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INSECTICIDE SUSCEPTIBILITY STATUS OF DENGUE VECTORS, *AEDES AEGYPTI(L.)* AND *AEDES ALBOPICTUS (S.)* IN SELECTED LOCALITIES OF DISTRICT DEHRADUN, UTTARAKHAND

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ABSTRACT

Dengue is one of the most common arthropod borne viral disease which is transmitted mainly by two vector species *Aedes aegypti* and *Ae. albopictus*. Immature stages were collected from selected localities of district Dehradun and incubated for hatching in insecterium for rearing upto adult stage. The adults were then identified by using the pictorial keys. The susceptibility status of both the species against different insecticides was assessed by using the WHO standard bioassay tests. Larval bioassays of both the population shows incipient resistance against temphos while adult susceptibility testing results showed that *Ae. aegypti* was resistant to DDT and Fenitrothion, susceptible for Endosulphan and probable resistant to Malathion, Deltamethrin and Permethrin, while *Ae. albopictus* is resistant to DDT and Probable resistant to other insecticides.

KEYWORDS: *Aedes aegypti*, larvicidal, insecticides, Resistance, mosquitoes.

INTRODUCTION

Mosquitoes are of very great importance to man as vector of dreaded human diseases such as malaria, dengue, chikungunya, filarial etc. Mosquito -borne diseases prevalent in more than 100 countries across the world, infecting over millions of individuals every year at the global level and are leading cause of human death (Ghosh, *et al*., 2012).

Dengue virus (DENV, Flaviviridae, Flavivirus) and Chikungunya virus (CHIKV, Togaviridae, Alphavirus) are mosquito borne viruses of medical concern in most tropical regions.

Aedes aegypti (Linnaeus, 1762) and *Aedes albopictus* (Skuse, 1894) are the main vectors of dengue and Chikungunya viruses worldwide.

As per WHO record, currently 47 countries are at the risk of severe dengue, while 60 countries are under the attack of chikungunya (WHO). In recent decades, the incidences of dengue have grown dramatically around the world. Moreover, under-reporting of actual number of dengue cases and their misclassification has made the disease serious and uncontrollable (Samal and Kumar, 2018).

Dengue contagion is one of the most probative *Aedes* – borne viral diseases of human in tropics. In India, diseases transmitted by *Ae. aegypti* and *Ae. albopictus* have shown a significant rise during the last decade. According to the data compiled by ministry of family and health welfare, India experienced a total of 1,01,192 cases of dengue with 172 fatalities in 2018 which substantially increased to 1.57,315 cases in 2019 leading to 166 deaths(NVBDCP accessed, may 2021). In addition, with the recent outbreak of chikungunya across India a total of 12,205 cases in 2019 and 6263 confirmed cases during 2020. *Ae. aegypti* has taken a huge attention of researchers(NVBDCP). Moreover, diseases like Zika are on the rise causing grave situation. Keeping in view the lack of an adequate and successful vaccine against these diseases, control of mosquito vector by large scale larval mortality is the only solution (Rajmohan and Ramswamy, 2007).

Although the use of insecticides poses a serious threat of the environmental pollution and pest resistance, yet in the developing countries including India control of vector borne disease is solely dependent upon chemical control (Bansal and Singh, 2004).

The most recommended plan to control mosquito- borne diseases primarily lies on mosquito management below threshold level and interrupting their disease

transmission cycle. Various control measures; elimination of their breeding places, use of several biological agents, sterile insect release method, etc have been devised and practiced till date (Kumar, *et al*., 2017). Insecticides belonging to different groups, especially DDT (Organochlorine) and Malathione (Organophosphates) have been in extensive use for the past few decades in vector control programmes in India. Though majorly all the organochlorines are banned by EPA for residential usage due to acute toxicity, they are still in use in agricultural fields (Moore, *et al*., 2009).

Devising a suitable mosquito management programme requires the latest reports on the susceptibility status of *Aedes* mosquito against different insecticides in use. As it is well known that injudicious pesticides application against that insect pest often leads to environmental pollution and harmful effects on human and non target species; it become imperative to evaluate the toxicities of different insecticides to formulate control strategy.

Lack of base line susceptibility data, continued rise of *Aedes* borne diseases and increase in the insecticide resistance in *Aedes* mosquito has forced us to assess the current insecticide susceptibility status. Present investigation, thus attempts to take a comprehensive view of susceptibility in dengue fever vector against various insecticides.

MATERIALS AND METHODS

Culture of mosquito

Dengue fever mosquitoes, *Ae. aegypti* and *Ae. albopictus* were collected from selected localities of Dehradun district viz Doiwala, Sahaspur, Vikash Nagar and ISBT locality during January to December 2020. The colony of *Aedes* mosquitoes was maintained in an insect rearing laboratory in deoartment of Zoology, under controlled conditioned of 28 ± 1^0C, 80 ± 5% RH, 14 h of light and 10 h of darkness (WHO, 2005). Adults kept in clothed cages were fed on sugary juice by supplying them raisins soaked in water. Female mosquitoes were provided with occasional blood meals for egg maturation by keeping albino rat in the cage. The eggs were collected in an ovitrap lined with whatman filter paper strips which were then transferred into the enamel trays filled with at least 1.5 -2.0 L of de-chlorinated water. The hatched larvae were fed on powdered dog biscuits and yeast in a ratio of 3:1 (Warikoo, *et al*., 2012). The pupae were collected on regular basis and were kept in clothed cages for adult emergence. Adults were identified by pictorial keys of Rueda (2004) and Barraud (1934).

Preparation of insecticidal solution

Larval bioassays were carried out by using WHO recommended diagnostic dosage of 0.02mg/l for temphos and adult bioassays by using control and test papers of three groups of insecticides. Among these; Organochlorines (DDT 4%, Endosulphan1%), Organophospahates (Malathin 5%, Fenitrothion 1%) and Pyrethroids (Deltamethrin 0.05% and Permethrin 0.75%) were used against one to two day old mosquitoes collected from different localities.

Larval Bioassays

The larval bioassays were conducted separately for both *Ae. aegypti* and *Ae. albopictus* by transferring 30 late III or earl IV instars larvae with the help of droppers in small disposable test cups which contains 99 ml of water and 1 ml of temphos diagnostic concentration of 0.02 mg/l. each bioassay was comprised of four replicates and one control group. Mortality was estimated after 24 h of temphos exposure. Larvae were considered dead when they were incapable of reaching to the water surface after being touched. Each bioassay was repeated four times on separate days and was repeated four times on separate days and was conducted at a temperature of 27 ± 2^0C, relative humidity of 80 ± 10% and a photoperiod of 12 : 12 h (WHO, 2005).

Interpretation of larval susceptible tests

For larval bioassays, the criterion of Davidson and Zahar (1973) was used to evaluate the qualitative modifications in susceptibility status of vector populations. A percent mortality > 98% against the diagnostic concentration indicates susceptible status; mortality between 80 to 98% indicates incipient resistance status; while percent mortality < 80% confirms the resistance status.

Adult Bioassays

About 150 active female *Aedes* mosquitoes of each species were transferred to six exposure tubes (100 in four exposures tubes lined with insecticides impregnated papers and 50 in two control tubes with oil impregnated papers) separately against WHO recommended diagnostic dosage of each insecticide for one hour. The four replicates of each vector species containing 25 female mosquitoes per replicate were set up simultaneously for each insecticide. Control replicates were also held concurrent to each test. After exposure for one hour the mosquitoes were transferred to six holding tubes for recovery. During this recovery time period, the holding tubes were kept in cool, dark and shady places immediately, at room temperature of 27 ± 2^0C and relative humidity of 80 % ± 10% (WHO, 2013). Cotton pads soaked in 10% glucose solution were provided as supplementary food during recovery time period of 24 h. The percent mortalities were computed by calculating the dead and alive mosquitoes after 24 h of recovery time period and Abbott's formula (Abbot, 1925), if needed was used for its correction.

Interpretation of adult susceptibility tests

Adult susceptibility tests were evaluated by following the WHO (2013) recommended criteria as: a) mortality in the range of 98-100% indicates susceptibility; b) mortality of < 98% is suggestive of the development of resistance and further investigation is needed;c) if the observed mortality (corrected if necessary) is between 90 and 97%, the presence of resistant genes in the vector

population must be confirmed; and d) if mortality is < 90% , resistance is confirmed.

Data analysis

The bioassays resulting in more than 20% larval mortality or pupae formation in control indicated the inappropriate selection of larvae and thus were discarded and run again. However, if 5-20% larval mortality was obtained in control assays, it was corrected by Abbott's formula (Abbott, 1925).

$$\% \text{ corrected mortality} = \frac{\% \text{ test mortality} - \% \text{ control mortality x } 100}{100 - \% \text{ Control mortality}}$$

RESULTS

Larval Bioassays

Table 1 presents the susceptibility status of *A. aegypti* and *Ae. albopictus* larvae collected from different localities of Dehradun district against WHO suggested diagnostic concentration of temphos (0.02 mg/l). The results of larval bioassays showed that percent mortality of *Ae. aegypti* and *Ae. albopictus* varied from 91.84 and 99.17 for Doiwala, 96.33 and 82.98 for Sahaspur, 97.92 and 93.75 for Vikash Nagar and 99.00 and 96.00 for ISBT area respectively. The results of diagnostic dose tests revealed that larvae of *Ae. aegypti* and *Ae. albopictus* collected from all localities of Dehradun showed incipient resistant according to the criterion of Davidson and Zahar(1973).

Adult Bioassays

After 24 hour post- exposure, all the field populations of *Ae. aegypti* showed resistance to DDT, with percent mortality rate 24.47 and 23.47 for *Ae. albopictus*. The percent mortality rates for Endosulphan, Malathion, Fenitrothion, Deltamethrin and Permethrin ranging from 99.17, 96.33, 82.98, 97.92 and 93.75 respectively, while percent mortality results of *Ae. albopictus* field population ranged from 97.40 for Endosulphan, 94,57 for Malathion, 97.34 for Fenitrothion, 90.96 for Deltamethrin and 92.19 for permethrin. So *Ae. aegypti* is resistant for DDT and probable resistant (PR) for other insecticides while *Ae. aegypti* shows susceptible for endosulphan (Table 2 and 3).

Table 1: Insecticide susceptibility status of the late III or early IV instar of *Aedes aegypti* and *Aedes albopictus* against temphos in different localities of District Dehradun, Uttarakhand.

Name of locality	Species	No. of mosquitoes larvae exposed		No. of mosquitoes larvae dead		Corrected % mortality	Susceptibility status
		Test	Control	Test	Control		
Doiwala	*Ae. aegypti*	250	50	230	1	91.84	IR
	Ae. albopictus	250	50	248	2	99.17	IR
Sahaspur	*Ae. aegypti*	250	50	241	1	96.33	IR
	Ae. albopictus	250	50	210	3	82.98	IR
Vikash nagar	*Ae. aegypti*	250	50	245	2	97.92	IR
	Ae. albopictus	250	50	235	2	93.75	IR
ISBT	*Ae. aegypti*	250	50	248	0	99.00	S
	Ae. albopictus	250	50	240	0	96.00	S

S= Susceptible, if 98-100% observed mortality; IR= 80-97% observed mortality suggests incipient resistance; WHO diagnostic concentration of 0.02 mg/l.

Table 2: Insecticide susceptibility status of the adults of *Aedes aegypti* in different localities of District Dehradun, Uttarakhand.

Insecticide Tested and concentration (%)	No. of mosquitoes exposed		No. of mosquitoes dead		Corrected % mortality	Susceptibility status
	Test	Control	Test	Control		
DDT	250	50	65	0	26.00	R
Endosulphan	250	50	248	2	99.17	S
Malathion	250	50	241	1	96,33	PR
Fenitrothion	250	50	210	3	82.98	R
Deltamethrin	250	50	245	2	97.92	PR
Permethrin	250	50	235	2	93.75	PR

S: Susceptible, if 98 - 100% observed mortality; PR: Probable resistance; if 90 - 97% observed mortality suggests the possibility of resistance that needs to be further confirmed; R: resistance, if < 90% observed mortality.

Table 3: Insecticide susceptibility status of the adult *Aedes albopictus* in different localities of district Dehradun, Uttarakhand.

Insecticide Tested and concentration (%)	No. of mosquitoes exposed		No. of mosquitoes dead		Corrected % mortality	Susceptibility status
	Test	Control	Test	Control		
DDT	200	50	50	1	23.47	R
Endosulphan	200	50	195	2	97,40	PR
Malathion	200	50	190	4	94.57	PR
Fenitrothion	200	50	195	3	97.34	PR
Deltamethrin	200	50	183	3	90.96	PR
Permethrin	200	50	185	2	92.19	PR

S: Susceptible, if 98 - 100% observed mortality; PR: Probable resistance; if 90 - 97% observed mortality suggests the possibility of resistance that needs to be further confirmed; R: resistance, if < 90% observed mortality.

DISCUSSION

Aedes aegypti has developed incipient resistance to commonly used larvicide and adulticide which necessitates continuous susceptibility monitoring for effective vector control programme. *Insecticide* resistance management (IRM) is crucial to maintain vector control sustainable. Studies have been undertaken by earlier investigators to assess insecticidal susceptibility status against dengue vectors in different parts of India (Samal and Kumar, 2018; Singh, *et al*. 2013; Mariappan, *et al*., 2017 and Kaushik, *et al*., 2019).

Temphos is organophospahate insecticide which is still effective as larvicide for controlling *Aedes* mosquito larvae (Mukhopadhaya, *et al*., 2006).Widespread use of temphos has led to the development of resistance in different countries including Thailand (Ponlawat, *et al*., 2005) and Rawalpindi (Pakistan) (Arslan, *et al*., 2016). Tolerance/resistance against temphos is reported from the field collected larvae in Delhi (Singh, *et al*., 2014) and Assam also (Dhiman, *et al*., 2014).

In the laboratory, the aquatic stages of *Ae. aegypti* developed induced resistance to temphos, which showed varying degree of cross resistance to Fenthion, Malathion and DDT. The expression of Temphos induced larval resistance was also observed in adult stages (Shetty, *et al*., 2010). The immature of *Aedes* mosquito have shown the tendency of developing induced resistance to Temphos under laboratory conditions (Tikar, *et al*., 2009). Our study is consistent with the study carried out in NCR Delhi, Ranchi city, Jharkhand and Assam in which immature stages is still susceptible to temphos, Fenthion and Malathion (Kaushik, *et al*., 2019; Das, *et al*, 2011 and Dev, *et al*., 2014).

DDT resistance in *Ae. aegypti* mosquitoes was recorded for the first time in 1967 from Jharia in Jharkhand state (Mourya, *et al.*, 1994). In 1970, DDT resistance was reported in *Ae. aegypti* strains from Banglore, Bellary, Delhi, Mettupalayam, Rajahmundrym Varanasi and Vellore, but the species was found to be susceptible to all organophosphorus insecticides except Malathion (Madhukar and Pillai, 1968).

In southern India, *Ae. aegypti* was resistance to DDT and Dieldrin but susceptible to Propoxin, Fenitrothion, Malathion, Deltamethrin, Permethrin and Lambdacyhalothrin (Singh, *et al*., 2013) which is consistent with our study against adult *Ae. aegypti* in Dehradun. Previous studies conducted in different parts of India have reported varying degree of resistance towards DDT and Pyrethroid (Kushwaha, *et al*., 2015; Singh, *et al*., 2011). In bioassay method, 100% adult *Ae. aegypti* mosquitoes were found to have resistance against DDT, about 8 % showed resistance against Pyrethroid and 4% towards Malathion (Dhiman, *et al*., 2014).

CONCLUSION

From the study it is concluded that *Aedes aegypti* and *Ae. albopictus* which are prevalent in District Dehradun, India have progressively started to developed resistance capability towards currently used insecticides which may bring an indication of major dengue outbreaks in this district. There is a need to test the insecticide susceptibility status time to time to monitor and manage resistance to insecticides used in public health for the prevention and control of dengue outbreaks.

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