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# SEASONAL VARIATION IN MOSQUITO LARVICIDAL POTENTIALITY OF *Holoptelea integrifolia*

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**AUTHORS' CONTRIBUTIONS**

This work was carried out in collaboration between both authors. Author SS contributed to methodology, data collection, statistical analysis, writing original manuscript. Author GC contributed supervision of total work, checking and editing of manuscripts. Both authors read and approved the final manuscript.

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## ABSTRACT

Japanese encephalitis (JE) which was previously known as Japanese B encephalitis, is caused by the Japanese encephalitis virus. Plant produced the photochemical mainly for their own defense mechanism is experimentally used to control varieties of insect pests or vectors. Crude extract (0.1%, 0.2%, 0.3%, 0.4% and 0.5%) of leaves of *Holoptelea integrifolia* when applied on third instars mosquito larvae in successive seasons i.e. Summer, Rainy and Winter, caused 100 percent mortality at 0.5% concentration in summer after 72 h of exposure. In Winter, also a 100 percent mortality of the third instars of *Cx. vishnui* group larvae were noticed after 48 h of exposure at 0.5% concentration. Out of three predominant seasons in West Bengal, variation in the mortality of *Cx. vishnui* group larvae by crude extract of *H. integrifolia* leaf are well established and mortality increases with higher extract concentration and time of exposure. Highest mortality was noted in Rainy season followed by Summer and Winter.

**Keywords:** *Holoptelea integrifolia*; *Culex vishnui*; mortality; mosquito; larvae; seasonal variation.

## 1. INTRODUCTION

Mosquito belonging to the family Culicidae is divided into three sub families namely Culicinae, Anophelinae, and Toxorhynchitinae, in which all the disease transmitting vectors are included. Female mosquito uses blood meal as protein and vitamin source for egg development. According to NVBDCP (National Vector Borne Disease Control programme) under The Ministry of Health and Family Welfare, Government of India. *Culex vishnui* is the chief vector of Japanese Encephalitis in different parts of India,

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including West Bengal. *Culex vishnui* Theoblad belongs to the *Cx. vishnui* group and is the most important vector of JE in India, Srilanka and Thailand [1].

Japanese encephalitis which was previously known as Japanese B encephalitis is caused by the Japanese encephalitis virus [2]. The Japanese encephalitis virus belongs to the Flaviviridae family. Symptoms of most JE virus infections are moderate fever and headache, but in some instances there are no 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 a new area has been associated with extensive rice cultivation, especially under an irrigation schedule [3].

A multitude of prevention and control strategies have been developed against Japanese encephalitis, such as proper treatment of affected persons, vaccination and control of vector population to inhibit the transmission of JE virus. Control of mosquitoes at their larval stage is more rational because during this time they are restricted in a particular habitat. So attempting different strategies to control larvae is more significant than adult control. Adverse effects on the environment with the application of synthetic insecticides shifted the research towards alternative sources that must be cheap and lack effects on the ecosystems.

In the recent past natural product of plant origin have been experimentally used to control varieties of insect pests or vectors. Plant produced phytochemicals as a mechanism mainly for their own defense. Different secondary metabolites i.e. alkaloids, steroids, terpenoids, tannins, and flavonoids have been reported for their insecticidal properties [4].

The mosquito *Holoptelea integrifolia* (Roxb.) is also known as *Ulmus integrifolia* (Roxb.) and belongs to the family Ulmaceae that have 15 genera and about 200 species distributed over tropical and temperate region of the Northern hemisphere, including from the Indian peninsula to Indo China and Srilanka [2].

## 2. METHODOLOGY

### 2.1 Preparation of Crude Extract

Fresh mature and green leaves of *Holoptelea integrifolia* were randomly harvested from Debipur, Burdwan, West Bengal, India. Leaves were initially rinsed with tap water followed by distilled water to remove dust and debris from leaves, then they were dried on paper towel. Finally dried leaves were chopped into small pieces of approximately 1cm size by a sharp razor and crushed with a mortar and pestle. The ground materials are progressively passing through cheese cloth and what man No 1 filter paper. Required concentration of crude extract was made by mixing stock solution with required amount of distilled water.

### 2.2 Bio Assay

The bioassay experiments were conducted according to standard WHO procedure [5] with slight modification.

### 2.3 Bio Assay with Crude Extract of Mature Leaves

Crude extract of mature leaves of *H. integrifolia* was applied for bioassay experiments at five different concentrations viz 0.1%, 0.2%, 0.3%, 0.4% and 0.5%.Prepared concentrations of crude extract were transformed into a sterile glass Petri dish (9 cm diameter /150 ml capacity) containing 100 ml of tap water. Twenty five third instar larvae of *Cx. vishuni* group mosquito were treated separately [5]. The five crude prepared extract concentrations were applied on third instars larvae. This particular stage was identified on the basis of the number of thoracic setae and plates to distinguish them from other instars [6]. All the experiments were repeated for three times on different days, and control experiment was also done at the same conditions. The test containers were held at 25-28$^0$C and subjected to a photoperiod of 12h light followed by 12 h dark. For long exposure, larval food was added to the test container, if high mortality was noted in the control group. Tap water was used in the control experiment and the experiment was conducted without supply of food to larvae due to insignificant mortality at control experiment.

Larvae were considered died if they were unrousable within a period of time, even when gently probed with the help of a needle. Larval mortality was recorded after 24, 48 and 72 h of exposures and was expressed by the addition of mortality at 24 and 48 h respectively. Percentage mortality was calculated from the average of three replication of each experiment.

### 2.4 Statistical Analysis

Statistical analyses of collected data were done through SPSS version 11, Microsoft Excel software.

## 3. RESULTS

One-hundred? percent mortality was observed at 0.5% concentration in summer after 72h of exposure where

as in Rainy season some result observed at same concentration just after 48 h of exposure.

In winter season also one-hundred? percent mortality of third instars *Cx. vishnui* group larvae were noticed after 48 h of exposure at 0.5% concentration (Table 1).

Log Probit analyses revealed that $LC_{50}$ values gradually decreases with increasing hour of exposure. From the Table 2, lowest $LC_{50}$ value obtained from winter season after 72h of exposure (0.2447). So winter season showed a more potent larvicidal activity. Result of regression analyses revealed that the mortality rate (Y) were positive correlated with concentration (X). (Table 2) Multivariate ANOVA (Table 3) revealed that hour, season and test concentration had significant effect on mortality of *Cx. vishnui* group of larvae. The Tukeys' test on seasonal effect on mortality rate revealed significant difference between summer, winter and rainy (Table 4). Significant difference on toxicity was found between 24h and 72h (Table 5), and between lowest and highest concentration (Table 6). For those effects mature leaves those collected during Rainy and Summer were used for further studies.

**Table 1. Seasonal variation in the efficacy of crude extract of *H. integrifolia* leaf on rice field mosquito *Cx. vishuni* group on third instars larval form**

| Season | Conc. (%) | Mortality of mosquitoes larvae | | |
|---|---|---|---|---|
| | | 24 h | 48 h | 72 h |
| Summer | 0.1 | 33.67±0.33 | 36.67±0.67 | 56.67±0.33 |
| | 0.2 | 46.67±0.33 | 53.67±0.67 | 72±1.00000 |
| | 0.3 | 60.67±0.33 | 66.67±0.33 | 83.67±0.33 |
| | 0.4 | 73.33±0.33 | 84.67±0.33 | 93.67±0.67 |
| | 0.5 | 90.67±0.67 | 095±1.000 | 100±0.0000 |
| Control | | 0.00±0.00 | 00.00±0.00 | 0.00±0.000 |
| Rainy | 0.1 | 42.33±0.67 | 51.67±0.33 | 72±1.00000 |
| | 0.2 | 53.33±0.33 | 66.67±0.33 | 74.33±0.67 |
| | 0.3 | 73.67±0.33 | 80.67±0.33 | 85.67±0.67 |
| | 0.4 | 76.67±0.33 | 83.67±0.33 | 92.33±0.89 |
| | 0.5 | 97.67±0.67 | 100±0.000 | 100±0.0000 |
| Control | | 00.00±0.00 | 00.00±0.00 | 00.00±0.00 |
| Winter | 0.1 | 03.67±0.33 | 04.67±0.33 | 9.33±0.670 |
| | 0.2 | 19.67±0.89 | 27.67±0.89 | 29.33±1.20 |
| | 0.3 | 43.33±0.67 | 48.33±0.67 | 57.67±0.89 |
| | 0.4 | 64.33±1.45 | 69.0±0.580 | 78.33±0.67 |
| | 0.5 | 93.33±0.67 | 100.0±0.00 | 100.0±0.00 |
| Control | | 00.00±0.00 | 0.00±0.00 | 0.00±0.000 |

**Table 2. Probit analysis and regression equation of seasonal variation in efficacy of *H. integrifolia* crude extract against third instars form of *Cx. vishnui* group of larvae**

| Larval instars | Hour of exposure | $LC_{50}$ | $LC_{90}$ | LCL-UCL($LC_{50}$) | Regression equation | $R^2$ value |
|---|---|---|---|---|---|---|
| Summer | 24 | 0.1866 | 0.7392 | 0.0759-0.2737 | Y=138.28x+19.278 | 0.9972 |
| | 48 | 0.1597 | 0.5321 | 0.0671-0.2269 | Y=150.06x+22.556 | 0.9929 |
| | 72 | 0.0965 | 0.3277 | 0.0186-0.1499 | Y=110.72x+48.222 | 0.9743 |
| Rainy | 24 | 0.1459 | 0.5575 | 0.0046-0.2386 | Y=131.11x+29.111 | 0.9606 |
| | 48 | 0.1086 | 0.4241 | 0.0031-0.1803 | Y=113.06+42.556 | 0.9622 |
| | 72 | 0.0587 | 0.3193 | 0.0128-0.2695 | Y=73.722x+62.722 | 0.9596 |
| Winter | 24 | 0.3023 | 0.5788 | 0.2246-0.395 | Y=220.39x-21.611 | 0.9893 |
| | 48 | 0.2732 | 0.5208 | 0.1618-0.3919 | Y=228.94x-19.056 | 0.9927 |
| | 72 | 0.2447 | 0.4854 | 0.1512-0.3361 | Y=231.17x-14.333 | 0.9954 |

**Table 3. Multivariate ANOVA of mortality of third instars larvae of *Cx. vishnui* group in different seasons, different hours and different concentrations as variables**

| Source | Sum of Squares | df | Mean Square | F -value | P- Value. |
|---|---|---|---|---|---|
| Season | 17440.933 | 2 | 8720.467 | 7404.170 | .000 |
| Hour | 5435.733 | 2 | 2717.867 | 2307.623 | .000 |
| Concentration | 65898.993 | 4 | 16474.748 | 13987.994 | .000 |
| Season * hour | 479.333 | 4 | 119.833 | 101.745 | .000 |
| Season * conc. | 7668.030 | 8 | 958.504 | 813.824 | .000 |
| Hour * conc. | 679.674 | 8 | 84.959 | 72.135 | .000 |
| Season * hour * conc. | 561.037 | 16 | 35.065 | 29.772 | .000 |
| Residual | 677257.000 | 135 | | | |
| Corrected Total | 98269.733 | 134 | | | |

**Significant at P < 0.05; N.S: not significant*

**Table 4. Multiple comparisons by Tukeys' HSD on seasonal variation on the Toxicity of *H. integrifolia* on *Cx. vishnui* group larvae**

| Multiple comparison | (I) season | (J) season | Mean Difference (I-J) | Significance |
|---|---|---|---|---|
| | 1.00(Summer) | 2.00 | -6.867* | .000 |
| | | 3.00 | 19.933* | .000 |
| **Tukeys HSD** | 2.00(Rainy) | 1.00 | 6.867* | .000 |
| | | 3.00 | 26.800* | .000 |
| | 3.00(winter) | 1.00 | -19.933* | .000 |
| | | 2.00 | -26.800* | .000 |

**Significant at P < 0.05; N.S: not significant*

**Table 5. Multiple comparisons by Turkeys' HSD on hours wise variation on the Toxicity of *H. integrifolia* on *Cx. vishnui* group larvae**

| Multiple comparison | (I) hour | (J) hour | Mean Difference (I-J) | Significance |
|---|---|---|---|---|
| | 1.00 (24h) | 2.00 | -6.400* | .000 |
| | | 3.00 | -15.467* | .000 |
| **Turkeys' HSD** | 2.00 (48h) | 1.00 | 6.400* | .000 |
| | | 3.00 | -9.067* | .000 |
| | 3.00 (72h) | 1.00 | 15.467* | .000 |
| | | 2.00 | 9.067* | .000 |

**Significant at P < 0.05; N.S: not significant*

**Table 6. Multiple comparisons by Turkeys' HSD on concentrations wise variation on the Toxicity of *H. integrifolia* on *Cx. vishnui* group larvae**

| Multiple comparison | (I) conc. of crude extract | (J) conc. of crude extract | Mean Difference (I-J) | Significance |
|---|---|---|---|---|
| | 1.00 (0.1%) | 2.00 | -14.741* | .000 |
| | | 3.00 | -32.185* | .000 |
| | | 4.00 | -45.037* | .000 |
| | | 5.00 | -62.889* | .000 |
| | 2.00 (0.2%) | 1.00 | 14.741* | .000 |
| | | 3.00 | -17.444* | .000 |
| | | 4.00 | -30.296* | .000 |
| | | 5.00 | -48.148* | .000 |
| **Turkeys' HSD** | 3.00 (0.3%) | 1.00 | 32.185* | .000 |
| | | 2.00 | 17.444* | .000 |
| | | 4.00 | -12.852* | .000 |
| | | 5.00 | -30.704* | .000 |

| Multiple comparison | (I) conc. of crude extract | (J) conc. of crude extract | Mean Difference (I-J) | Significance |
|---|---|---|---|---|
| | 4.00 (0.4%) | 1.00 | 45.037* | .000 |
| | | 2.00 | 30.296* | .000 |
| | | 3.00 | 12.852* | .000 |
| | | 5.00 | -17.852* | .000 |
| | 5.00 (0.5%) | 1.00 | 62.889* | .000 |
| | | 2.00 | 48.148* | .000 |
| | | 3.00 | 30.704* | .000 |
| | | 4.00 | 17.852* | .000 |

**Significant at P < 0.05; N.S: not significant*

## 4. DISCUSSION

In recent years insecticides of botanical origin are much preferred over synthetic insecticides because they are cheap, biodegradable, and have no harm on ecosystems [7,8,9]. Larvae are restricted in their breeding habitats, so they are the softest target for control programmes. Different secondary metabolites which are mainly obtained from plant source have wide range of biological activities. According to Jackson, 1958 [10] plant products are used more traditionally for pest or vector control programmes. Different products of plant origin are used more successfully for biocontrol [11,12,13,14,15]. The present study revealed that mature leaves of *H. integrifolia* have good larvicidal effect on third instar larvae of *Cx. vishnui* group mosquito, vectors of Japanese Encephalites. Seasonal variation in the activity of crude extract of *H. integrifolia* leaf was also noticed in three prevalent seasons (Summer, Rainy and Winter) in West Bengal. It may be due to seasonwise variation in the amount of primary and secondary biochemicals of leaves of *H. integrifolia*. This variation may be due to seasonwise change of temperature, humidity, amount of supplied water, pathogen attack. According to Singha et al. [16], acetone extract of leaf of *H. integrifolia* have very good larvicidal activity on *Cx. vishnui* mosquito. In that present study only crude extract was used for established of seasonal variation in mosquito larvicidal potential.

## 5. CONCLUSION

Season wise variation in the mortality of *Cx. vishnui* group larvae by crude extract of *H. integrifolia* leaf are well established where mortality incerases with increasing concentration of extract and time of exposure. Highest mortality was noted in Rainy season followed by Summer and Winter. Period of exposure, season and test concentration were considered, they have significant effect on ( $p < 0.05$) mortality rates of larvae of *Cx. vishnui* group.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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