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Research Article

# Dose-dependent Pupicidal, Adulticidal and Ovicidal activities of leaf extracts of *Tiliacora acuminata* on Japanese encephalitis vector *Culex vishnui* group

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**Abstract**: Vector of Japanese encephalitis is *Culex vishnui* group of mosquito and control of that mosquito is facing threat due to emergence of resistance to synthetic insecticides. Insecticides of plant origin now act as suitable alternate for control of JE vector. To investigate dose-dependent pupicidal, adulticidal and ovicidal activities, crude and acetone extracts of leaf of *Tiliacora acuminata* were used against *Cx. vishnui* group of mosquito. In case of dose-dependent pupicidal activity, highest mortality observed at 1.5% concentration of crude extract and 75 ppm of acetone extract with 45.67% and 67.67% mortality respectively after 36 h of exposure followed by 24h and 12h. In case of adulticidal activity, highest mortality in crude extract was observed at 2.5% concentration with 73% of adult mosquitoes were dead, but in acetone extract at 120 ppm concentration shows nearly about 67% mortality of adult mosquitoes after 24 h of exposure. While in ovicidal activity at 0.5% crude extract have 11.67% egg hatching so nearly about 88.33% ovicidal activity takes place at this concentration. In acetone extract, there was nearly about 93.33% ovicidal activities at 55 ppm concentration. So leaf extracts of *T. acuminata* may be used as better pupicidal, adulticidal and ovicidal plant origin insecticide for control of *Cx. vishnui* group of mosquito. Further research is required to isolate and characterize the active principle of *T. acuminata* plant leaf extract.

**Keywords**: *Tiliacora acuminate*, *Culex vishnui* group, Pupicidal, Adulticidal and Ovicidal activity.

## 1. Introduction

Mosquito, the term derived from the Sanskrit word ″Masak″, is a notorious creature because they pierce the host's skin for consumption of blood.

According to NVBDCP (National Vector Borne Disease Control Programme) under The Ministry of Health and Family Welfare, Government of India, *Culex vishnui* group (*Culex tritaeniorhynchus*, *Culex vishnui* and *Culex pseudovishnui*) is the chief vectors of JE in different parts of India. *Cx. vishnui* breeds preferably in the stagnant water of rice fields.

Japanese encephalitis which is previously known as Japanese B encephalitis caused by the mosquito-borne Japanese encephalitis virus.[1]

Symptoms of most JE virus infections are moderate fever and headache but in some instances without any significant symptoms. The extreme symptoms of the disease are characterized by quick start of high fever, headache, neck stiffness, coma, seizures, spastic paralysis and death. Spreading of JE in new area has been associated with extensive rice cultivation especially by irrigation schedule.[2]

A multitude of prevention and control strategies have been developed against Japanese encephalitis such as proper treatment of affected person, vaccination and control of vector population to inhibit the transmission of JE virus. We must take special emphasis on the third strategy to eradicate the spreading of JE.

From very ancient period's practice of using different synthetic insecticides to control mosquito population is a common phenomenon in Cosmo tropical areas. But in the recent year, their use declines drastically in many countries due to the appearance of insect resistance, biomagnification through food chain and non-biodegradable properties.

In the recent past, natural product of plant origin is experimentally used to control varieties of insect pest or vectors.

The extract which bears the active components of plants is applied for mosquito control programming. Repellent, ovipositional attractants, deterrent and insect growth hormone regulator activity of different plant extracts have been reported from the work of different authors.[3,4,5] Some phytochemicals act as growth, development inhibitor, chemosterilant and repellent or

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attractant to mosquitoes.[6] Nearly about 200 plants belonging to following families, i.e. Rutaceae, Miliaceae, Brassicaceae, Labiatae, Asteraceae, Solanaceae, Cladophraceae and also some member of the family Solanaceae have been reported for mosquitocidal activity.[7, 8, 9, 10, 11]

*Tiliacora acuminata* (Lam.) Hook. f. & Thom. (Menispermaceae) is a very useful plant where all of the parts are efficiently used for various medicinal activities. It is used as an antidote for snake bite by tribal population. Juice of plant leaf paste is applied to cuts and bitten area. Crude and solvent extract of *T. acuminata* flowers shown high activity against the larval form of *Culex quinquefasciatus*.[12] In this study, the possible inhibitory effect of dose-dependent pupicidal, adulticidal and ovicidal effect of crude and acetone extract of leaf of *T. acuminata* were tested against Japanese encephalitis vector *Cx. vishnui* group.

## 2. Material and Methods

### 2.1 Collection of larvae

The present study was conducted at Vivekananda Mahavidyalaya, Burdwan, Department of Zoology, West Bengal, India. The eggs of *Culex vishnui* group of mosquitoes were collected from rice fields. They were reared in plastic trays of (12.6 x 10 x 6 inches$^3$) volume, containing dechlorinated tap water. The larvae were fed with artificial food, i.e. dog biscuits and dried yeast, in the ratio of 3:1. When larvae of *Cx. vishnui* group mosquito transformed into pupae and the pupae were separated from the tray to a 500ml beaker containing tap water. The transformation procedure was done manually with the help of a glass dropper. The beaker was kept in an insect cage of 12.6″ x 10″ x 6″ for emergence of adult mosquitoes. Adult mosquitoes were fed with 10% glucose soaked in cotton ball for glucose meal. Blood feeding of these mosquitoes was done after second day of their emergence and every 3rd day thereafter from an albino rat. A filter paper was placed in a beaker in the cage for mosquitoes to lay their eggs. The filter paper containing laid eggs was transformed into a larval basin for maintenance of colony.

### 2.2 Preparation of crude extract

Fresh mature and green leaves of *T. acuminata* were randomly harvested from Debipur, Burdwan, West Bengal, India. Leaves were initially rinsed with tap water followed by distilled water to remove dust, debris from leaves, and dried on a paper towel. Finally, dried leaves were chopped into small pieces of approximately 1cm size. The ground materials are progressively passing through cheesecloth and Whatman No. 1 filter paper. Required concentration of crude extract was made by mixing of stalk solution with required amount of distilled water.

### 2.3 Preparation of solvent extracts

Collected mature leaves were dried in the shade at room temperature and were crushed into fine particles with Jankel and Kunkel model A10. Weighed (250g) finely grounded leaves were put into a Soxhlet apparatus for solvent extraction procedure. Plant extracts were prepared using solvents of increasing polarity (nonpolar to polar) namely petroleum ether, benzene, ethyl acetate, chloroform: methanol (1:1, V/V), acetone and absolute alcohol, applying successively (extraction period 72 hours for each solvent) with the same leaves. The extracts were collected separately and acetone extract was used for further experimentation. Eluted material of acetone extract was concentrated below 40°C temperature to 100ml of solution by evaporation using rotary evaporator. The solid residue was weight and then dissolved in a suitable amount of sterilized distilled water to make the different graded concentrations.

### 2.4 Dose-dependent pupicidal bioassay

The pupicidal bioassay was performed by the standard method as prescribed by WHO (1975).[13] Crude extract of mature leaves (0.5, 1 and 1.5%) and acetone solvent extract (25 ppm, 50 ppm and 75 ppm) was transferred into a glass beaker of 250ml capacity volume. Ten early pupae of *Cx. vishnui* group was introduced into different beaker containing appropriate concentrations. Mortality of mosquito larvae was counted after 12 h, 24 h and 36 h respectively. The experiments were conducted on three different days with 3 replicates on each day (n=9) at 25-30°C temperature and 80-90% relative humidity. A set of control experiments (without the test solution) using tap water for pupae was also parallel on each day of experiments (n=3).

### 2.5 Adulticidal activity

Adulticidal bioassay was performed by the standard method as prescribed WHO (1981).[14] For the adulticidal activity, the concentration of the crude extract (1, 1.5, 2 and 2.5%) and acetone solvent extract (30, 60, 90 and 120 ppm) of mature leaves of *T. acuminata* were prepared. 4ml of each crude and acetone extract concentration were used for impregnating the filter paper (140mm x 115mm). For control experiment, only acetone was used to impregnate the paper. According to WHO, adulticidal bioassay was conducted by using 3-7 days old adult female mosquitoes of *Cx. vishnui* group as in batches of 15 in number. Each test specimen was held for three hours of exposures and mosquitoes were placed in tube and 10% sugar solution was supplied as the food. The test was repeated for three times. Mortality of mosquito was recorded after 8 h, 16 h and 24 h of exposure was used when control experiment shows minimum mortality.

### 2.6 Ovicidal activity

For ovicidal activity, freshly laid eggs were collected using ovitraps in mosquito cage. In ovitraps used 2 days old blood-fed female mosquitoes. The eggs were laid on filter paper that kept in the ovitrap. Hundred gravid female mosquitoes of *Cx. vishnui* group were kept in a cage where ten oviposition cups were positioned for oviposition and oviposition start at dusk period. Five cups were treated with test solution of crude extract (0.1, 0.2, 0.3, 0.4 and 0.5% concentration) and another five were treated with acetone extracts (15, 25, 35, 45 and 55 ppm concentration). In case of control experiments, two cups were used, one was filled with 100ml of distilled water and another was with respective solvent. 100 eggs were used for each experiment and the experiment was replicated five times. Eggs were collected through muslin cloth and kept in plastic cups filled with distilled water for hatching experiment after counting the eggs under microscope. The percent egg mortality was measured on the basis of non-hatchability of eggs.[15] The hatching rate of eggs was assessed after 72 h post-treatment.[16]

### 2.7 Statistical analysis

Statistical analyses of the experimental data were performed using the computer software "STAT PLUS 2007 (trial version)" and "MS EXCEL 2002" to find out the $LC_{50}$ and $LC_{90}$ lethal values, regression equations and regression coefficient values.

## 3. Results and Discussion

### 3.1 Dose-dependent pupicidal activity

The effect of crude and acetone solvent extract of mature leaves of *T. acuminata* tested against pupae of *Cx. vishnui* group. The highest mortality observed at 1.5% concentration of crude extract and 75 ppm of acetone extract with 45.67% and 67.67% mortality, respectively, after 36 h of exposure followed by 24 h and 12 h (Table 1).

Probit analysis of mortality rates in crude and acetone extract are shown in (Table 2). The lowest values of $LC_{50}$ for both crude and acetone extract were 3.908% and 1.3488 ppm, respectively, after 36h of exposure, whereas $LC_{90}$ of both extracts were 4.916% and 49.121 ppm respectively for crude and acetone extract after 36 h of exposure.

Result of regression analysis represented that the mortality rates (Y) were positively correlated with concentration (X) and regression coefficient value are close to one (Table 2).

Two ways ANOVA of dose-dependent pupicidal activity of *Cx. vishnui* group mosquito pupae by *T. acuminata* leaf crude extract had significant effect on mortality rates of pupae. There was also no significant difference arisen when factors were interacted such as hour and concentration (Table 3). But two Ways ANOVA of pupal mortality by acetone extract have significant effect on mortality when factors, i.e. hour and concentration interacted (Table 4).

### 3.2 Adulticidal activity

In adulticidal activity, both adult mosquitoes i.e. male and female of *Cx. vishnui* group were exposed to 1%, 1.5%, 2% and 2.5% concentration of crude extract and 30, 60, 90, 120 ppm concentration of acetone extract (Table 5). Highest mortality in crude extract was observed at 2.5% concentration with 73% of adult mosquitoes were dying after 24 h of exposure.

But in acetone extract of *T. acuminata* at 120 ppm concentration show nearly about 67% mortality of adult mosquitoes after 24 h of exposure.

Probit analysis revealed the $LC_{50}$ and $LC_{90}$ values of crude and acetone extracts gradually decrease from 8 to 24 hours. The lowest $LC_{50}$ values of crude and acetone observed after 24 h of exposure were 1.121% and 67.4109 ppm respectively. But the lowest $LC_{90}$ values were 5.5116% after 24 h and 4.99 ppm after 16 h of exposure. The regression analyses revealed the positive correlation between mortality (Y) and concentration of exposed (X) having regression coefficient value close to one (Table 6).

Two ways ANOVA of adulticidal activity of crude extract of *T. acuminata* on *Cx. vishnui* group where hour and concentration act as variables had significant effect on adult mortality. There was also significant difference arisen when the factors hour and concentration interacted (Table 7).

Two ways ANOVA of adulticidal activity of acetone extract had also significant result when factors hour and concentration interacted and have significant *p*-value of their variables (Table 8).

### 3.3 Ovicidal activity

Ovicidal activity of crude and acetone extracts of *T. acuminata* mature leaves were done against the eggs of *Cx. vishnui* group mosquitoes. In case of crude extract of *T. acuminata* mature leaf, 0.1% concentration exhibited 77.33% eggs hatching potentiality that means there was only 22.67% ovicidal activity. While at 0.5% crude extract has 11.67% egg hatching. So nearly about 88.33% ovicidal activities take place at this concentration.

In acetone extract, there was nearly about 93.33% ovicidal activities at 55 ppm concentration of extract (Table 9). So acetone extract possesses greater ovicidal activity than crude extract.

**Table 1. Mortality rates or Dose dependant pupicidal bioassay of pupae of *Cx. vishnui* group in crude and acetone extracts of mature leaves of *T. acuminate.***

| Crude extract | | | Acetone | |
|---|---|---|---|---|
| **Time of exposure** | **Conc. (%)** | **% Mortality** | **Conc. (ppm)** | **% Mortality** |
| **12h** | 0.5 | 27.67±0.33 | 25 | 031.67±0.67 |
| | 1 | 32.33±0.67 | 50 | 037.67±0.67 |
| | 1.5 | 35.67±0.33 | 75 | 536.67±0.33 |
| **Control** | | 00.00±0.00 | Control | 000.00±0.00 |
| **24h** | 0.5 | 29.33±0.33 | 25 | 049.33±0.67 |
| | 1 | 34.33±0.67 | 50 | 052.33±0.67 |
| | 1.5 | 38.33±0.67 | 75 | 061.67±0.33 |
| **Control** | | 0.00±0.000 | Control | 000.00±0.00 |
| **36h** | 0.5 | 40.33±0.33 | 25 | 00063±1.00 |
| | 1 | 43.00±0.58 | 50 | 065.33±0.33 |
| | 1.5 | 45.67±0.67 | 75 | 067.67±0.67 |
| **Control** | | 00.00±0.00 | Control | 000.00±0.00 |

**Table 2. Probit analyses and regression analyses of mortality rates of pupae of *Cx. vishnui* group in crude and acetone solvent extract of mature leaves of *T. acuminate.***

| Solvent used | Hour of exposure | $LC_{50}$ (%) | $LC_{90}$ (%) | LCL-UCL ($LC_{50}$) | Regression equation | $R^2$ value |
|---|---|---|---|---|---|---|
| **Crude** | 12 | 9.2145 | 15.467 | 2.9207-8.297 | Y=8X+23.889 | 0.9515 |
| | 24 | 5.8475 | 7.5238 | 2.398-12.448 | Y=9X+25 | 0.9492 |
| | 36 | 3.9081 | 4.9160 | 0.3157-3.859 | Y=5.3333X+37.667 | 0.8889 |
| **Solvent used** | **Hour of exposure** | **$LC_{50}$ (ppm)** | **$LC_{90}$ (ppm)** | **LCL-UCL ($LC_{50}$)** | **Regression equation** | **$R^2$ value** |
| **Acetone** | 12 | 73.547 | 101.21 | 60.38-104.51 | Y=0.4385X+18.977 | 0.923 |
| | 24 | 29.759 | 38.882 | 10.093-41.01 | Y=0.2457X+42.107 | 0.8911 |
| | 36 | 1.3488 | 49.121 | 0.0039-46.65 | Y=0.0936X+60.633 | 0.783 |

**Table 3. Two ways ANOVA of dose-dependent pupicidal activity of *Cx. vishnui* group mosquito pupae by *T. acuminata* crude extract.**

| Source | Sum of Squares | df | Mean Square | *F*-value | *p*-value |
|---|---|---|---|---|---|
| **Hour** | 1784.063 | 2 | 892.031 | 6.598 | .006 |
| **Conc.** | 977.526 | 2 | 488.763 | 3.615 | .046 |
| **Hour* conc.** | 465.887 | 4 | 116.472 | .86200 | .504 NS |
| **Total** | 53772.000 | 29 | | | |
| **Corrected Total** | 6890.759 | 28 | | | |

*Significant at $p<0.05$; NS: Not significant

**Table 4. Two ways ANOVA of dose-dependent pupicidal activity of *Cx. vishnui* group mosquito pupae by *T. acuminata* acetone extract.**

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Hour** | 2674.296 | 2 | 1337.148 | 1128.219 | .000 |
| **Conc.** | 804.963 | 2 | 402.481 | 339.594 | .000 |
| **Hour* conc.** | 251.926 | 4 | 62.981 | 53.141 | .000 |
| **Total** | 81301.000 | 27 | | | |
| **Corrected Total** | 3752.519 | 26 | | | |

*Significant at $p<0.05$; NS: Not significant

**Table 5. Adulticidal activity of crude and acetone extract of leaf of *T. acuminata* on rice field mosquitoes *Cx. vishnui* group.**

| Extract | Conc. | % Mortality ± SE | | |
|---|---|---|---|---|
| | | **8 h** | **16 h** | **24 h** |
| **Crude extract** | 1% | 27.67±0.67 | 40.67±0.33 | 47.67±0.67 |
| | 1.5% | 33.67±0.33 | 47.33±0.33 | 56.33±0.33 |
| | 2% | 53.67±0.33 | 60.67±0.33 | 67.67±0.67 |
| | 2.5% | 67.33±0.33 | 70.67±0.67 | 73.00±0.58 |
| **Control** | | 00.00±0.00 | 00.00±0.00 | 00.00±0.00 |
| **Acetone extract** | 30 ppm | 11.67±0.33 | 18.00±1.00 | 27.33±0.33 |
| | 60 ppm | 21.67±0.67 | 28.33±0.67 | 46.67±0.33 |
| | 90 ppm | 37.67±0.67 | 44.00±1.00 | 57.67±0.33 |
| | 120 ppm | 47.67±0.67 | 58.33±0.67 | 67.33±0.33 |
| **Control** | | 00.00±0.00 | 00.00±0.00 | 00.00±0.00 |

**Table 6. Probit and regression analyses of adulticidal activity of crude and acetone extract of leaf of *T. acuminata* on rice field mosquitoes *Cx. vishnui* group.**

| Solvent used | Hour of exposure | $LC_{50}$ (%) | $LC_{90}$ (%) | LCL-UCL ($LC_{50}$) | Regression equation | $R^2$ value |
|---|---|---|---|---|---|---|
| Crude | 8 | 1.8365 | 5.5118 | 1.1639-7.1533 | Y=27.8X-3.0667 | 0.9632 |
| | 16 | 1.4271 | 6.4398 | 1.2849-1.5553 | Y=20.667X+18.667 | 0.9828 |
| | 24 | 1.1210 | 6.1791 | 0.931-1.2648 | Y=17.467X+30.6 | 0.9764 |
| **Solvent used** | **Hour of exposure** | **$LC_{50}$ (ppm)** | **$LC_{90}$ (ppm)** | **LCL-UCL ($LC_{50}$)** | **Regression equation** | **$R^2$ value** |
| Acetone | 8 | 133.2644 | 605.74 | 117.292-158.468 | Y=0.4133X-1.333 | 0.987 |
| | 16 | 103.4148 | 499.0313 | 93.145-117.972 | Y=0.4556X+3 | 0.9873 |
| | 24 | 67.41090 | 606.8954 | 61.0475-74.4388 | Y=0.4367+17 | 0.9698 |

**Table 7. Two way ANOVA of adulticidal activity of crude extract of leaf of *T. acuminata* on rice field mosquitoes *Cx. Vishnui* group where hour and concentration as variables.**

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Hour** | 1474.056 | 2 | 737.028 | 1020.500 | .000 |
| **Conc.** | 5524.750 | 3 | 1841.583 | 2549.885 | .000 |
| **hour* conc.** | 268.167 | 6 | 44.694 | 61.885 | .000 |
| **Total** | 111721.000 | 36 | | | |
| **Corrected Total** | 7284.306 | 35 | | | |

*Significant at $p<0.05$; NS: Not significant

**Table 8. Two ways ANOVA of adulticidal activity of acetone extract of leaf of *T. acuminata* on rice field mosquitoes *Cx. Vishnui* group where hour and concentration as variables.**

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Hour** | 2471.722 | 2 | 1235.861 | 1034.674 | .000 |
| **Conc.** | 7684.972 | 3 | 2561.657 | 2144.643 | .000 |
| **hour* conc.** | 114.944 | 6 | 19.157 | 16.039 | .000 |
| **Total** | 64667.000 | 36 | | | |
| **Corrected Total** | 10300.306 | 35 | | | |

*Significant at $p<0.05$; NS: Not significant

**Table 9. Ovicidal activity of crude and acetone extract of mature leaves of *T. acuminata* on eggs of *Cx. vishnui* group mosquitoes.**

| Extract used | Conc. | % of egg hatching (Ovicidal activity) |
|---|---|---|
| Crude | 0.1% | 77.33±0.33 (100-77.33=22.67 Ovicidal activity) |
| | 0.2% | 53.33±0.33 (100-53.33=46.67 Ovicidal activity) |
| | 0.3% | 33.67±0.33 (100-33.67=66.33 Ovicidal activity) |
| | 0.4% | 26.67±0.33 (100-26.67=73.33 Ovicidal activity) |
| | 0.5% | 11.67±0.33 (100-11.67=88.33 Ovicidal activity) |
| Acetone | 15 ppm | 65.67±0.67 (100-65.67= 34.33 Ovicidal activity) |
| | 25 ppm | 50.33±0.33 (100-50.33= 49.67 Ovicidal activity) |
| | 35 ppm | 30.33±0.33 (100-30.33=69.67 Ovicidal activity) |
| | 45 ppm | 17.67±0.67 (100-17.67=82.33 Ovicidal activity) |
| | 55 ppm | 6.670±0.33 (100-6.67=93.33 Ovicidal activity) |

## 4. Conclusion

In this research work, only two extracts, i.e. crude and acetone extracts of leaf of *T. acuminate* was used against pupae, adult and on eggs of *Cx. vishnui* group of mosquito to observe dose-dependent pupicidal, adulticidal and ovicidal activity of extracts. Here acetone extract shows better potentiality rather than crude extract on three life cycle stage of *Cx. vishnui* mosquito i.e. egg, pupae and adult. So further research is necessary to isolate and characterized the active principle of acetone extract of that plant leaf for better application in future to control Japanese encephalitis disease.

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## Conflict of interest

The author declares no conflict of interest.

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