ABSTRACT

Hematophagous arthropods pose danger to humans owing to their ability of ingesting the disease causing pathogens from an infected individual and inject it into a healthy being causing the transmission of the disease/infection. Of the total diseases suffered globally, around 16.6% are due to vector borne diseases. Globally, more than 1 billion people get infected by vector borne diseases and 1% die primarily by Malaria, Dengue, Leishmaniasis, Chagas Disease, Yellow Fever, Filariasis and Onchocerciasis. Globally, climatic changes are increasing the numbers and distribution of many disease vectors. Additionally, poverty related issues, *i.e.* lack of access to adequate housing, malnutrition, lack of proper sanitation and drainage and unavailability of safe drinking water also contribute towards the increased risk of such diseases.

Mosquitoes, one of the most successful hematophagous arthropods are responsible for the transmission of numerous dreadful diseases, such as Malaria, Dengue, Japanese encephalitis, Yellow fever, West Nile fever, Zika, Chikungunya, Filariasis causing several million deaths throughout the world annually. The most rapidly spreading mosquito borne viral disease is Dengue which has expanded to previously unexplored regions increasing its incidence rate approximately 30 fold in the last 50 years. Another mosquito borne viral disease affecting Africa, Asia and the Indian subcontinent is Chikungunya. Recently this disease resurgence occurred in Asia, Africa, Europe as well as North America.

India owing to its subtropical climate is subjected to regular infections of various vector borne diseases. It is estimated that approximately 3.9 billion people residing through 128 countries inhabit Dengue risk areas. An estimated 390 million infections of

Dengue occur every year, out of which only 25% show clinical manifestations. Around 1.9 million Chikungunya infections have been reported to occur in five Asian countries viz., India, Indonesia, Maldives, Myanmar and Thailand.

India is reported to acquire the largest number of Dengue infections annually with approximately 100 million asymptomatic and 33 million clinically manifested infections). The presence of both the urban vectors and ideal climatic conditions aggravate the factors for major outbreak of Dengue. More than 0.1 million cases of Dengue occur every year in India, the trend towards an increase in infection rates every year.

In 2017, among the Indian states the highest numbers of Dengue infections were reported in West Bengal. The warm and humid temperature and climatic conditions, rapid urbanisation, high vegetation cover, lack of sanitation, hygiene and drainage in majority of the state together provide the ideal ambience for *Aedes* and other mosquito growth and proliferation. Additionally, the high population density of West Bengal supports the efficient circulation of disease causing pathogens.

Aedes mosquitoes pose severe threat to human race because of its capability to transmit several arboviruses, *i.e.* Dengue virus (DENV), Chikungunya virus (CHIKV), Zika virus (ZIKV), Yellow fever virus, *etc*. These diseases have increased severely in the past five decades and expanded itself many folds and spread throughout the globe. *Aedes* mosquitoes are closely associated with the human colonies and occur near such dwellings, commonly laying eggs in and around human houses. Additionally, human made products, *i.e.* tyres, tanks, plastic containers *etc* serve as egg laying sites and becoming its breeding and proliferating site, thereby increasing their abundance and establishing colonies in human inhabiting areas.

For none of the arboviruses transmitted by *Aedes* mosquitoes, any treatment or vaccination exist. In absence of specific medications and vaccines for all the above mentioned disease, the prevention becomes the only option to restrict disease transmission in humans. Disease prevention for *Aedes* transmitted arbovirus mainly involves vector control and personal prophylactic measures to minimise mosquito biting.

Since the discovery of insecticides, they have been used heavily for mosquito control. In India, Organochlorines (DDT, Dieldrin, Aldrin *etc*), Organophosphates (Malathion, Temephos, Dichlorvos, Chlorpyrifos), Synthetic pyrethroids (Lambdacyhalothrin, Deltamethrin, Permethrin, Cypermethrin and its derivatives, Cyfluthrin) and Carbamates (Propoxur, Bendiocarb) have been widely used for both mosquito control as well as agricultural pest control.

But due to the uncontrolled heavy use of these chemicals/ insecticides, both target as well as non-target species have evolved to resist the actions of those chemicals in their body through different mechanism. This phenomenon interrupting the chemicals to manifest their planned actions is known as Insecticide resistance. Insecticide resistance results in the failure of mosquito control programmes to achieve their planned targets, thereby increasing the risk of DENV infection even after insecticide spray during severe disease outbreaks.

Resistance to insecticides can be caused by an range of modifications within a mosquito, such as behavioural alteration, physiological modifications within the cuticle reducing the insecticide penetration, biochemical changes within the activity of major insecticide detoxifying enzymes or structural modification within the target of the insecticide thereby blocking the insecticide binding and subsequent action. Metabolic detoxification of insecticides refers to the degradation of the chemicals into non-toxic and water soluble forms by the action of gut enzymes. The enzymes carrying on the task of xenobiotic detoxification generally belong to large families of multigenes, the most notable being the Carboxylesterases (CCEs), Cytochrome P450s (CYP450s) and Glutathione S-transferase (GSTs). Increased synthesis of one such enzyme, *i.e.* CCEs through gene amplification have been reported to confer resistance against organophosphate, carbamates and pyrethroid insecticides in insects .

Target site modification refers to the loss of sensitivity of the active site of the protein targeted by the insecticide. The most notable and commonly found is insensitivity of voltage gated sodium channel gene (VGSC) by synthetic Pyrethroids (SPs) or Organochlorines (OCs), thereby providing resistance against these insecticides, commonly called as knockdown resistance (kdr). Two of the most commonly detected point mutations in resistant *Ae. aegypti* are V1016G/I and F1534C in the IIS6 segment of VGSC.

So, in an attempt to gain knowledge on the prevalence of different species of *Aedes* mosquitoes, their levels of insecticide susceptibility and underlying mechanisms, *Aedes* mosquitoes were randomly sampled from five districts of northern West Bengal. The collected mosquitoes were subjected to insecticide susceptibility testing against 0.0200 and 0.0125 ppm of Temephos, 4% DDT, 5% Malathion, 0.05% Deltamethrin, 0.05% Lambdacyhalothrin, 0.75% Permethrin, and 0.1% Propoxur. The mosquitoes were assayed for the activity of major insecticide detoxifying enzymes *i.e.* Carboxylesterases

(CCEs), Cytochrome P450s (CYP450S) and Glutathione S-transferase (GSTs). Qualitative study for the presence of Carboxylesterase isozymes were also performed. Moreover, synergistic assay with the use of 4% PBO and 10%TPP were also performed to confirm the role of Cytochrome P450s and Carboxylesterases respectively behind the observed resistance. Additionally, the mosquitoes were also screened for two kdr mutations, *i.e.* V1016G/I and F1534C.

It was observed that, throughout the study region, a dominance of *Ae. albopictus* over *Ae. aegypti* was noticed. It was also revealed that for both the *Aedes* species, discarded tyres were the most preferred breeding habitat followed by uncovered cemented tanks. Majority of the studied *Ae. aegypti* populations possessed low resistance levels against temephos but one population NDP^{ae} was found to possess altered susceptibility against 0.02ppm and 0.0125ppm temephos. All the tested populations of *Ae. aegypti*, possessed widespread resistance against DDT with the lowest mortality recorded 47.9% for DAR^{ae} population followed by APD^{ae} (55.4%), NDP^{ae} (56.6%), COB^{ae} (70%) and JPG^{ae} (72.0%). One population of *Ae. aegypti* possessed moderate resistance against malathion, *i.e.* APD^{ae} with 72.5% mortality. Most of the studied population were revealed to be susceptible or incipiently resistant to lambdacyhalothrin and deltamethrin with the mortality ranging from 80.9-100% and 89.2-100% respectively. Against permethrin, very low mortality percentage *i.e.* 50% for NDP^{ae} to incipiently resistant for APD^{ae}, 83.3% were reported. Three of the tested *Ae. aegypti* populations were found to be severely to moderately resistant against propoxur, DAR^{ae}, JPG^{ae} and NDP^{ae}:

From the results of synergistic assay, it was observed that, prior exposure to 4% PBO before DDT was found to increase susceptibility to it in APD^{ae} population, restoring the mortality rate partially, thus a part of the observed resistance might be conferred by detoxification through Cytochrome P450s. In the same population, Carboxylesterases were revealed to drive the partial resistance against malathion, in APD^{ae} population restoring the mortality from 72.5% to 94.0% when exposed to 10% TPP. In APD^{ae}, JPG^{ae} and NDP^{ae}., Cytochrome P450s were revealed to be accountable for partial resistance against deltamethrin and lambdacyhalothrin. In NDP^{ae}, Carboxylesterase linked pathways were revealed to be involved in propoxur resistance, as use of 10% TPP could restore its mortality from 45.4 to 70.4%. Through kdr genotyping, both susceptible and mutant kdr allele were revealed to be present amongst the wild populations of *Ae. aegypti* indicating the possible role of these mutations behind observed resistance against permethrin and DDT. The study of qualitative analysis of Carboxylesterase in *Ae. aegypti* mosquitoes revealed the presence of around five different isozymes of α-Carboxylesterase (Rf values 0.62, 0.68, 0.73, 0.82, 0.97) and three isozymes of β-Carboxylesterase (Rf values 0.62, 0.80 and 0.96) were found. The intensity of the band izozymes depicting the overexpression of enzyme were found to be linked with resistance against the tested organophosphate in some of the field collected *Ae. aegypti* mosquitoes.

Similar pattern of resistance was also noted in *Ae. albopictus* mosquitoes, one population NGK^{al} exhibited incipient resistance against temephos at 0.0200 ppm dosage and two populations, NGK^{al} and SLG^{al} .possessed incipient resistance against 0.0125 ppm temephos. Severe to moderate resistance against DDT was revealed in the tested *Ae. albopictus* mosquitoes, namely SLG^{al}, JPG^{al} and NGK^{al}. However, complete susceptibility was recorded among the wild *Ae. albopictus* mosquito populations against malathion, deltamethrin and lambdacyhalothrin. In two of the tested *Ae. albopictus* population moderate level of resistance against permethrin was found with mortality percentages 75.4 (APD^{al}) and 75.0 (JPG^{al}). Severely resistant population of Indian *Ae. albopictus* against propoxur was revealed for the first time in this study with very low mortality rate, 42.5%.

Populations NGK^{al}, JPG^{al} and SLG^{al} were reported to possess Cytochrome P450s linked resistance mechanism against DDT, since prior exposure to PBO restored the mortality/susceptibility in these populations. CytochromeP450s were also revealed to confer resistance against permethrin in APD^{al} and JPG^{al}. The results of kdr genotyping revealed that, all but one (SLG^{al}) tested *Ae. albopictus* population were found to possess the 1534C mutant allele, which might be linked to the resistance against permethrin and DDT.

From the study of qualitative analysis of Carboxylesterases, two different isozymes for both α- Carboxylesterases (Rf values 0.81, 0.91) and β- Carboxylesterases (Rf values 0.63 and 0.95) were found amongst the different field caught mosquito populations. The presence of more than one band and the higher intensity of the expressed isozyme were found to be linked with resistance against organophosphates in the field collected *Ae. albopictus* population.

The knowledge gained through this study will help the personnel engaged in dengue vector control for designing of an effective strategy throughout the study region. As a part of habitat destruction, safe disposal of tyres and covering of open cemented tank or water holding containers should be aimed throughout the studied region. For successful control of *Ae. aegypti* throughout the districts of northern part of West Bengal, deltamethrin and lambdacyhalothrin seem to be the most effective. Similarly for *Ae. albopictus,* deltamethrin and lambdacyhalothrin and malathion were found to be the most potent for dengue vector control. Temephos can also be used throughout the region for *Aedes* vector control with exception for North Dinajpur and Nagrakata where use of synergist along with the larvicide seems promising. A detailed knowledge on the prevalence of other kdr mutations might also provide an insight for effective dengue vector control.