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TOXICITY OF *TARENNA ASIATICA* (L.) KUNTZE EX K. (SCHUM) ON MOSQUITO PUPAE

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ABSTRACT
Mosquitoes are highly populated vectors adapted to different socioeconomic environment has established its strength to transmit various diseases; therefore to control its population using a rigorous mosquito vector control programme is essential. Plant extracts can be potentially used to control mosquitoes. The plant *Tarenna asiatica* has been known to have medicinal use and reported to exhibit anti microbial activity, hence the insecticidal activity of this plant was studied. The plant extracts were prepared using solvents such as ethyl acetate, chloroform and hexane and each solvent extracts were used for the pupicidal assay at different concentrations. It has exhibited maximum toxicity at 400ppm of ethyl acetate followed by hexane and chloroform extracts to pupae of three mosquito species such as *Anopheles stephensi*, *Culex quinquefasciatus*, and *Aedes aegypti*. The LC_{50} values indicate that 50% mortality can be achieved between 40.7ppm to 60.2 ppm among three solvent extract for three mosquito species.

KEYWORDS: crude extract, pupicidal activity, *Anopheles stepensi*, *Culex quinquefasciatus*, *Aedes aegypti*.

INTRODUCTION

Mosquitoes have efficiently adapted to unpredictable milieu and evolved robustly to transmit disease like Dengue, Chikangunaya, Zika virus, Yellow fever, Japanese encepahilites, Filariasis and Malaria, globally. The Aedes, Culex and Anopheles species are involved in causing millions of death in human population every year. The increase in mosquito population, dense human habituation with low socioeconomic conditions aid in more human and mosquito interactions, immigration of diseased vector are important factors of disease transmission[1]. Apart from anthropronotic disease transmission the mosquitoes are involved in spreading of zoonotic diseases which are least affected by socioeconomic conditions[2]. Hence there is often a threat of re-occurrence of mosquito transmitted anthropronotic or zoonotic diseases to human population. In the area where high standards of living is pursued the mosquitoes shift its host preference to closely associated house hold animals and birds hence constantly the diseases are circulated in local environment and all set for vulnerable human infection[3]. In the country like India with diverse socioeconomic groups and increased human population has to encounter complex disease transmission cycle. Therefore intensive vector control programmes are crucial to reduce the pathogen mobility, spread of its stereotypes and emergence of new mosquito transmitted diseases.

There are several methods have been developed to control mosquitoes; even today it is a challenging task to target different phases of mosquito life cycle. The different stage of the mosquito is a potential target of pest control. In mosquito life cycle the pupal stage is known to be a non feeding stage. However it is the most active stage of disintegrating larval tissue and development of adult organs inside the pupal structure involved in high rate of physiological function facilitate in metamorphosis of adult from pupa[4] [5]. Hence this phase is highly susceptible to meagre change in the pupal environment. Earlier research studies have depicted that promising alternative against synthetic pesticides are plant phytochemicals[6]. India can have significant growth of phytopharma industry as it treasures medicinal plants of large varities. Bioprospecting these plants could bring in economic favour and alternatives against synthetic chemical use. The plant extracts containing phytocompounds are used as larvicides as well as pupicides, repellents, and oviposition deterrants against mosquitoes[7,8]. *Tarenna asiatica* is an evergreen plant belongs to Rubiaceace family. The plant also has antibacterial[9], antioxidant and antiviral properties[10]. The plant was known for its wound healing properties by the indigenous people of Maharastra[11]. This plant was in use for wound healing in animals[12] and for treating eye infection by tribes of Sirumalai hills[13]. The earlier reports depict that this plant holds potential medicinal values; thus selected for the study.

MATERIALS & METHODS

Plant collection

The plant raw materials were obtained from the hill named Pullian Solai situated in Nammakkal District of Tamil Nadu, India. The plant identification was authorised by Botanical Survey of India, Regional centre of South India, located in TNAU campus, Coimbatore, India. The plant specimen was stored in Insect Plant Herbarium of Entomology laboratory of PG & Research Department of Zoology in Government Arts College (Autonomous), Coimbatore, Tamil Nadu, India.

Preparation of crude plant extract

The collected leaves which are thoroughly washed were shade dried (room temperature (25- 27^{o}C) until the leaves become crispy in nature. The leaves were minced for extracting with suitable solvent. The hexane, chloroform, and ethylacetate crude extracts were obtained by soaking method with slight modification[14]. The solvent extracts

were vacuum evaporated and further stored in refrigerator for bioassay.

Mosquito pupa rearing

The larvae of three species namely *Aedes aegypti*, *Culex quinquefasciatus*, and *Anopheles stephensi*, were collected from National Centre for Communicable Diseases Control, Government of India, Ministry of health and family welfare field station, Mettupalayam, Coimbatore, Tamil Nadu, India. The procured larvae were cultured in separate plastic trays containing tap water covered by mosquito nets. The mixture containing ratio of 0.3:0.1gm of yeast and dog biscuit was used for feeding the larvae until IV instar then transferred to the oviposition cage for moulting into pupa and emerging into adult. The male adults were given sucrose solution of 10% dipped in cotton and females were fed with blood meal from a healthy hen to nourish egg production. Inside the cage the oviposition was facilitated by placing bowls with filter paper lined along the rim. The laid eggs were reared until pupal stage. The freshly moulted pupae's were used for the pupicidal bioassay. The mosquito culture was sustained at relative humidity (65-75%) under 25-27ºC room temperature.

Pupicidal bioassay

The pupicidal assay was executed in concurrence with the protocol of WHO[15] for bioassays with slight modifications. In the 48hrs bioassay; at various concentrations of 400, 200, 100, 50, 25ppm were used for each solvent extracts. Each individual concentration were prepared by mixing 400mg, 200mg, 100mg, 50mg, 25mg with 1ml of emulsifier (Tween 20-Polyoxyethylene sorbitan monolaurate) and dissolving in 1000ml of tap water. About 200ml per replication for five replications were followed for each concentration. Each replication was introduced with live pupae of 20 numbers. Five duplications were maintained for control with the same water volume with emulsifier and number of pupae. The morphological observations were done using Binocular Microscope (WESWOX).

Data Analysis

The mortality of pupae was calculated using Abbotts' formula[16] and the probit analysis was used to determined lethal concentration. The variation in pupal mortality was assessed using SPSS version 16.0 with One Way ANOVA followed by Tukey's test.

Phytochemical screening

The crude ethyl acetate, hexane and chloroform extracts of *Tarenna asiatica* were screened for the presence of phytochemicals [14] [17] [18]. About 0.2g of crude extract was prepared as stock solution for each assay. The flavonoids, terpenoids, phenols, pytosterols, tannins, catechins, saponins, alkaloids, quinones, carbohydrates, cardiac glycosides, and proteins were screened for each solvent extracts.

RESULTS & DISCUSSION

In pupa the adult transformation involves formation of new cuticular structures, and growth of adult organs governed by high physiological functions. These functions inside the pupa depicts the creamy delicate nature of this tiny creature; also the tracheal scheme is insufficiently developed in pupa because only the spiracle of the first abdominal segment is open hence the scantily formed tracheal system inclined to modest supply of air [4]. Hence any external agent interfering the flimsy pupal body or blocks in the spiracle will affect physiological functions and air supply in pupa.

All these physical and chemical barriers were reflected in the pupal movements. Initially when introduced into the experimental setup the pupa showed faster movements for 5 to 10mins and as the time exceeds the movement slows down. After 24 hrs the pupa strives to float to the surface and displays slow tumbling movements from bottom to the middle of experimental medium finally the movements are restricted to the bottom before demise. In this experiment the plant extract has taken possible routes to influence the survival of pupa. The figures (Fig 1) B, C, D, E, & F indicates the extract has been adsorbed to the pupal skin and penetrated into the pupa have produced physiological discomfort. The respiratory trumpet (Fig.1 C) filled with the plant extract materials must have reduced the air supply leads to suffocation and death. The dried body of treated pupa shown in figure E and F also informs the presence of extract inside the pupal body. Any foreign agent absorbed into the body and inability of the pupa to clean or excrete the absorbed material is utterly disastrous for the survival of the pupa. Overall pupa showed mortality more rapidly. The table 1 indicates that all the three extracts showed above 90% mortality for the three mosquito species.

TABLE 1: Pupicidal activity of *T.asiatica* crude extracts against three mosquito species

	Concentration				
Crude Extracts	25ppm	50ppm	100ppm	200ppm	400ppm
Aedes aegypti					
Hexane	38.8±3.6^{a}	51.1±1.1^{b}	64.1±4.0^{c}	78.1±3.8^{d}	94.3±2.8^{e}
Chloroform	40.3±1.1^{a}	49.1±2.0^{b}	59.1±2.3^{bc}	74.3±4.9^{d}	91.5±3.2^{e}
Ethylacetate	39.7±2.5^{a}	51.3±4.7^{b}	65.3±4.1^{c}	79.73±5.7^{d}	97.3±1.0^{e}
Culex quinquefasciatus					
Hexane	40.1±1.9^{a}	51.9±2.0^{b}	64.5±1.2^{c}	79.9±4.2^{d}	97.1±2.7^{e}
Chloroform	38.7±2.4^{a}	50.3±2.0^{b}	60.7±4.4^{bc}	77.9±2.7^{d}	93.5±2.2^{e}
Ethylacetate	39.9±3.7^{a}	52.9±1.9^{b}	67.9±2.7^{c}	81.3±3.4^{d}	98.3±1.0^{e}
Anopheles stephensi					
Hexane	41.7±2.1^{a}	52.3±3.7^{ab}	65.5±3.3^{c}	80.3 ±1.8^{d}	97.9±1.5^{e}
Chloroform	38.9±3.1^{a}	49.7±1.1^{ab}	63.5±2.0^{c}	78.7±3.1^{d}	96.1±1.4^{e}
Ethylacetate	44.1±1.4^{a}	52.7±3.1^{b}	63.5±2.7^{c}	82.1±2.9^{d}	99.1±0.4^{e}

The values marked with superscript letters indicate statistically significant difference in P values <0.05 calculated by one way Anova and Tukey's test. The '±' indicates the values with standard deviation.

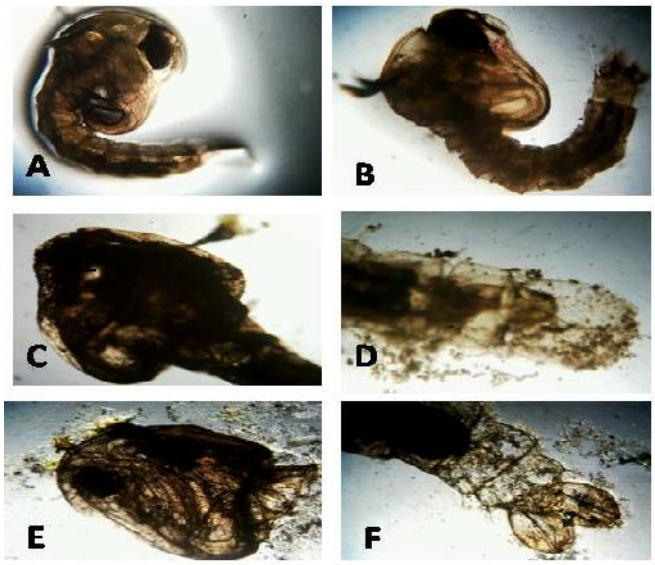

Figure 1: A- Control pupa, B- treated pupa, C- treated pupa head, D-treated pupa tail, E- -dried pupa head (treated), F-dried pupa tail (treated)
Symbols: %- percentage, ^{o}C- degree Celsius, ± - plus or minus

At 400ppm ethyl acetate extract showed 99.1% of mortality for *A. stephensi* followed by *C. quinquefasciatus* at 98.3% and *A. aegypti* at 97.3%. The hexane extract has produced above 70% mortality for all three mosquito species. The lowest mortality was produced at 25ppm for all the three extracts. The chloroform extract has produced mortality lower than other two extracts. The table 2 shows the LC_{50} and LC_{90} of crude extract exhibited on three mosquito pupae. The ethyacetate extract showed LC_{50} of 40.7ppm for *A. stephensi*, 46.1ppm for *C. quinquefasciatus*, and 50.8ppm for *A. aegypti.* Similarly hexane and chloroform extract showed LC_{50} at 46.4ppm and 55.9ppm for *A. stephensi*, 49.8ppm and 57.2ppm for *C. quinquefasciatus*, 52.6 ppm and 60.2ppm for *A. aegypti.*

TABLE 2: The lethal concentration for 50% and 90% mortality of pupa exerted by the crude extracts of *T. asiatica*

Crude extracts	*Aedes aegypti*		*Culex quinquefasciatus*		*Anopheles stephensi*	
	LC_{50} LCL-UCL	LC_{90} LCL-UCL	LC_{50} LCL-UCL	LC_{90} LCL-UCL	LC_{50} LCL-UCL	LC_{90} LCL-UCL
Hexane	52.6	313.0	49.8	277.4	46.4	266.7
	19.5-78.6	269.2-379.7	20.4-73.0	239.3-335.0	17.2-69.1	229.8-322.6
Chloroform	60.2	360.8	57.2	352.2	55.9	292.6
	23.2-89.2	307.9-443.7	24.1-83.6	279.6-394.9	26.5-79.5	252.8-352.7
Ethyl acetate	50.8	274.8	46.1	254.6-219.6-	40.7	250.4
	22.0-73.6	237.2-331.4	18.3-67.8	307.6	11.5-62.9	215.2-304.7

LCL- Lower concentration limit, UCL-upper concentration limit, LC_{50}- lethal concentration caused 50% mortality, LC_{90}-lethal concentration caused 90% mortality.

Similar research work has been reported for methanolic extract of *Alantia monophylla* against *A. stephensi, A. aegypti* and *C. quinquefasciatus* pupicidal activity. The *C. quinquefasciatus* and *A. aegypti* showed mortality at LC_{50} values of 0.07 ppm and for *A. stephensi* at 0.05 ppm[19]. The crude toxin of *B. subtilis* was highly effective against pupae of *A. aegypti, C. quinquefasciatus* and *An. stephensi*[20]. The results of this research work indicates that the plant extract has produced mortality at slightly different magnitudes for the three mosquito pupae which might be due to the fundamental dissimilarity in the cuticle or the absorption properties of the respiratory structures[5]. The *Catharanthus roseus* solvents extracts showed remarkable pupicidal activity against *A. stephensi* and *C. quinquefasciatus.* The aqueous, ethyl acetate and methanol extracts had LC_{50} values of 118.08, 182.47and 143.80mg /mL against the pupae of *A. stephensi* and 146.20, 226.84and 156.62mg/mL against the pupae of *C. quinquefasciatus*[21]. Mahesh kumar *et al.* [22] has stated highest pupicidal activity of *Solanum xanthocarpum* against *C. quinquefasciatus* at 650ppm. The compounds present in *Pogostemon cablin* oil showed remarkable pupicidal activity against Aedes, Culex and Anopheles species[23]. Hence the pupal physiology and anatomical structures are exploited favourably in this research work indicating the presence of mosquitocidal phytochemicals in the plant extract (Table 3). The crude extract of *T. asiatica* screening exposed the presence of important phytochemicals in ethyl acetate extract where as the hexane and chloroform extracts showed presence of only few phytochemicals. Hence ethyl acetate has shown maximum mortality than the hexane and chloroform extracts. However the various phyto compounds have different way of affecting the insect body. The flavonoids were found to inhibit the enzyme activity in mosquito larvae[24]. The teripenoids have potential to exert a

repellent activity against insects[25]. The saponins have been reported to have larvicidal activity for *A. aegypti* and *C. pipiens*[26] and the cardiac glycosides have shown inhibitory effect on the physiology of *Pomacea canaliculata*[27]. The phenolic acids present in a sea weed *Chaetomorpha antennina* was reported to shown larvicidal and repellent activity against *A.aegypti*[28]. The phytosterols play major role in mosquito larval development, although some phytosterol compounds from *Abutilon indicum*[29] and *Cestrum diurnum*[30] are turned to be toxic to mosquito larvae. The catechins compound of green tea epigallocatechin gallate was reported to control chikungunya virus [31]. The aqueous extract of tannic acid from the plant *Rhizophora mangle* has been reported to show larvicidal and ovicidal activity in *A.aegypti*[32]. These reports on phytochemical effects on insects and the presence of various phytochemicals in this plant uphold its insecticidal potentiality.

TABLE 3: Phytochemicals present in three different extracts of *T. asiatica*

Sl. No	Phytochemicals	Hexane extract	Chloroform extract	Ethylacetate extract
1	Flavonoids	+	+	+
2	Tannins	-	+	+
3	Glycosides	-	-	+
4	Catcehins	-	-	+
5	Quinones	+	-	+
6	Proteins	-	+	+
7	Phenols	-	+	+
8	Phytosterols	-	+	+
9	Alkaloids	-	-	+
10	Terpenoids	-	-	+
11	Saponins	+	+	+

'+' indicates the presence of compounds, '-'indicates the compounds were not detected

CONCLUSION

T. asiatica has proved to be toxic to the pupae of three mosquito species and this is the first report of *T. asiatica* applied against mosquito pupa hence further investigation of the plant potentiality to control the mosquito species at different stages are under study. However the comprehensive study on the function of phyto compounds of this plant against the mosquito species and its field application are required to substantiate the employment of this plant product against mosquito species.

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