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Original Article

Mosquito Larvicidal and Pupicidal Activity of *Tephrosia purpurea* Linn. (Family: Fabaceae) and *Bacillus sphaericus* against, Dengue Vector, *Aedes aegypti*

Ramesh Venkadachalam[1], Vijayakumar Subramaniyan[1]*, Manogar Palani[1], Mahadevan Subramaniyan[1], Prabhu Srinivasan[1] and Murugan Raji[2]

ABSTRACT

Objective: The bio-efficacy of *Tephrosia purpurea* leaf extract and bacterial insecticide, *Bacillus sphaericus* larvicidal activity was assessed against the first to fourth instars larvae of *Aedes aegypti,* under the laboratory conditions. **Methods**: The plant material was shade dried at room temperature and powdered coarsely. *T. purpurea* and *B. sphaericus* show the various concentrations of larvicidal and pupicidal activity against various instars larvae of *A. aegypti*. **Results**: The LC_{50} of *T. purpurea* against the first to fourth instars larvae were 139.24, 176.24, 219.28, 256.27, and 326.29 ppm and the 480.72, 541.21, 580.34, 672.20, and 762.80 ppm, respectively. *B. sphaericus* against the first to fourth instars larvae the LC_{50} values were 46.16, 56.23, 69.82, 80.81 and 96.12 ppm and the LC_{90} values 141.68, 172.46, 184.21, 193.31 and 218.16 ppm, respectively. However, the combined treatment of *T. purpurea* + *B. sphaericus* (1:2) material shows highest larvicidal activity of the LC_{50} values 80.08, 82.21, 88.00, 92.21 and 98.16 ppm; The LC_{90} values of 108.39, 118.71, 136.75, 149.02 and 153.24 ppm, against *A. aegypti* in all the tested concentrations than the individuals and clearly established that there is a substantial amount of synergist act. **Conclusion**: The present study reported that both *T. purpurea* and *B. sphaericus* materials could serve as a potential larvicidal agent. Since, *A. aegypti* is a container breeder vector mosquito this user and eco-friendly and low-cost vector control strategy could be a viable solution to the existing dengue disease burden. Therefore, this study provides first report on the mosquito larvicidal activity the combined effect of *T. purpurea* leaf extract and *B. sphaericus* against as target species of *A. aegypti*.

Key words: *Tephrosia purpurea, Bacillus sphaericus, Aedes aegypti,* Dengue vector, Larvicidal activity.

Ramesh Venkadachalam[1], Vijayakumar Subramaniyan[1], Manogar Palani[1], Mahadevan Subramaniyan[1], Prabhu Srinivasan[1] and Murugan Raji[2]

[1]P.G. and Research Department of Botany and Microbiology, A.V.V.M. Sri Pushpam College (Autonomous), Poondi-613503, Thanjavur district, Tamil Nadu, INDIA.
[2]Department of Botany Govt Arts College (Autonomous), Kumbakonam 612002. Tamil Nadu, INDIA.

Correspondence

Vijayakumar. S.

P.G. and Research Department of Botany and Microbiology, A.V.V.M.
Sri Pushpam College (Autonomous),
Poondi-613503, Thanjavur district,
Tamil Nadu, INDIA

Phone no: +91-9003311921

E-mail: svijaya_kumar2579@rediff.com

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INTRODUCTION

Mosquitoes are the root cause of many diseases, especially dengue, malaria, and Chikungunya. These are endemic diseases in Asia and Africa, which also affect severely on human health.[1,2] *Aedes aegypti* is responsible for vector diseases.[3,4] According to world health organization (WHO) postulated globally 400 million peoples are currently affected by dengue. Day today in India dengue fever is increased due to the water pollution, especially Delhi, a city in North India, is endemic for dengue infection with last reported in 2014.[5]

Mosquitoes spread dengue fever by way of biting. This kind of dengue virus is known as Flavi virus. It has four types they are DENV 1, DENV 2, DENV 3, DENV 4.[6,7] Thsese four are some way or other have distinct *Aedes aegypti* transmit dengue in large number. This type of mosquitoes breeds only still waters. In general, it bites a human being at day time for 2-7 days in the infected person's blood this virus is being circulated. Gradually mosquito transforms dengue from one person to another in the way of bitting infected persons.[8]

One of the method to control the growth of dengue causing mosquito by synthetic insecticides.[9] This method have some disprove such as environmentally contaminated by air, water and soil. There is best way to replace by a synthetic method by green evolution. Current scenario has proved the plant extracts are alternative larvicides because their derivatives were used to kill the mosquitoes. *Tephrosia purpurea* is a flowering plant and its belongs to the Pea family, this plant containing many properties.[10] This is used to kill the mosquitoes, which cause dengue. And also it has been recommended as a blood purifier.[11] It cures diseases apart from dengue like bronchitis, bilious febrile attacks, liver obstructions, spleen and kidney.[12,13] They are two important types of mosquitoes existing in the world, namely nuisance and vector mosquitoes. For the past twenty years, bacterial larvicides like *Bacillus thuringiensis* and *Bascillus sphaericus* are being used in order to control these two mosquitoes. *Bacillus sphaericus*, a soil bacterium that kills mosquito larvae in water. It is developed

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as commercial larvicide. But in several countries that is not used in out-number.[14-16]

In this research, the methanolic leaf extract of *T. purpurea* and bacterial insecticide of *B. sphaericus* were tested against larval and pupal of Dengue vector for analyzing their inhibitory activity. The larvicidal and pupicidal activities were checked by the under laboratory as well as field conditions.This plant was selected based on their ethnobotanical literatures.[17] Therefore this study provide first report mosquito larvicidal and pupicidal activity, combined effect of *T. purpurea* leaf extract *B. Sphaericus against A. aegypti*.

MATERIALS AND METHODS

Collection of plant and preparation of extract

T. purpurea plant was collected from in and around the A.V.V.M.Sri Pushpam College, Campus, Poondi, Thanjavur. The plant was identified at The Rapinat Herbarium, St. Joseph's College, Trichy, India. *T. purpurea* leaves were washed with tap water and shade dried at room temperature. The dried plant materials (leaves) were powdered by an electrical blender. From the powder 500 g of the plant sample was extracted with 1.5 L of organic solvents of methanol for using a Soxhlet apparatus boiling point 60-80°C for h.[18] The extracts were filtered through a Buchner funnel with Whatman number 1 filter paper. The crude plant extracts were evaporated to dryness in rotary vacuum evaporator. 1g of the plant residue was dissolved in 100 mL of acetone (stock solution) considered as 1% stock solution. From this stock solution concentrations were prepared ranging from75, 150, 225, 300 and 375 ppm, respectively.

Collection of eggs and maintenance of larvae

The eggs of *A.aegypti* were collected from National Centre for Disease Control field station at Mettupalayam, Tamil Nadu, India, using an "O"-type brush. These eggs were brought to the laboratory and transferred to 18×13×4 cm enamel trays containing 500 mL of water for hatching. The mosquito larvae were pedigree dog biscuits and yeast at 3:1 ratio. The feeding was continued until the larvae transformed into the pupal stage.

Maintenance of pupae and adults

The pupae were collected from the culture trays and that are transferred into the to plastic containers (12×12 cm) containing 500 mL of water with the help of a dipper. The plastic jars were kept in a 90×90× 90 cm mosquito cage for adult emergence. Mosquito larvae were maintained at (27 ±2) °C, 75% - 85% relative humidity, under a photoperiod of 14:10 (light/dark). A 10% sugar solution was provided for a period of 3 days before blood feeding.

Blood feeding of adult *A.aegypti*

The adult female mosquitoes were allowed to feed on the blood of a rabbit (a rabbit per day, exposed on the dorsal side) for 2 days, to ensure adequate blood feeding for 5 days. After blood feeding, enamel trays with water from the culture trays were placed in the cage as oviposition substrates.

Microbial bioassay

B. sphaericus was obtained from K.K Biotech, Lab Service, Chennai, Tamil Nadu, India. The organism was grown in a liquid medium containing (in g per liter of distilled water): $FeSO_4 \cdot 7H_2O$,0.01; $MnSO_4$, 0.1; $MgSO_4 \cdot 7H_2O$, 0.2; $CaCl_2$, 0.08; K_2HPO_4, 0.025; yeast extract, 2; peptone, 4; and D-glucose, 1 and casein, 5. Solutions of yeast extract, peptone casein, D-glucose, K_2HPO_4 and $CaCl_2$ were separately prepared, sterilized, and added before inoculation. The pH of the medium was adjusted to 7.1 before sterilization. The required quantity of *B. sphaericus* was thoroughly mixed with distilled water and prepared at various concentrations ranging from 10, 20, 40, 60 and 80 ppm, respectively.

Larval/pupal toxicity test

Laboratory colonies of mosquito larvae/pupae were used for the larvicidal/pupicidal activity. Twenty-five numbers of first to fourth instars larvae and pupae were introduce into 500 mL glass beaker containing 249 mL of de-chlorinated water and 1 mL of desired concentrations of plant extract and *B. sphaericus* were added. Larval food was given for the test larvae. At each tested concentration two to five trials were made and each trial consisted of five replicates. The control was setup by mixing 1 mL of acetone with 249 mL of dechlorinated water. The larvae and pupae were exposed to dechlorinated water without acetone served as control. The control mortalities were corrected by using Abbott's formula.[19]

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{Control mortality}} \times 100$$

$$\text{Percented mortality} = \frac{\text{Number of dead larvae/pupae}}{\text{Number of larvae/pupae introduced}} \times 100$$

The LC_{50} and LC_{90} were calculated from toxicity data by using probit analysis.[20]

Field trail

For the field trial, the quantity of plant extract residues and *B. sphaericus* (*Bs*) required (based on laboratory LC_{50} and LC_{90} values) quantity for each treatment was determined by calculating the total surface area of drinking water bodies in each habitat. The required quantities of *T. purpurea* and *B. sphaericus* were mixed thoroughly with water in a bucket with constant agitation. Teepol was used as emulsifying agent (0.05%). Field applications of the *T. purpurea* leaf extracts and *B. sphaericus* were done with the help of a knapsack sprayer (Sujatha Products, India, Private Limited, 2010) and uniformly on the surface of the drinking water bodies in each habitat. Dipper sampling and counting of larvae monitored the larval density before 24, 48 and 72 h after the treatment. A separate sample was taken to determine the composition of each larval habitat. Six trails were conducted for *T. purpurea* of the plant extracts and *B. sphaericus* alone and combined the treatment. The percentage of reduction was calculated by the following formula:

$$\text{Percentage of reduction} = \frac{C - T}{100} \times 100$$

Where C is the total number of mosquitoes in control, T is the total number of mosquitoes in treatment.

Statistical analysis

All data's were subjected to analysis of variance; the means were separated using Duncan's multiple range tests by Alder and Rossler.[21] The average larval mortality data were subjected to probit analysis for calculating, LC_{50}, LC_{90} and other statistics at 95% confidence limits of upper confidence limit (UCL) and lower confidence limit (LCL) and chi-square values calculated using the (Statistical software package). The values were expressed as mean ± standard deviation of five replicates. Results with $P < 0.05$ were considered to be statistically significant.

RESULTS

Table 1 shows the phytochemicals present in the methanolic leaf extract of *T.purpurea* contains alkaloids, carboxylic acid, coumarins, flavanoids, quinines, proteins and resins.Larval and pupal mortality of *A.aegypti* after the treatment of methanolic leaf extract of *T.purpurea* was observed. Table 2 provides the results of larval and pupal mortality of *A.aegypti*

(I and II instars) at different concentrations (75 to 375 ppm). Forty two point four percent mortality was noted at I instar larvae by the treatment of *T.purpurea* at 75 ppm, whereas it has been increased to 83.4 at 375 ppm of *T.purpurea* leaf extract treatment. Similar trend has been noted for all the instars of *A.aegypti* at different concentrations of *T.purpurea* treatment. The lethal concentrations ($LC_{50\ and}$ LC_{90}) were represented as LC_{50} value of Ist instar was 139.24 ppm, IInd instar was 176.24 ppm, IIIrd instar was 580.34 ppm and IVth instar was 256.27 respectively. Whereas LC_{90} value of Ist instar 480.72, IInd instar 541.21, IIIrd instar 580.34 and IVth instar 672.20 respectively. The pupa value of LC_{50} and LC_{90} is 326.29 and 762.80 ppm respectively.

The different concentrations of (10 to 80 ppm) *B. sphaericus* to treat the larval mortality of *A.aegypti* (Table 2). The treatment of Ist instar larvae by *B. sphaericus* at 10 ppm, 31% was recorded, whereas it has been increased to 68.2% at 80 ppm of *B. sphaericus* treatment. Eighteen percentage of mortality was observed at pupal by the treatment of *B. sphaericus* at 10 ppm and it has been increased to 45% at 80 ppm. Likewise all the different concentrations have been noted. The LC_{50} and LC_{90} values were represented as Ist instar was 46.16, 141.68 ppm followed by IInd instar 56.23, 172.46 ppm, IIIrd instar 69.82, 184.21 ppm and IVth 80.81, 193.31 ppm respectively. The pupa value of LC_{50} and LC_{90} was 96.12 and 218.16ppm respectively Table 3.

The combined larval mortality after treatment of *T.purpurea* and *B. sphaericus* for all the larval instars was presented in Table 4. The concentration at 75+40 combined *T.purpurea* and *B. sphaericus* treatment for IVth instar mortality was 65.6% recorded. The LC_{50} and LC_{90} values are represented as Ist instar was 80.08: 108.39ppm followed by IInd instar 82.21; 118.21 ppm, IIIrd instar 88.0; 136.75ppm and IVth instar 92.21;149.02ppm respectively. The pupa value of LC_{50} and LC_{90} was 98.16; 153.24 ppm respectively. The X^2 values are significant at $P < 0.05$level. The 95% confidence limits at $P < 0.05$ level. The 95% confidence limits at LC_{50}, LC_{90} (LCL-UCC) values were calculated. Larval and Pupal mortality was observed after 24 h exposure. In observation of control mortality was absent.

In drinking water body systems totally 409 *A. aegypti* larvae have been noted. *T. purpurea* extract was treated against *A .aegypti* the larval density was reduced by 17.8%, 40% and 87% at 24, 48 and 72 h respectively Table 5.

As well as the reduction of *A.aegypti* larval densities after treatment with *B. sphaericus* were 9.5%, 31.5% and 79.46% respectively. Combined effect of *T .purpurea* and *B. sphaericus* were 44.74%, 79.95% and 100% at 24, 48 and 72h respectively Table 6.

DISCUSSION

Plants based organic products are preferred for the control of insect vectors of human diseases. The effectiveness of secondary metabolites such as alkaloids, isoflavonoids, saponine and steroids has potential mosquito larvicides.[22-24] According to Pedro *et al*[25] phytocompounds such as flavonoids, alkaloids and saponins are responsible for insecticidal activity. Kotkar *et al*[26] reported that flavonoids isolated from *Annona squamosa* are effective as insecticides against *Callosobruchus chinensis*. In the recent study, the methanolic leaf extract of *T.purpurea* contain good amount of flavonoids and alkaloids were recorded Table 1.

From 19th century plant extract treated for the control of vector mosquitoes has been reported.[27] While subsequently plants for identifying the potential activity not only against the larval stages but also include all other stage of mosquitoes.[28] Taken from plants are successful and vital source of several elements which are used in distinct ways.[29]

Larvicidal and pupicidal actions of methanol leaf extract *E .hirta* against the malaria inducing vector, *A. stephensi* was shown the LC_{50} values in initial larva instars to till fourth larval instars respectively 172.65, 217.5, 269.37 and 332.39 ppm. The results obtained show that this plant material exhibited significant activity and could be considered as potential natural larvicidal agent.[16] Similarly Maheshkumar *et al*[30] reported that the efficacy of *Solanum xanthocarpum* leaf extract in the larval and puppal of *A. stephensi* with LC_{50} value of initial instars to fourth instars respectively 155.29, 198.32, 271.12, 377.44 and 448.41 ppm. Likewise, LC_{90} value of first to fourth instars larvae and pupae 687.14, 913.10, 1011.89, 1058.85 and 1141.65 ppm, respectively.

In the present study, *T.purpurea* leaf extract against first to fourth instars larvae and pupae of *A.aegypti* has been studied in the laboratory condition. The lethal concentrations (LC_{50}/LC_{90}) of *T. purpurea* were 139.24, 176.24, 219.28, 256.27, and 326.29ppm respectively. The LC_{90} values of 480.72, 541.21, 580.34, 672.20, and 762.80 respectively.

Aloe vera methanolic leaf extracts and the insecticide of bacteria *B.sphaericus* for the mosquitocidal property was screened as potential species of mosquito vectors.[31] This is a peculiar biodegradable method for inhibiting the vector programs. In the proposed study, the *B. sphaericus* at various concentrations were carried out against the various larval instars of dengue vector, *A.aegypti.* Likewise 85% mortality was observed

Table 1: Phytochemicals present in the methanolic extracts *T. purpurea*.

Phytochemical constituents	*T. purpurea*
Alkaloids	++
Carboxylic acid	+
Coumarins	+
Flavanoids	+++
Quinones	+
Phenols	-
Saponins	-
Xanthoproteins	-
Proteins	+
Resins	+
Steroids	-
Tannins	-
Sugars	-

Absents; ++ Medium; +++ High; + Low

Table 2: Larval and pupal toxicity effect of *T. purpurea* against dengue vector *A. aegypti*.

Mosquito larval instars and pupa	% of Larval and pupal mortality ± SD					LC_{50} (LCL- UCL)	LC_{90} (LCL-UCL)	x^2(*df*=4)
	75 ppm	150 ppm	225 ppm	300 ppm	375 ppm			
1St Instar	42.4 ± 0.6	52.4 ± 1.3	65.6 ± 1.2	72.0 ± 1.1	83.4 ± 1.4	139.24 (92.37-173.02)	480.72 (410.31 - 592.00)	0.05*
2nd Instar	38.2 ± 1.1	49.4 ± 1.3	60.2 ± 10	64.3 ± 1.6	79.4 ± 0.3	176.24 (136.12-208-62)	541.21 (457.23 - 684.17)	0.23*
3 rd Instar	29.3 ± 1.3	38.4 ± 1.2	58.4 ± 0.7	62.4 ± 1.4	70.4 ± 1.6	219.28 (192.62-262.76)	580.34 (487.23 - 743.20)	0.44*
4 th Instar	25.1 ± 1.7	34.2 ± 0.7	43.5 ± 1.8	50.2 ± 1.6	61.4 ± 2.0	256.27(228.56-312.18)	672.20 (542.16 - 913.82)	0.21*
Pupa	21.4 ± 1.4	29.6 ± 0.7	37.0 ± 1.2	46.2 ± 0.7	52.6 ± 0.8	326.29(272.23-403.79)	762.80 (612.32 - 1009.29)	0.11*

Control-Nil mortality, LCL - Lower confidence Limit, UCL - Upper confidence Limit, x^2 - Chi-square value, *df* - degrees of freedom. *Significant at $P < 0.05$ level.

Table 3: Larval and Pupal toxicity effect of *B. sphaericus* against dengue vector *A.aegypti*.

Mosquito larval instars and Pupa	% of Larval and Pupal mortality ± SD					LC_{50} (LC-UCL)	LC_{90} (LC-UCL)	x^2 (*df*=4)
	10 ppm	20ppm	40ppm	60ppm	80ppm			
1St Instar	31.1 ± 1.1	39.3 ± 1.3	48.3 ± 0.9	55.4 ± 0.7	68.2 ± 1.0	46.16 (34.26 - 52.02)	141.68 (113.27 - 196.00)	0.13*
2nd Instar	29.0 ± 1.2	35.3 ± 0.7	43.6 ± 1.3	50.8 ± 1.2	60.7 ± 1.4	56.23 (49.61 - 68.03)	172.46 (136.81 - 247.43)	0.20*
3rd Instar	24.3 ± 1.2	29.6 ± 1.1	37.8 ± 1.2	45.0 ± 0.7	57.8 ± 1.2	69.82 (52.71 - 83.80)	184.21 (141.32 - 263.81)	0.35*
4th Instar	21.6 ± 0.7	27.6 ± 1.3	31.5 ± 0.7	38.6 ± 1.3	49.6 ± 1.1	80.81 (68.23 - 117. 08)	193.31 (151.81 - 308.39)	1.07*
Pupa	18.0 ± 1.3	21.3 ± 1.1	27.4 ± 0.7	33.6 ± 1.2	45.0 ± 0.7	96.12 (75.16 - 136.81)	218.16 (172.81 - 341.36)	0.24*

Control-Nil mortality, LCL - Lower confidence Limit, UCL - Upper confidence Limit, x^2 - Chi-square value, *df* - degrees of freedom.*Significant at P < 0.05 level.

Table 4 : Combined effect of larval and pupal mortality of methonal extract of *T. purpurea* and *B. sphaericus* against dengue vector *A.aegypti*.

Mosquito larval instars and pupa	% of Larval and pupal mortality ± SD					LC_{50} (LC-UCC)	LC_{90} (LC-UCC)	x^2 (*df*=4)
	75 + 10	75 + 20	75 + 30	75 + 40				
1st	48.3 ± 1.2	59.3 ± 0.7	68.1 ± 1.7	77.8 ± 1.3	91.5± 0.4	80.08 (66.07 - 89.24)	108.39 (99.29 - 128.33)	6.15*
2nd	42.8 ± 1.3	51.6 ± 11	60.6 ± 1.6	69.7 ± 1.4	82.4 ± 1.3	82.21 (78.12-90.51)	118.71 (106.21-131.26)	3.72*
3rd	37.2 ± 0.7	46.3 ± 1.9	53.7 ± 1.0	61.6 ± 11	73.0 ± 1.6	88.00 (86.81-98.43)	136.75 (121.31-156.12)	1.64*
4th	31.3 ± 1.9	41.6 ± 1.0	48.6 ± 0.7	56.3 ± 1.1	65.6 ± 1.9	92.21 (89.71-102.50)	149.02 (132.58-168.91)	3.16*
pupa	26.3 ± 1.2	37.4 ± 1.2	41.7 ± 1.4	49.2 ± 1.8	61.9 ± 1.3	98.16 (92.27-105.62)	153.24 (134.21-186.68)	2.59*

Control-Nil mortality, LCL - Lower confidence Limit, UCL - Upper confidence Limit, x^2 - Chi-square value, *df* - degrees of freedom.*Significant at P < 0.05 level.

Table 5: Field trial by using plant extract of *T. purpurea* and *B. sphaericus* drinking water tanks against *A. aegypti*.

Sample No.	Before treatment	After treatment					
		T. Purpuria			B. Spheerious		
		24 hrs	48hrs	72 hrs	24 hrs	48 hrs	72 hrs
1.	83	64	43	12	77	51	18
2.	75	61	57	10	69	70	16
3.	46	33	26	9	41	32	8
4.	62	51	43	7	54	49	17
5.	93	85	40	8	8	42	13
6.	50	42	36	7	41	36	12
Total	409	336	245	53	370	280	84
Average	68.2	56.0	40.8	8.8	61.7	46.7	14

Table 6: Field trial by using combined effect of drinking water tanks 0.5x 0.5 x1.0 against *A. aegypti*.

Sample No.	Before treatment	After treatment		
		24 h	48 h	72 h
	83	53	18	-
	75	42	10	-
	46	25	8	-
	62	28	13	-
	93	48	24	-
	50	30	9	-
Total	409	226	82	0
Average	68.2	37.7	13.7	0

using bacterial pesticides of *Spinosad* against the malarial vector, *A. stephensi*. Based on the above mentioned mortality rate indicate that the extract can be used as bio-pesticides. The LC_{50} values of second, third and fourth instars larvae *A. stephensi* were 0.27%, 0.28% and 0.30% observed respectively.[32-35] According to Kamalakannan and Murugan[36] reported that control the mosquito larval and pupal population in the lab and field trials using ten microbial products. The lethal concentrations (LC_{50} and LC_{90}) at 0.25 and 0.5 at 24 h for *A.aegypti* suggest that highest larval motility in the lab. Kalfon *et al* 1984[37] reported that *B. sphaericus* which is highly toxic to dipteran larvae has opened the possibility of its use as a potential biolarvicide in mosquito eradication programe. In Amazonia strains of *B. sphaericus* against treated for the larvicidal activity in different concentration levels.[38] In the present study also were supports with Subramanian *et al*[31]

Various concentrations like 10, 20, 40, 60 and 80 ppm were used in laboratory bioassays for *A. stephensi*.[16] Similarly, various concentrations of *B. sphaericus* were treated for *C. quinquefasciatus* after 24h it was observed. The percentage of mortalities were different for the different

instars of *C.quinquefasciatus, B. sphaericus* against the first to fourth larvae and pupae was 0.051%, 0.057%, 0.062%, 0.066% and 0.073% respectively. The outcome of the result gives that B. sphaericus which has rich and powerful properties to control mosquito. And also it acts as eco-friendly. The previous report of Kovendan is that *B.sphaericus* is used to control *A.stephensis*, malarial vector if it is isolated from soil sample. But the recent outcome shows that *B.sphaericus* reduced the activity of larvicidal . Previously, Kovenden *et al*[39] reported that *B. sphaericus* isolated from soil sample and used to control the malarial vector *A. stephensi.* In the present results showed that *B. sphaericus* dealing the decrease of larvicidal activity.

Field trials was conducted by Sharma *et al* a and b[40,41] at Sundergarh district of Odisha, they are used the drug deltamethrin in the form of tablet for controlling malaria. The effective results were observed from field as well as laboratory conditions against *A. culicifacies* and *A. stephensi*. Similarly stiles *et al*[42] reported that both cement and mud surfaces, found 100% motility was observed residual activity of deltamethrin against *A. gambiae.* On the other hand emulsified neem oil formulation was observed against *A. stephensi* larvae in tank and pits. Hundred percent of reductions were observed by V.K Dua *et al.*[43] However in the present study combined activity of plant extract of *T. purpurea* and *B. sphaericus* in the field were 44.74%, 79.95% and 100% at 24 hrs, 48 hrs and 72 hrs respectively. This results show that *T. purpurea* and *B. sphaericus* can control the dengue vector, *A. aegepti*. Likewise Panneerselvam *et al*[16] were conducted combined activity of plant extracts of *E .hirta* and *B. sphaericus* in the field were 44.23%, 81.64% and 100% at 24 hrs, 48 hrs and 72 hrs respectively.

CONCLUSION

The outcome of the present research obviously shows that both *T.purpurea* and *B. sphaericus* have astonished mosquito properties against *A.aegypti*. This is the first study on the joined mosquito larvicidal and pupicidal acttivities. The research confirms that *T. purpurea* and *B. sphaericus* are two vital biological agents that could control the dengue incidence. Besides, there is a scope and agte way for further research on the activities of larvicidal and pupicidal.

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CONFLICT OF INTEREST

The authors are declared that there is no conflict of interests regarding this manuscript publication.

ABBREVIATION USED

DENV: Dengue virus; **LC:** Lethal concentration.

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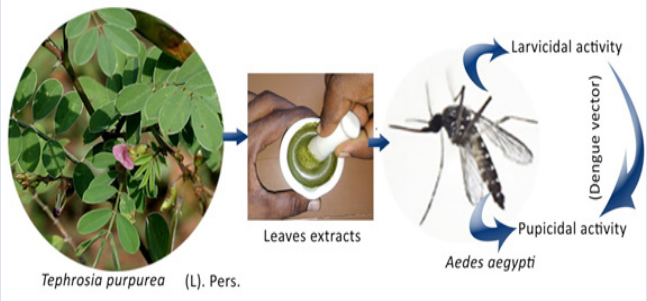
GRAPHICAL ABSTRACT



SUMMARY

- Dengue virus is transmitted to human through by the bite of *Aedes aegypti*. Generally, the dengue vector *Aedes aegypti* is highly active during the day time. Dengue virus is mainly found in tropical regions particularly in rural areas. The dengue affected people are face lots health complications like physical weakness, severe headache, retro- orbital pain, joint and muscle pain. It does not produce long term complications. Currently, there is no specific treatment available for dengue. In recent day, people are emerging to seek effective treatment without any side effects and escaping that disease related health complications.

ABOUT AUTHORS

Dr. S. Vijayakumar has received his doctorate degree from Barathidhasan University and has more than 15 years of teaching experience at U.G. and P.G. levels. He has 15 years of research experience. He is working as Assistant Professor in the Department of Botany and Microbiology, A.V.V.M. Sri Pushpam College (Autonomous), Poondi-613 503, Thanjavur district, Tamil Nadu. He has got vast experience in research especially on cyanobacteria, Medicinal plants, Environmental Microbiology and Computer Aided Drug Design (CADD). He has guided P.G. students for their projects in the field of fresh water Algae, Medicinal plants and Bioremediation. At present he is completed 10 Ph.D and guiding 8 Ph.D. programme in this line. So, far he has guided 22 M. Phil., candidate for their degree and published 68 research papers in the international level journals. He has got best paper award from National Symposium on "Algae, Man and Biosphere" and International Medicinal Plants and Pharmacological Research, Columbo University, Srilanka. Recently, he got YOUNG SCIENTIST AWARD from Wasinghton University, USA. He has visited more than 8 countries which include Spain, Dubai, Sri Lanka, Singapore, etc. He also completed One UGC Minor and UGC Major Project. Right now one DST-SERB major project is ongoing in the area of Computer Aided Drug Design and Modelling.

Mr. V. Ramesh has received graduation and post graduation in Botany from Barathidasan University. Presently he is a Research scholar in Department of Botany and Microbiology, A.V.V.M. Sri Pushpam College (Autonomous), Poondi-613 503, Thanjavur district, Tamil Nadu. Currently, he is studying about dengue serotypes and their effects in human and he is finding vaccine candidate for dengue vector.

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