands. The first cases confirmed in Brazil was in May 2015 and the country is currently experiencing the largest epidemic ever recorded with 440,000 to 1,300,000 suspected cases reported by the Brazillian health authorities. There are 3174 cases and 28 deaths from microcephaly in Brazil as of 21 October 2015 in the world. Zika virus is enveloped and icosahedral and has a nonsegmented, single-stranded, positive-sense RNA genome. It is most closely related to the Spondweni virus and is one of the two viruses in Spondweni virus clade (Kinpe *et al.*, 2007).

Zika virus is transmitted by day-time active *Ae. aegypti*. Studies show that the extrinsic incubation period in mosquitos is about 10 days (Hayes, 2009). Zika virus can migrate between humans through sexual contact and it can also cross the placenta, affecting an unborn fetus. A mother already infected with Zika virus near the time of delivery can pass on virus to her newborn around the time of birth, but this is rare. These symptoms are headache, fever, skin rashes (exanthema), pink eye, conjunctivitis, muscle and joint pain, malaise. Symptoms are similar to that dengue or chikungunya.

Epidemic outbreaks of dengue fever have also been reported in India. For instance, in 1980 a total of 4,601 cases were recorded (Park and Park, 1987). In October 2001, an outbreak of dengue resulting in 16 deaths

was reported in Chennai (Tamil Nadu) India (The Hindu, 2001). 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 (The Hindu, 2006). In 2010, there were a total of 28,292 cases and 110 deaths (NVBDCP, 2011). In 2012 a total of 9,000 cases and 50 deaths were reported in Madurai, Tirunelveli and Kanyakumari districts (Tamil Nadu)(The Hindu,2012). According to the Central Health Ministry of India in 2013, 75,454 people affected by this disease, in Tamilnadu alone 4,000 affected by dengue which led to 167 deaths (The Dinamani, 2013). According to Health Minister of Tamilnadu in Sivagangai district nearly 1,400 people affected by dengue fever (The Dinamalar, 2014a). In Coimbatore Corporation alone 2014, 172 people affected by dengue fever (The Dinamalar, 2014b). In 2015, nearly 2000 people affected by dengue fever in Tamilnadu (The Dinakaran, 2015).

Chikungunya, a febrile disease is caused by Chikungunya virus. 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 (Park and Park,1987). 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 (The Hindu, 2006). In 2013, a total of 500 cases were reported in Thirunelveli district (Uthakulam village) Tamilnadu, India (The Dinamani, 2013). These diseases devastate Indian economy every year (Jaswanth *et al.*, 2002).

At present, no effective vaccine is available for these diseases; therefore, the only way of reducing the incidence of this disease is mosquito control (Sarita *et al.*, 2012). 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 (Rutledge *et al.*, 2003).

Mosquito control has been becoming increasingly difficult because of the indiscriminate uses of synthetic chemical insecticides such as malathion, DDT, deltamethrin (Yang *et al.*, 2005) 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 (Ghose, 1991). Thus, the Environment protection Act in 1969 has formed a number of rules and regulation to check the application of chemical control agents in nature (Bhatt and Khana, 2009). It has prompted researchers to look for alternative approaches ranging from provision of or promoting the adoption of effective and transparent mosquito management strategies that focus on public education, monitoring and surveillance, source reduction and environment friendly least-toxic larval control. These factors have resulted in an urge to look for environment friendly, cost-effective, biodegradable and target specific insecticides against mosquito species.

During the last decade, various studies on natural plant products against mosquito vectors indicate them as possible alternatives to synthetic chemical insecticides (Mittal and Subbarao, 2003 and Promsiri *et al.*, 2006). In this regard, India has a rich flora that is widely distributed throughout the country. More than 2000

plant species have been known to produce chemical factors and metabolites of value in the pest control programmes (Ahmed *et al.*, 1984) and among these plants, products of some 344 species have been reported to have a variety of activities against mosquitoes (Sukumar *et al.*, 1991). Phytochemicals are advantageous due to their eco-safety, target- specificity, non-development of resistance, reduced number of applications, higher acceptability and suitability for rural areas. Plants being rich source of bioactive chemicals (Rajkumar and Jebanesan, 2004) and so far there is no report of resistance to plant extracts (Sharma *et al.*, 1992). The use of different parts of locally available plants and their various products in the control of mosquitoes have been well established globally by numerous researchers.

The phytochemicals derived from plant sources possess a complex of chemicals with unique biological activity. The phytochemicals derived from plant resources can act as larvicidal, ovicidal, oviposition deterrence, growth and reproduction inhibitors, repellents, growth regulation, fecundity suppression, male sterility (Elimam *et al.*, 2009a,b). Some of the plant leaf extract tested for their diverse insecticidal properties on the medically important mosquitoes are: methanolic extract of *Derris elliptica* leaves (Prempree and Sukhapanth, 1990); aqueous extract of *Senna didymobotrya* leaves (Ojewole *et al.*, 2000); aqueous extract of *Solanum nigrum* leaves (Singh *et al.*, 2001); acetone extract of *Solanum trilobatum* leaves (Rajkumar and Jebanesan, 2004); aqueous extract of *Gymnema sylvestre* and *Eclipta prostrate* leaves (Khanna and Kannabiran, 2007); methanol, benzene and acetone leaf extracts of *Cassia fistula* (Govindarajan, 2009); petroleum ether extract of *Azadirachta indica*, *Ocimum gratissimum* and *Hyptis suaveolens* leaves (Okigbo *et al.*,

2010); aqueous and chloroform extracts of *Leucas aspera* leaves (Ramanibai *et al.*, 2011); ethanolic extract of *Datura stramonium* leaves (Swathi *et al.*, 2012); aqueous extract of *Spathodea campanulata* leaves (Saranya *et al.*, 2013 a, b,c); methanolic extract of *Spathodea campanulata* leaves (Karthika Devi *et al.*, 2013); acetone extract of *Spathodea campanulata* leaves (Pravin *et al.*, 2014); aqueous extract of *Pithecellobium dulce* leaves (Mahendran *et al.*, 2015); acetone extract of *Spathodea campanulata* leaves (Pravin *et al.*, 2015); methanol extract of *Annona reticulata* leaves (Mohankumar *et al.*, 2016); hexane, acetone, ethanol and aqueous extracts of *Agave angustifolia* leaves (Kajla *et al.*, 2016); water extract of *Andrographis paniculata* leaves (Wannee *et al.*, 2016); hexane extract of *Ocimum basilicum* leaves (El-Akhal *et al.*, 2016); water, chloroform, acetone and diethyl ether extracts of *Nerium oleander* leaves (El-Sayed and El-Bassiony, 2016); hexane, benzene, chloroform, ethyl acetate and methanol extracts of *Pithecellobium dulce* (Rajeswary and Govindarajan, 2016); petroleum ether, chloroform, ethanol and acetone extracts of *Gmelina asiatica* leaves (Augustian and Jeeva, 2016); hexane, ethyl acetate, chloroform and methanol extracts of *Pedaliium murex* leaves (Gokulakrishnan *et al.*, 2016); methanol extract of *Ricinus cummunis* and *Argemone mexicana* leaves (Saroj *et al.*, 2016); methanol extract of *Callistemon lanceolatus* leaves (Ilango *et al.*, 2016); ethanol extract of *Hyptis suaveolens* and *Chenopodium ambrosoides* leaves (Dawet *et al.*, 2016); hexane, dichloromethane, ethyl acetate, acetone and methanol extracts of *Spondias mombin* leaves (Ajaegbu *et al.*, 2016).

As for as our literature survey that there was no information available on the ovicidal, ovipositional deterrence effects of the leaf extract of the *T.stans*.

The present study was therefore carried out to evaluate mosquitocidal properties *T.stans* aqueous leaf extract against the vector mosquito, *Ae. aegypti*.

Tecoma stans (Plate-1) is a promising species in the trumpet vine family, Bignoniaceae that is native to the Americas with many synonyms and common name. Synonyms include *Bignonia stans* L., *Gelsemium stans* (L.) Kuntze, *Stenolobium stans*(L.) Seem and Common names, yellow trumpet bush, yellow bells, yellow elder, ginger-thomas, and *Esperanza*. In India, it is known as Sonapatti in Tamil language, Panchalogotla in Telugu language and Korenekalar in Kannada language (Parrotta, 2001). *Tecoma stans* is the official flower of the United States virgin islands and the national flower of the Bahamas. It is a flowering perennial shrub or small tree, 5.7-6 m in height. Bark is pale brown to grey and roughens with age. Leaves are opposite, compound and imparipinnate with 2 to 5 pairs of leaflets and a larger single terminal leaflet. Leaflets are lanceolate, up to 10 cm long, with serrated margins, mid-green above and soft to the touch. Flowers occur in clusters at the ends of the branches and are trumpet shaped with 5 rounded lobes, 6cm long, pale to bright yellow, with faint orange stripes at the throat. Fruits are narrow, slightly flattened to pointed capsules, up to 20 cm long, containing many winged seeds; green when young, pale brown on ripening and remain on the tree in untidy clusters for many months (Parrotta,2001). *Tecoma stans* leaves bark, and roots contain many biologically active chemicals, and extracts from those tissues have been used in traditional folk medicine to treat many diseases and conditions (Liogier, 1990). Leaves are used throughout Mexico and Central America for antimicrobial, antidiabetic, antispasmodic activities and urinary disorder control (Winkelman, 1986; Lozoya *et al.*,1987 and

Shapiro and Gong,2002). Roots are used as diuretic, vermifuge (Khare, 2007)

Chemical constituents of this botanical species are well known; numerous monoterpenic alkaloids have been identified (Hammouda *et al.*, 1963; Arlete and Joana, 1993) and among them, tecomanine and tecostanine possess hypoglycemic effects according to observations performed in animals (Hammouda *et al.*, 1964; Hammouda and Amer, 1966). The biosynthesis of these monoterpene alkaloids in callus tissues of *T. stans* has been studied, together with the identification of the presence of lapachol and other primary and secondary plant metabolites such as: sugars (glucose, fructose, sucrose and xylose), triterpenoids(ursolic and oleanolic acids and α -amyrine), p-sitosterol and phenolics (chlorogenic, caffeic, vanillic, o-cumaric and sinapicacids). All of these compounds have already been identified in the whole plant at different concentrations (Dohnal,1976). The presence of iridoid glycosides, (Bianco *et al.*, 1980) indolic compounds (Satya *et al.*, 1984) in the leaves was established in *T.stans* (Marzouk *et al.*, 2006).

The aim of the present study is therefore to find out: Ovicidal activity of the aqueous leaf extract *T.stans* of on mosquito eggs. Ovipositional preferences of adult mosquitoes to different concentrations of aqueous leaf extract of *T.stans*.

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 (Lymio *et al.*, 1992) *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 inside 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 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.

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

T. stans leaves (Plate 2) were collected from Periyanaickenpalayam village, Coimbatore District, Tamilnadu, India. The identification of the plant was authenticated at BSI (Botanical Survey of India), Coimbatore (Reference number – BSI/SRC/5/23/2015/Tech/41).

Preparation of Plant Extract

The fresh leaves of *T. stans* were collected in the Periyanaickenpalayam village, Coimbatore District, Tamilnadu, India. 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 ($27 \pm 2^\circ\text{C}$) for about 2 weeks till they dried completely. The leaves were finely powdered using electric blender. Powdered plant material (100g) was soaked in double distilled water (1000 ml) in airtight wide mouth bottle and kept for 4 days with periodic shaking. After that, the cold extract was filtered using Whatman No.1 filter paper and kept in petri dishes for drying at room temperature (Kongkathip, 1994). Dried extract was used for the preparation of stock solution.

Preparation of Stock Solution and Different Concentrations of Leaf Extract

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

Ovicidal Assay

Freshly laid eggs obtained from the general stock of mosquitoes were tested for their hatching ability in relation to the different concentrations of aqueous leaf extract of *T.stans*. Hatching rate was calculated on the basis of non-hatchability of eggs according to Sahgal and Pillai (1993).

Five replications were conducted at each concentration of test compound.

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

Oviposition Bioassay

The effect of aqueous leaf extract of *T.stans* on oviposition of *Ae. aegypti* was determined under two set of conditions (Choice oviposition test, No-choice oviposition test) as suggested by Beehler and Mulla (1993), Chen *et al.*, (1996) and Dhanakkodi *et al.*, (1999).

Oviposition Active Index (OAI) was calculated as detailed by Vasuki (1991) 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 whether 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 Dimetry *et al.*, (1995).

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

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

Effect of Aqueous Leaf Extract of *T.stans* 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 aqueous leaf extract of *T.stans*. Percent hatch of eggs placed in control medium was 90 % where as in 0.1, 0.5, 0.10, 0.15 concentrations it was 75, 55, 45 and 10. 0.20 % dose completely arrested hatching eggs (Table- 1). The decrease in hatchability was found to be dose dependent.

Effect of Aqueous Leaf Extract of *T.stans* on Oviposition of *Ae.aegypti*

Choice Oviposition Test

Mosquitoes showed more preference towards control ovitrap for oviposition, though; media of different concentrations of aqueous leaf extract of *T.stans* were

available along with control (Choice oviposition test). The total number of eggs laid in ovitraps containing any concentration of the aqueous leaf extract of *T.stans* was always less than that in the control. Among the total number of eggs laid, 49.58 % was present in control medium when placed along with ovitraps with 0.1, 0.5, 0.10 and 0.15 % aqueous leaf extract of *T.stans* in which appeared 30.57, 13.22, 5.785 and 0.826 % of eggs respectively. This was also indicated by ODI values (23.17, 57.89 and 79.10 and 96.72). Rate of oviposition in ovitraps with any concentration of test compounds was significantly ($P < 0.001$) less than in control (Table-2).

No - choice Oviposition Test

The ovipositional deterrence of aqueous leaf extract of *T.stans* 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 0.1, 0.5, 0.10 and 0.15% of aqueous leaf extract of *T.stans* was 33.33, 23.68, 8.510, and 2.803 which were significantly ($P < 0.001$) less compared to their control counterparts 66.66, 76.31, 91.48 and 97.19%, respectively. The data of no - choice oviposition test clearly exhibited interference of aqueous leaf extract of *T.stans* on the oviposition preference of mosquitoes (Table-3).

The extensive use of synthetic chemical insecticides has resulted in environmental degradation, hazards and resistance to these insecticides in major vector species. This has necessitated the formulation of a more potent and environment-friendly insecticide. Since last few years, the researchers have focused their interest towards the control of mosquito larvae with the use of plant extracts. Plants have been known to produce various chemicals, many of which have reported to possess medicinal, insecticidal

and pesticidal properties. More than 2000 plant species have been known to produce chemical factors and metabolites of value in pest control programmes. A number of plant extracts has been reported to possess larvicidal, adulticidal or repellent activities against different species of mosquitoes (Ghosh *et al.*, 2012).

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 (Govindarajan, 2011b); 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 (Rajkumar and Jebanesan, 2004); ovicidal activity with ethyl acetate, aqueous solution, ethanol leaf extracts of *Nerium oleander* against *An. 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 extracts (Roni *et al.*, 2013); 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 *Ae.aegypti* was 65, 46, 40 and 20%. 0.8% dose completely arrested hatching eggs (Saranya *et al.*, 2013b); it has been noticed that higher concentrations of *Pongamia pinnata* leaves extracts possesses strong ovicidal activity 100% at 400 ppm concentration against *Ae.aegypti*, *An.stephensi* and *Cx.quinquefasciatus*. In the same way, methanol extracts showed maximum ovicidal activity at 240, 320, and 400 ppm

followed by hexane, dichloromethane and ethyl acetate against vector mosquitoes (Anitha *et al.*, 2014); maximum percentage of *Ae.aegypti*, *An. stephensi* and *Cx.quinquefasciatus* eggs was killed when they were exposed to 500 ppm concentration of *Clausena excavate* leaves of the methanol extract (Thangarasu *et al.*, 2014); the diethyl extract of *Coleus aromaticus* leaves exerted 100% ovicidal activity against *Ae. aegypti* at 200ppm (Baranitharan and Dhanasekaran, 2014); at 100ppm concentration of *Dodonaea viscosa* and *Lantana camara* leaves had the most severe effect on *Ae. aegypti* egg hatching rate which were reduced by about 35% and 33.5% compared with 26% for *Ruta chalepensis*. At their highest concentrations (500 ppm), the plant extract reduced egg hatchability percentages by about 90%, 87% and 69% for *Dodonaea viscosa* and *Lantana camara* and *Ruta chalepensis* respectively (Madkour *et al.*, 2014) ; the higher concentrations of *Polygala arvensis* leaf methanol extract possesses strong ovicidal activity at 200 ppm concentration against *Ae.aegypti*, *An.stephensi* and *Cx.quinquefasciatus*, no egg hatchability was recorded (Deepa *et al.*, 2014); one hundred per cent egg mortality was obtained in ethyl acetate extract of *Ageratum houstonianum* leaves at 20.0 mg/L against *Ae. aegypti* (Samuel *et al.*, 2015); the methanol extract of *Ageratum conyzoides* leaves exerted 100% egg mortality at 250 ppm against *An. stephensi* (Janakan and Ramakrishnan, 2014); the acetone leaf extract of *Argemone mexicana* was found to exhibit the highest ovicidal potential against *Ae.aegypti* with a significantly reduced egg hatch of 27.32% when exposed to 400 ppm extract as compared to control treatment; this extract causing the lowest percent hatch of 18.35% at 1000 ppm (Radhika and Sarita, 2015); both plant extract (acetone) exhibited excellent oviciding effect as 92.33% of eggs failed to be hatched when treated with 705.0

mg/L of *Melanochyle fasciculiflora* and 86.67% with 562.5 mg/L of *Gluta renghas* leaves (Zuharah *et al.*, 2015).

In the case of ovicidal activity, exposure of freshly laid eggs was more effective than that of the older eggs (Miura *et al.*,1976).

The aqueous 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 (Rajkumar *et al.*,2011). Eggs and egg shells treated with plant extracts become damaged probably due to endosmosis. After the initial phase of swelling, eggs become desiccated, followed by shrinkage and death of larvae trapped within (Arivoli and Samuel 2011d). The treated eggs contained developed embryos the eclosion of the egg was incomplete (Miura *et al.*,1976).

The findings of the present investigation were comparable with other ovicidal studies and revealed that the aqueous *T.stans* leaf extract possesses ovicidal activity against the eggs of *Ae. aegypti*.

One of the approaches to control the expanding mosquito population is to deter oviposition of female adults. In the absence of a suitable site for oviposition, the female avoid oviposition leading to its regression. Such an event can strongly influence vector population distribution and dynamics as well as provide additional understanding into the factors affecting population regulation (Kweka *et al.*, 2010). It has also been reported that reduce exposure to predators and competitors or increase access to food. In fact, in the presence of oviposition deterrents, female mosquitoes move towards and land upon a site, assess site quality, but lay few or no eggs before flying away (Xue *et al.*, 2003).

Table.1 Altration in the Hatchability of *Aedes aegypti* Eggs Exposed to Differen Concentrations of Aqueous Leaf Extract of *T.stans* and Control

Parameters	Control	Concentrations (%)				
		0.1	0.5	0.10	0.15	0.20
Number of eggs introduced	20	20	20	20	20	20
Mean number of eggs hatched #	18.66	15.33	11.66	9.00	2.66	0
S.D	± 0.010	± 0.102	± 0.020	± 0.012	± 0.210	± 0.011
Percent hatchability	90	75*	55*	45*	10*	0*
Percent reduction over control		16.66	38.88	50.00	88.88	100

#, Mean (± SD) of 5 replicates.

*, Significantly different from control, P < 0.001

Table.2 Changes in the Indices of Oviposition Deterrence at Different Concentrations of Aqueous Leaf Extract of *T.stans* against *Aedes aegypti* under Choice Oviposition Test

Parameters	Control	Concentrations (%)			
		0.1	0.5	0.10	0.15
Total number of eggs laid*	600	370	160	70	10
Percent oviposition	49.58	30.57#	13.22#	5.785#	0.826#
Percent reduction in oviposition over control		61.66	26.66	11.66	1.666
Percent in number of eggs compared to control		230	440	530	590
Oviposition active index (OAI)		- 0.237	- 0.578	- 0.791	- 0.967
Oviposition deterrent index (ODI)		23.17	57.89	79.10	96.72

*, Total number of eggs in 10 replicates.

#, Significantly different from control, P < 0.001

Table.3 Changes in the Indices of Oviposition Deterrence of Different Concentrations of Aqueous Leaf Extract of *T.stans* against *Aedes aegypti* under No-choice Oviposition Test

Parameters	Concentrations (%)							
	C	0.1	C	0.5	C	0.10	C	0.15
Total number of eggs laid*	300	150	290	90	430	40	520	15
Percent oviposition	66.66	33.33#	76.31	23.68#	91.48	8.510#	97.19	2.803#
Percent reduction over control		50		31.03		9.302		2.884
Reduction in number of eggs compared to control		150		200		390		505
Oviposition active index (OAI)		- 0.333		- 0.526		- 0.829		- 0.943
Oviposition deterrent index (ODI)		33.33		52.63		82.97		94.39

*, Total number of eggs in 3 replicates.

#, Significantly different from control, P < 0.001

Plate.1 Shows the Tree *Tecoma stans* from which Leaves Collected for this Study



Plate.2 Shows the Leaves of *Tecoma Stans* with Which the Extract was Prepared



It has been reported that the oviposition activity index represents the relative preference of a substrate by egg-laying females (Waliwitiya *et al.*, 2009). Positive index values indicate more oviposition in the test cups than in control cups, whereas more eggs in control cups as compared to the test cups results in negative index values. The negative OAI values, thus, indicate the preference of distilled water as the medium for egg deposition over the treated medium which proved repulsive or unsuitable.

Oviposition active index for both ethanolic leaf extract of *Ocimum kilimandscharicum*

(OK) and *Ocimum 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* (Kweka *et al.*, 2010); 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%) (Swathi *et al.*, 2010); the ethanolic leaf extract of *Solanum 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% (Rajkumar and Jebanesan, 2005 a); 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 (Elango *et al.*,2009); 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. quinquefasciata* (Vijaya kumar *et al.*,2011a); 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 (Vijaya kumar *et al.*,2011b); acetone extract of *Prosopis juliflora* leaf found to be strong oviposition deterrent which inhibited egg laying (OAI-0.466) at 100 ppm (Yadav *et al.*, 2014); the maximum oviposition deterrent activity was recorded from the highest concentration of methanol, hexane,dichloromethane and ethyl acetate extracts 400 ppm and the least oviposition deterrent activity was recorded from the 80 ppm concentration of *Pongamia pinnata* leaves extracts against mosquitoes (Anitha *et al.*, 2014); the maximum oviposition deterrent potential of the petroleum ether extract of *Argemone mexicana* leaves against *Ae.ageypti* with 31% effective deterrence at 200 ppm

which rose by 66% at 400 ppm (Radhika and Sarita,2015).

The oviposition is one of the most important events in the life cycle of mosquitoes (Xue *et al.*,2001). If oviposition is prevented the mosquito life cycle is disrupted and population growth is reduced. The present oviposition study shows that the aqueous leaf extract of *T.stans* 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 aqueous 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 (Davis and Bowen,1994)

T.stans aqueous leaf extract used for the first time in this study to assess their ovicidal and oviposition deterrent activities against the dengue vector *Ae. aegypti* and the extract exhibited remarkable results.

To avoid the detrimental effects caused by the chemicals for the control of dengue vector, natural and nontoxic bioactive compounds of plant origin can be used as an alternate control measure (Das *et al.*, 2007). This study finally proposed new alternative potential biopesticides from local flora, which is easily available with low technology and can easily be integrated into the ongoing mosquito management rather than using conventional chemical control, which is more expensive than using the biological control comprising of plant extracts and is more effective and target specific (Jaenson

et al., 2006). Our investigations have indicated the potential of *T.stans* leaves as the possible and effective control agent against *Ae. aegypti*. The studies recommend that this plant could form safe and eco-friendly alternative to eco-enemy synthetic pesticides. Further studies regarding enhancing effectiveness, constancy, noxious effects and impacts on non-target population and the environment, along with field trials are required to recommend *T.stans* as an anti-mosquito agent.

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