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BIOCONTROL EFFICACY OF OPERCULINA TURPETHUM (L.) (CONVOLVULACEAE) LEAF EXTRACTIVES AGAINST LARVAL FORM OF MALARIAL MOSQUITO ANOPHELES STEPHENSI (LISTON 1901)

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ABSTRACT

Vector control is the foremost step to diminish the mosquito borne diseases as the proper vaccinations are yet to develop against those obnoxious diseases. After many resistant cases, it is important to develop other sources of eco-friendly insecticides. The present piece of study was designed to evaluate the mosquito larvicidal efficacy of *Operculina turpethum* against malarial vector *Anopheles stephensi.* Hot and cold aqueous leaf extracts, and two different solvent extracts were treated against all the larval instars. LC_{50} and LC_{90} values were determined. Statistical justifications were made by log-probit, regression and ANOVA analyses. Cent percent mortality was found against 1st instar larvae at a concentration of 0.5% hot aqueous and 160 ppm solvent extracts after 72 h of exposure. Non-target organisms were nonresponsive after treatment with the same. From the present study it can be concluded that the bioactive fractions from *O. turpethum* leave extracts have remarkable mosquito larvicidal potentiality.

KEYWORDS: ***Operculina turpethum, Anopheles stephensi,*** **non-target organisms, larvicide**

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INTRODUCTION

Mosquito is a small midge like arthropod belonging to family culicinae which plays a decisive role as vector for many nauseating diseases such as malaria, dengue, Japanese encephalitis, chikungunya, filariasis around the whole world that sustains in nature by biological transmission of disease causing pathogen by blood feeding of inclined vertebrate host [1]. To prevent the transmissions of such obnoxious diseases it is a crying need to prevent vector populations. It is a threat that about half of the World's population is at a high risk of malaria. Sub-Saharan Africa is most prevalent with many malarial cases and deaths [2]. Malaria was found in 108 countries and territories in the year 2008. Amongst 460 recognised species 100 can transmit human malaria of which 30-40 species can transmit the parasites of *Plasmodium* genus cause malaria that affect human of endemic area. Nine *Anopheles* species are the vector of malaria among 53 Anopheline species present in India. In urban areas of India malaria is transmitted by *An. stephensi* [3]. In India, about 2-3 million new malarial cases are reported every year which ensures that malaria is an important cause of morbidity and mortality along with enormous financial loss [4]. About 10,000 malarial cases were found each year in Europe and about 1500 in United States [5]. About 77% of total malaria is found from India amongst the whole Southeast Asia [6]. Malaria is transmitted usually by biting of infected female *Anopheles* mosquito which is previously being infected with a blood meal of an infected person. So the disease is avertable only if the control of this vector is possible. Various strategies have been utilized worldwide to diminish the predominance of various vectors responsible for such diseases. However, malaria or mosquito borne diseases cannot be totally diminished, mosquito control faces considerable challenges now a day due to ever increasing resistance against daily exploitable synthetic insecticides [7, 8]. Larvicides play a major role in the breeding sites of mosquito, however, many adverse effects against environment and some non target fauna inhabiting with mosquito larvae are reported due to utilization of synthetic insecticides [9]. A major human health problem is also noticed. The problem stimulated to search for an undisruptive and efficient alternative larvicide [10]. Insecticides from botanical origin can utilize as a high-quality insecticide alternative to the chemical one [11, 12]. Essential oils and phytochemicals from plants or plant part extracts can be used as a potential larvicidal agent because it is filled with many bioactive compounds which are biodegradable and not detrimental to environment [13]. *Operculina turpethum* belongs to the morning glory family Convolvulaceae, commonly known as 'dudhkalmi', is endemic, perennial herbs, exudes a milky juice when cut, hairy vines growing 4 to 5 meter in length. The leaves are ovate, alternate, very variable in shape, oblong and truncate at the base. The large flowers are solitary and axillary. Fruit is a capsule with prominent distended sepals and thickened pedicles. *O. turpethum* is commonly used in the Indian traditional system of medicine to treat various diseases including peptic ulcer, inflammation, and pain. Roots are anthelmintic, purgative, alexiteric and antipyretic; constructive to ascites, constipation, leucoderma, bronchitis, piles, pain in chest, joints and muscles. The root extracts of *O. turpethum* has been used as an anti-inflammatory and hepato-protective agent [14]. Stem bark extracts of Operculina turpethum shows ulcer protective activities in experimental rats [15]. Previously antibacterial activity was found against *Shigella dysenteriae, Bacillus subtilis, Escherichia coli* and *Sarcina lutea* [16]. From the aerial parts of *O. turpethum*, four new dammarane-type saponins namely operculinosides A–D (1–4), were isolated of which two dammarane-type triterpenoids have an oxymethyl group at C-24. By spectroscopic analysis and acid hydrolysis the structures of the compound were determined. Two compounds showed significant protective activities against D-galactosamine-induced toxicity in L-02 human hepatic cells [17]. However, this is the first ever report on its mosquito larvicidal role under laboratory conditions.

MATERIALS AND METHODS

i) Collection of plant material

O. turpethum is endemic recurrent basil to India. Fresh, young, green leaves of *O. turpethum* were collected during June - July 2013 from plants inhabiting outer reaches of Mahammad Bazar (23°59′31″N , 87°34′19″E), Birbhum, West Bengal, India. The plant was identified appositely and a voucher specimen is deposited as herbarium in the Department of Zoology named GCK-12.

ii) Test mosquito

Larva of *Anopheles stephensi* was collected from the underground and overhead tanks of Kolkata metropolis, Kolkata, West Bengal, India. The mosquito colony was maintained in Mosquito research unit, Parasitology laboratory, Department of Zoology, The University of Burdwan. With subtle changes of the existed procedure of Sharma and Saxena (1994)[18] the mosquito colony was maintained in an insectary (45× 30×10) containing tap water at 28^0C and in 82% relative humidity with 14L: 10D photoperiod cycles. The larvae were fed with an alternative diet with a mixture of well minced dog biscuits and Brewer's yeast in a ratio of 3:1. The colony was maintained under laboratory condition without any pathogen, repellent and insecticide contamination.

iii) Preparation of solvent extracts of leaves of O. turpethum

Fresh young leaves of *O. turpethum* were rinsed well with tap water and soaked in a paper towel.

a) *Cold aqueous extract*

Finely chopped 50 g leaves of *O. turpethum* were weighed out and soaked into 200 ml cold water in a stoppered conical flask with rubber corks and left uninterrupted for 24 h, then filtered off using sterile filter paper (Whatman no. 1) into a clean conical flask and subjected to rapid evaporation at 100^0 C water using water bath. Then the standard extracts were stored in a refrigerator at 4^0 C for further use [19].

b) *Hot aqueous extract*

50 gm of finely chopped leaf samples of *O. turpethum* were weighed out and soaked separately into 200 ml hot water which was then boiled for 30 min [19] and reserved for 24 h incessantly in a conical flask. The other steps were the same as followed in case of cold aqueous extract.

c) *Differential solvent extraction*

Unsoiled and unspotted leaves of *O. turpethum* were desiccated in shed for few days. Then the dried leaves were severed into tiny pieces and weighed properly in digital balance. 200 g of finely chopped, unsoiled and unspotted leaves were put into the 'thimble' of the Soxhlet apparatus. Following the standard of 1:10 material: solvent ratio, 2000 ml of each solvent, one after another, was poured into the 'still pot'. Two different solvents viz. petroleum ether (highly non-polar) and acetone (highly polar) were eluted through the same column one after another. A maximum 8 hours/ day extraction period was fixed for each solvent keeping the total extraction period 72 hours/ solvent. Solvent extractives were intensified following complete evaporation of the solvent through rotary evaporator and the congregated deposit was stored at 4°C in a refrigerator.

iv) Larvicidal Bio-assay

Dose dependent larvicidal bioassay was done in the laboratory as per the WHO (2009)[20] protocol. Each larval instar was treated with the hot and cold aqueous extracts and solvent extracts. Twenty five larvae of each instar were transferred from the insectary to the glass Petri dish containing 100 mL of tap water. 0.1% to 0.5% hot and cold aqueous extracts were applied against all the larval instars of *An. stephensi* under laboratory condition. Two other solvent extracts i.e. petroleum ether and acetone extracts were applied in the concentration of 40 ppm to 200 ppm against all larval instars. Each test was done in triplicate with a set of control where leaf extracts were applied. Petri dishes were kept at room temperature for 72 h of total experimental observation. 88 ± 2% relative humidity was maintained at the laboratory. Mortality rate after 24 h, 48 h and 72 h of

exposure were recorded. The larvae were supposed to be dead when they failed to move to the water surface or after probing with needle in the cervical area of it [21].

viii) Costing the impacts on a non-target population

The little creatures sharing the equivalent environment with mosquitoes are considered as the most terminal risk group. Susceptibility of these non-targets to leaf extractives was experimented on *Chironomus circumdatus* larvae (insect). *C. circumdatus* larvae were exposed to concentration of LC_{50} value of 24 h of 3rd instars larvae to examine the mortality and other irregularities such as tardiness of swimming activity for a total period of 72 h of post exposure.

ix) Statistical analyses

The percentage mortalities (%M) were précised by Abbott's formula[22] during the observation of larvicidal potentiality of the plant extracts. Determination of LC_{50} and LC_{90} values of crude and solvent extracts were carried out through Log-probit and regression analyses. Statistical validation amongst three completely randomized independent variables (different instars, different time exposures and different concentrations) and larval mortality were detailed through ANOVA analyses assuming homoscedasticity.

RESULTS

Operculina turpethum was found to have remarkable mosquitocidal property against *An. stephensi* in our laboratory observation. The highest mortality (100.00%) was achieved in 1st instars larvae at 0.5% concentration of hot aqueous extract at 72 h of exposure (Table 1). The mortality rate gradually increased in all instars for every concentration with increase of the time of exposure. It was highest in 72 h of exposure and lowest in 24 h of exposure. There was no mortality rate after treatment with cold aqueous extract. In case of petroleum ether extracts 1st instars larvae exhibit 80% mortality in 200 ppm concentrations after 72 h of exposure (Table 2). In case of acetone extracts 1st instars larvae exhibit 100% mortality in 160 ppm and 200 ppm concentrations after 72 h of exposure (Table 3). Also, 100% mortality was found in 200 ppm concentration at 72 h of exposure in case of 2nd and 3rd instars larvae. In case of 4th instars larva highest mortality (88%) was found in 200 ppm concentration at 72 h of exposure. The results of log probit analysis (95% confidence level) revealed that LC_{50} and LC_{90} values gradually decreased with the exposure periods having the lowest value at 72 h of exposure to first instar larvae followed by second, third and fourth instars larvae. LC_{50} and LC_{90} values of 1st instars larvae after 72 h of exposure were 18.79 and 68.03 respectively (Table 4). The result of the three-way factorial ANOVA of *O. turpethum* acetone extract of leaves carried out at different concentrations, different time intervals and different instars revealed significant difference in larval mortality ($p<0.05$). The results of regression analysis of *O. turpethum* acetone extract revealed that the mortality rate (Y) is positively correlated with the concentration of exposure (X) having a regression coefficient (R^2) close to 1 in each case (Table 5).

Table 1

Larvicidal bioassay using hot aqueous extract of Operculina turpethum leaves against Anopheles stephensi larvae

Larval Instars	Concentration (%)	Mortality rate (Mean ± SE)		
		24h	48h	72h
First	0.1	30.67 ± 0.33	45.33 ± 0.00	53.33 ± 0.00
	0.2	38.67 ± 1.12	53.33 ± 1.44	60.00± 56.00
	0.3	49.33 ± 0.00	62.67 ± 0.56	66.67 ± 0.33
	0.4	58.67 ± 0.56	69.33 ± 0.39	80.00 ± 0.00
	0.5	65.33 ± 0.77	70.67 ± 0.93	100.00 ± 0.00
Second	0.1	25.33 ± 0.53	33.33 ± 0.33	36.00 ± 0.89
	0.2	34.67 ± 1.12	40.00 ± 1.12	42.67 ± 1.44
	0.3	52.00 ± 0.89	52.00 ± 0.00	60.00 ± 0.39
	0.4	56.00 ± 0.39	61.33 ± 0.39	66.67 ± 0.93
	0.5	62.67 ± 0.56	65.33 ± 0.89	76.00 ± 0.00
Third	0.1	22.67 ± 0.00	28.00 ± 0.53	36.00 ± 0.77
	0.2	32.00 ± 0.53	37.33 ± 0.56	46.67 ± 1.12
	0.3	49.33 ± 0.00	53.33 ± 0.93	58.67 ± 0.00
	0.4	53.33 ± 1.44	58.67 ± 0.33	64.00 ± 0.39
	0.5	57.33 ± 0.53	62.67 ± 0.75	68.00 ± 0.00
Fourth	0.1	1.33 ± 0.89	8.00 ± 1.77	24.00 ± 0.93
	0.2	2.67 ± 0.93	9.33 ± 0.33	30.67 ± 1.77
	0.3	6.67 ± 0.33	13.33 ± 0.77	34.67 ± 0.66
	0.4	9.33 ± 1.44	17.33 ± 0.67	36.00 ± 0.53
	0.5	12.00 ± 0.33	25.33 ±0.00	38.67 ± 0.89

Table 2

. Larvicidal bioassay using petroleum ether extract of Operculina turpethum leaves against Anopheles stephensi larvae

Larval Instars	Concentration (%)	Mortality rate (Mean ± SE)		
		24h	48h	72h
First	40	16.00 ± 0.33	28.00 ± 0.00	30.00 ± 0.00
	80	22.67 ± 1.12	34.67 ± 1.44	39.33 ± 56.00
	120	26.00 ± 0.00	41.33 ± 0.56	46.00 ± 0.33
	160	42.67 ± 0.56	56.67 ± 0.39	60.00 ± 0.00
	200	68.00 ± 0.77	73.33 ± 0.93	80.00 ± 0.00
Second	40	12.00± 0.53	21.33 ± 0.33	28.00 ± 0.89
	80	16.67 ± 1.12	28.67 ± 1.12	37.33 ± 1.44
	120	22.00± 0.89	33.00 ± 0.00	51.33 ± 0.39
	160	39.33 ± 0.39	47.33 ± 0.39	58.67 ± 0.93
	200	57.33 ± 0.56	66.67 ± 0.89	70.00 ± 0.00
Third	40	06.00 ± 0.00	18.33 ± 0.53	21.33 ± 0.77
	80	11.33 ± 0.53	23.67 ± 0.56	34.67 ± 1.12
	120	18.00 ± 0.00	31.67 ± 0.93	40.00 ± 0.00
	160	36.67 ± 1.44	33.33 ± 0.33	49.33 ± 0.39
	200	43.33 ± 0.53	51.33 ± 0.75	62.00 ± 0.00
Fourth	40	05.00 ± 0.89	12.67 ± 1.77	18.67 ± 0.93
	80	09.00 ± 0.93	19.33 ± 0.33	27.67 ± 1.77
	120	15.33 ± 0.33	29.00 ± 0.00	37.33 ± 0.66
	160	24.00 ± 1.44	31.33 ± 0.67	45.33 ± 0.53
	200	39.33 ± 0.33	42.00 ±0.00	58.00 ± 0.00

Table 3
Larvicidal bioassay using acetone extract of Operculina turpethum leaves against Anopheles stephensi larvae

Larval Instars	Concentration (%)	Mortality rate (Mean ± SE)		
		24h	48h	72h
First	40	56.00 ± 0.33	68.00 ± 0.00	80.00 ± 0.00
	80	62.67 ± 1.12	74.67 ± 1.44	89.33 ± 56.00
	120	76.00 ± 0.00	81.33 ± 0.56	96.00 ± 0.33
	160	82.67 ± 0.56	86.67 ± 0.39	100.00 ± 0.00
	200	88.00 ± 0.77	93.33 ± 0.93	100.00 ± 0.00
Second	40	49.33 ± 0.53	57.33 ± 0.33	68.00 ± 0.89
	80	54.67 ± 1.12	67.67 ± 1.12	77.33 ± 1.44
	120	62.67 ± 0.89	72.00 ± 0.00	85.33 ± 0.39
	160	69.33 ± 0.39	77.33 ± 0.39	98.67 ± 0.93
	200	77.33 ± 0.56	86.67 ± 0.89	100.00 ± 0.00
Third	40	36.00 ± 0.00	41.33 ± 0.53	61.33 ± 0.77
	80	41.33 ± 0.53	58.67 ± 0.56	74.67 ± 1.12
	120	48.00 ± 0.00	66.67 ± 0.93	80.00 ± 0.00
	160	66.67 ± 1.44	73.33 ± 0.33	89.33 ± 0.39
	200	73.33 ± 0.53	81.33 ± 0.75	100.00 ± 0.00
Fourth	40	32.00 ± 0.89	38.67 ± 1.77	58.67 ± 0.93
	80	37.00 ± 0.93	53.33 ± 0.33	70.67 ± 1.77
	120	45.33 ± 0.33	60.00 ± 0.00	77.33 ± 0.66
	160	64.00 ± 1.44	65.33 ± 0.67	85.33 ± 0.53
	200	69.33 ± 0.33	72.00 ±0.00	88.00 ± 0.00

Table 4
Assessment of LC_{50} and LC_{90} values through log-probit and regression analyses using acetone extract of Operculina turpethum leaves

Larval Instars	Period of Exposure	LC_{50}	LC_{90}	Regression	R^2- value
1st	24	37.63	313.41	0.05 x + 11.96	0.98
	48	18.79	252.42	0.04 x + 15.50	0.99
	72	19.78	68.03	0.03 x + 19.46	0.89
2nd	24	55.30	984.11	0.04 x + 10.36	0.99
	48	32.76	474.44	0.04 x + 12.79	0.99
	72	28.31	119.20	0.05 x + 15.06	0.96
3rd	24	95.38	877.40	0.06 x + 5.78	0.95
	48	62.14	453.19	0.06 x + 8.97	0.96
	72	34.50	156.04	0.06 x + 13.36	0.99
4th	24	110.20	901.23	0.06 x + 4.80	0.95
	48	75.78	761.69	0.05 x + 8.57	0.95
	72	33.96	268.86	0.05 x + 13.50	0.96

x = concentration of acetone extractives (in ppm)

Table 5
Completely randomized three way ANOVA analyses of the larvicidal activity using concentration (C), hour (H) and instars (I) as three independent parameters

Source of variations	Sum of squares	df	Mean squares	F value	P value
Hour	538.563	2	269.281	172.121	0.005
Instars	11615.281	5	2323.056	1637.408	0.000
Conc.	319.983	3	1173.328	837.122	0.000
Hour * Instars	48.438	10	4.744	3.456	0.000
Hour * Conc.	8.382	6	1.397	0.977	0.128
Instars * Conc.	306.955	15	20.464	13.600	0.001
Hour * Instars * Conc.	95.618	30	3.187	2.274	0.000
Residual	302.750				
Total	**16435.969**				

DISCUSSION

Due to lack of potentiality of controlling the mosquito vector, it persists as a great challenge to diminish the mosquito borne diseases till now. It is very essential to control the mosquito to put a stop to the propagation of mosquito borne diseases and to get better quality of community health and environment [23]. Mosquito can be pre-eminently controlled at their wriggler phase [24]. Plant derived eco-friendly and biodegradable pesticides are gaining much attention due to its target specificity nature in recent years [25]. In comparison with synthetic insecticides, herbal products are least phototoxic and do not build up any chemical residues in the environment. Various researchers promisingly reported the plant derived products as mosquito control agents having the larvicidal [26], pupicida [27], repellent and smoke toxicant [28] properties against different mosquito species. The present study well documented the mosquito larvicidal activity of *O. turpethum* leaf extractives against malarial vector *An. stephensi.* Rahuman et al. (2000)[29] showed that the acetone extract of *Feronia limonia* dried leaves as a potent mosquito larvicide against fourth instar larvae of *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti*, with LC50 values of 129.24, 79.58 and 57.23 ppm, respectively. However, in this study acetone extracts of *O. turpethum* leaf showed highest larval mortality and the LC_{50} and LC_{90} values of the 1st instars larvae was 18.79 and 68.03 respectively after 72 h of exposure. Singha Ray *et al.* (2014) [30] established that *Nelumbo nucifera* seed coat acts as mosquito larvicide promisingly against 3rd instar larvae of *An. stephensi*. Singh et al., (2015) [31] stated that ethyl acetate extract of *Nicotiana plumbaginifolia* leaves have larvicidal potential against 1st instar larvae of *An. stephensi* where LC_{50} and LC_{90} values against 1st instars larvae after 72 h of exposure was 9.25 and 19.42 ppm respectively. Shahi *et al.* (2010) [32] reported that the alcoholic extract of leaves of *Calotropis procera* showed remarkable mosquito larvicidal effect against *An. stephensi*. LC_{50} and LC_{90} values were 109.71 and 234.61 ppm respectively after 24 h of exposure for the 3rd instars. In an experiment of Kamaraj *et al.*, (2009) [33] leaf acetone extract of the plant *Tridax procumbens* showed remarkable larvicidal activity against 4th instar larvae of *An. subpictus* after 24 h of exposure where LC_{50} value was 51.57 mg/mL determined by the log-probit analysis. Kaushik and Saini (2008) [34] tested the larvicidal activity of the leaf acetone extract of *Milingtonia hortensis* against *Anopheles stephensi* and found that the LC_{50} and LC_{90} values of leaf extract for 24 h of exposure against *An. stephensi* were 83.18 and 190.5, 147.9 and 257, 138 and 275.4 ppm for 2nd, 3rd and 4th instar mosquito larvae. The results of toxicity test on non target organisms indicated no mortality after 24 h.

CONCLUSION

This study reveals the plant *O. turpethum* has excellent larvicidal properties against the crucial malarial vector *An. stephensi.* This bioactive portion can be used as a potent larvicidal agent in near future. Further experiments is mandatory to determine the proper bioactive fractions responsible for larval death.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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