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USE OF TURPENTINE OIL AGAINST THE LARVAE OF *ANOPHELES, AEDES* AND *CULEX* (CULICIDAE: DIPTERA) MOSQUITOES

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ABSTRACT

The study has been made against the larvae of *Anopheles vector* of malaria, *Aedes;* vector of chikungunya and dengue and Culex; vector of filariasis and Japanees encephalitis. Indiscriminate use of synthetic insecticides as Abate, Functional and immunohistochemical characterization of CCEae3a, a carboxylesterase associated with temephos resistance in the major arbovirus vectors *Aedes aegypti* and *albopictus* studied[8] and Mosquito (*Aedes taeniorhynchus*) resistance to methoprene in an isolated habitat the resistant among adult mosquito and its larvae Transcriptome profiling and genetic study reveal amplified carboxylesterase genes implicated in temephos resistance, in the Asian tiger mosquito, *Aedes albopictus.* Therefore; it's our intuition to use the plants extracts for making pollution free environment and wellbeing for human health. In this study, antilarval activities were studied using turpentine oil solution with methanol and ether. The bioassay has been made with the third / early fourth instars larvae of each mosquito genera These were exposed in control solution and treated also with the turpentine solution 50ppm, 75ppm and 100ppm, to check the mortality interval after 24 hrs. The bioassay test has been made for the first time to know the anti larval efficacy and mortality was calculated and corrected by Abbott's formula.

Figure : 00 References : 20 Tables : 04
KEY WORDS : Abbott's formula, Auto-disposable syringe, Ether, Methanol, Micro pipette, Turpentine oil

Introduction

A number of serious human life threatening diseases were investigated, some of them were transmitted by different mosquito species causing millions of human deaths every year, disability, burden on the large poor group of society. Culicidae is the most important largest group of arthropod which transmit nine dreadful diseases to human beings; over 100 countries, causing morbidity and mortality of nearly two millions peoples every year. *Anopheles, Aedes* and *Culex* are the vectors for pathogens of different diseases like malaria, Dengue, Chikungunya and Japanase encephalitis. These diseases are burden, social stigma and devaste Indian Economy. The intensive and indiscriminate use of synthetic insecticides cause resistance rebounding vectorial capacity and spread other parasitic diseases. It is necessary to intervene the proliferation of mosquito borne diseases and prevent the quality of public health environment. In recent years, use of many synthetic insecticides for mosquito control program has been limited due to the non-biodegradable nature. Resistance has been developed in larvae of *Aedes* and *Anophelese* against Phenthion and temebhos revealed[19] while determing the susceptibility and resistance mosquito laevae to insect development inhibitor recorded[20]. Plant products may be the alternative sources of mosquito control. Earlier researchers[5,6,11] investigated the alternative substitute of the insecticides while, working on the vector borne disease control aspect. The insecticidal properties of indigenous plant products recorded[11-14] while significant reduction in

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TABLE-1 : Mortality Rate of *Anophelese, Aedes* and *Culex* Larvae exposed with Alocohol solution of Turpentine Oil

S.N.	Time	Solution with Alcohol	Larvae Exposed with Different Concentrations														
			No of Larvae in Control Sol.		Solution in ppm	*Anophelese* Larvae in Sol.				*Aedes* Larvae in Sol.				Culex Larvae in Sol.			
			Larvae Exposed	Observe Mortality		Larvae Exposed	No of dead Larvae	% LCL	% HCL	Larvae Exposed	No of dead Larvae	% LCL	% HCL	Larvae Exposed	No of dead Larvae	% LCL	% HCL
1	24 hrs	TPO with Alc	25	0	50	25	12-14	48	56	25	11-13	44	52	25	11-12	44	48
2		TPO with Alc	25	0	75	25	18-20	72	80	25	17-19	68	76	25	17-18	68	72
3		TPO with Alc	25	0	100	25	22-24	88	96	25	21-23	86	92	25	20-23	84	92

LCL-lower confidence limit., HCl -Highestconfidence limit. TpO- Turpentine oil

TABLE: 02 Shows the Mortality Rate of *Anophelese, Aedes* and *Culex* Larvae exposed with Ether solution of Turpentine Oil

S.N.	Time	Solution with Ether	Larvae Exposed with Different Concentrations														
			No of Larvae in Control Sol.		Solution in ppm	*Anophelese* Larvae in Sol.				*Aedes* Larvae in Sol.				Culex Larvae in Sol.			
			Larvae Exposed	Observe Mortality		Larvae Exposed	No of dead Larvae	% LCL	% HCL	Larvae Exposed	No of dead Larvae	% LCL	% HCL	Larvae Exposed	No of dead Larvae	% LCL	% HCL
1	24hrs	TPO with Ether	25	0	50	25	15-17	56	64	25	13-15	52	60	25	12-14	48	56
2		TPO with Ether	25	0	75	25	20-22	80	88	25	18-20	72	80	25	18-20	72	80
3		TPO with Ether	25	0	100	25	24-25	96	100	25	23-24	92	96	25	23-24	92	96

LCL-lower confidence limit., HCl -Highest confidence limit. TpO- Turpentine oil

TABLE - 03 : Mortality Rate of *Anophelese, Aedes* and *Culex* Larvae exposed with Alocohol and Ether solution of Turpentine Oil

S.N.	Time	Solution with Alcohol	Larvae Exposed with Different Concentrations													
			No of Larvae in Control Sol.		Solution in ppm	*Anophelese* Larvae in Sol.				*Aedes* Larvae in Sol.				Culex Larvae in Sol.			
			Larvae Expo-sed	Obs-erve Mort-ality		Larvae Expo-sed	No of dead Larvae	% LCL	% HCL	Larvae Expo-sed	No of dead Larvae	% LCL	% HCL	Larvae Expo-sed	No of dead Larvae	% LCL	% HCL
1	24 hrs	TPO with ALc	25	0	50	25	12-14	48	56	25	11-13	44	52	25	11-12	44	48
		TPO with Ether	25	0	50	25	15-17	56	64	25	13-15	52	60	25	12-14	48	56
2		TPO with ALc	25	0	75	25	18-20	72	80	25	17-19	68	76	25	17-18	68	72
		TPO with Ether	25	0	75	25	20-22	80	88	25	18-20	72	80	25	18-20	72	80
3		TPO with ALc	25	0	100	25	22-24	88	96	25	21-23	86	92	25	20-23	84	92
		TPO with Ether	25	0	100	25	24-25	96	100	25	23-24	92	96	25	23-24	92	96

LCL-lower confidence limit., HCl -Hughest confidence limit. TpO- Turpentine oil

TABLE- 04 : Comparasion with earlier workers.

S.No	Name of Mosquito genera	Solution with	Workers[15]		Workers[16]		Workers[06]		Present study	
			Concentration of Solution		Concentration of Solution		Concentration of Solution		Concentration of Solution	
			LCL/LC_{50}	UCL/LC_{90}	LCL/LC_{50}	LCL/LC_{90}	LCL/LC_{50}	UCL/LC_{90}	LCL/LC_{50}	UCL/LC_{90}
1.	*Anopheles*	Methanol	82.57	112.69	219.14	50	50	100	50	100
		Ether	0	0	0	50	50	100	50	100
		Ehtylacetate	124.06	149.76	221.26	0	0	0	0	0
2	*Aedes*	Methanol	109.24	131.73	162.76	52	52	100	52	100
		Ether	0	0	0	50	50	100	50	100
		Ehtylacetate	139.79	174.61	221.26	0	0	0	0	0
3	*Culex*	Methanol	121.76	156.64	232.07	52	52	100	52	100
		Ether	0	0	0	50	50	100	50	100
		Ehtylacetate	132.11	180.64	271.62	0	0	0	0	0

density of dengue and chikungunya disease vectors *Aedes* by phytoproduct *Azadirachtin*[5]. Experiments have been made on the repellency of *Lantana camara* (Verbenaceae) flower against *Aedes* mosquitoes[11]. Larvicidal and repellent action of *Dalbergia sissoo oil (Roxb)* (*F.Laguminasae*) studied[2].

Culex quinquefasciatus,a domestic mosquito mainly found in urban areas, is a vector of human filariasis in India. *Cx. quinquefasciatus* acts as a vector for *Wuchereria bancrofti, Brugia malayi and Brugia timori*, which are responsible for lymphatic filariasis, a prevalent disease in India. . There is a renewed interest in plant essential oils products as sources of new insect controlling agents, because they may be biodegradable to nontoxic compounds, thus minimizing the accumulation of harmful residues, leading them to be more environmental friendly compared to synthetic compounds. Research on the use of plant-derived chemicals to control mosquitoes and other insects has increased in recent years. It is true that the use of natural products based on plant essential oils (EOs) as insecticides and repellents[3,4,8,9,13,14] .

Materials and Methods

The larvae were collected from clean stagnant and also from polluted water bodies, an ideal breeding sites, coolers (Fig- 02), domestic containers, Cement tanks, discarded plastic tubs and buckets and from different drains of the various locality of Jhansi city around Avas Vikas, Suryapuram, Vardhan Vihar , Mayur vihar colony, M.L.B. Medical college Campus , K.K. Puri and also from Rajghat colony.

PREPARATION OF SOLUTION

Solutions of turpentine were prepared with auto-disposable syringes/ micro pipette of 0.1ml, 0.5ml and 1.00ml for actual purities. The oil of turpentine bought from market. Thereafter, the solution has been prepared with alcohol and ether in different concentrations likewise auto disposable syringes/ micro pipettes of 0.1ml, 0.5ml and 1.00ml were used for maintaining purity and then kept in the refrigerator as stock to maintain its original potency.

TEST ORGANISMS

Larvae of *Anopheles, Aedes* and *Culex ;* (Diptera: Culicidae) were brought to the Entomological laboratory. These were held at room temperature and 75-85 relative humidity with dark and photo period.

BIOASSAY TEST

The crushed dog biscutes dissolved in the water where larvae were added in it. In each study 12 Jars/ bowls were used. One liter of water filled in each Jar. Twenty five larvae of each mosquito genera were poured in control and in each solution of turpentine oil with concentration 50ppm, 75ppm and100ppm and check in 24 hrs in control, methanolic and ether solutions.

STATISTICAL ANALYSIS

Lower confidence limit and upper confidence limit has determined for the calculation. The mortality rate has been observed, calculated and corrected.

$$\% \text{ Mortality} = \frac{\% \text{ Test motility—\% control mortality}}{100 \text{ — \% control mortality,}} \times 100$$

Result and Discussion

Study has been made in different concentrations of methanolic and ether solutions of turpentine oil for various genera of.*Anopheles* ,*Aedes* and *Culex.*

25 Larvae of Anopheles exposed with the methanolic solution of turpentine oil showed 48-56% mortality with 50ppm, 72- 80% mortality with 75ppm while; 88 -96% mortality were recorded with 100ppm solution. 25 Larvae of *Aedes* exposed with the methanolic solution of turpentine oil showed 44-52% mortality with 50ppm, 68- 76 % mortality with 75ppm while; 86 -92% mortality with 100ppm solution. 25 Larvae of *Culex* larvae exposed with the methanolic solution of turpentine oil showed 44-48 % mortality 50ppm, 68- 72 % mortality with 75ppm while; 84 -92% mortality with 100ppm solution (Table- 01).

25 Larvae of *Anopheles* exposed with the ether solution of turpentine oil showed 56-64% mortality with 50ppm, 80- 88% mortality with 75ppm while; 96 -100% mortality were recorded with 100ppm solution of ether solution of turpentine oil . 25 Larvae of *Aedes* exposed with the ether solution of turpentine oil showed 52-60% mortality with 50ppm, 72- 80 % mortality with 75ppm while; 92 -96% mortality with 100ppm solution. and the same no. of *Culex* larvae exposed with ether solution of turpentine oil showed 48-56 % mortality with 50ppm, 72-80 % mortality with 75ppm while; 92 -96% mortality with 100ppm solution (Table-02).

In this study comparision has been made with the methnolic and ether solutions of TpO and found that the larval mortality were better with the turpentine solution of ether than that of methanolic solution. It is also observed that the mortality rate was high in Anopheles larvae which is decreasing towards *Aedes* and *Culex* respectively in the higher confidence limit as well as lower confidence limit (Table-03).

Discussion

The study has been made with the methanolic solution of turpentine oil showed higher to lower larvicidal activity against *Anopheles, Aedes* and *Culex* at LCL was

48,44 and 44 with 50ppm and the same way at 75 ppm lower confidence limit was observed 72, 68 and 68 and with 100ppm, 88,86 and 84 respectively.

The same number of larvae of exposed with the ether solution of turpentine oil showed higher to lower larvicidal activity against *Anopheles, Aedes* and *Culex* at higher confidence limit was 56,52 and 48 at 50ppm and the at 75 ppm HCL was observed 84,76 and 72 and with 100ppm, 96, 96 and 92 respectively (Table-04).

Our study is better than the previous studie[5,6,10-12] with the methanolic solution of *Azadirachitin*. The pioneer scientist[15] showed the larvicidal efficacy with the methanolic solution of *Ficus bengalensis* which is same as our study. Our study is better than the previous studies[10-12] which postulated larvicidal activity with *Casia fistula* against *Aedes* and *Culex* .

The result of our study showed very good mortalities in 100ppm solution of ether and much better results than that of earlier studies [5,6,10-12]. Longevity is observed in the developing time of different larval stages and also paralyses the body of the larvae of *Aedes albopictus* Skuse in very low concentration as well as in high concentration it showed quick mortality. More research work is needed in this field to save the poor population from vector borne disease, social stigma and burden.

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