DISCUSSIONS

5. DISCUSSION:

5.1 Surveying of Aedes mosquitoes:

Throughout the study region, a dominance of *Ae. albopictus* over *Ae. aegypti* was noticed. Majority of the part of the studied area had been rural region covered with dense vegetation for a long time span. The urbanisation in this region is very recent in this region which might explain the abundance of rural vector of dengue throughout the sampling area (Gratz, 2004). It might also be the case that some kind of competition might be occurring between these two *Aedes* species since both occupy similar niche resulting in the establishment of one species with considerable competitive advantage over the other in the studied area. *Ae. albopictus* have been reported to outcompete *Ae. aegypti* occupying same habitat owing to its higher mating competitiveness over *Ae. aegypti* (Bellini *et al.*, 2013).

In this study, it was found that for both the *Aedes* species, discarded tyres were the most preferred breeding habitat resulting highest positivity indices (Table 12). Similar inferences have been observed in other such studies (Vijayakumar *et al.*, 2014; Ferede *et al.*, 2018). The advantage of tyres used as breeding habitats by mosquitoes lie on the fact that the water inside it is not easily observable and thus is less prone to climatic disturbances, maintaining ideal environmental conditions for *Aedes* development (Ferede *et al.*, 2018). Owing to the structure of tyres, some amount of water is always carried in it and even if it dries completely, it may serve as a good egg preserving surface, which when refilled with water may again become the mosquito breeding habitat (Rao *et al.*, 2010). The second most preferred habitat for *Ae. aegypti* in this study was revealed to be uncovered cemented tanks, mainly used by garage workers to check tyre leakage. In India, cemented tanks and plastic containers are regarded as the major breeding habitats of *Ae. aegypti* (Balakrishnan *et al.*, 2006). Thus use of discarded tyres for *Ae. aegypti* breeding might have taken place fairly recently in India. Other breeding habitats such as bamboo stumps and plant axils were also found to be preferred mainly by *Ae. albopictus,* owing to its behaviour and history of being the sylvatic mosquito thus preferring the natural habitats (Gratz, 2004).

From the result of larval density index, it was found that higher densities were noted for *Ae. aegypti* in ISL followed by NMZ and SLG. Similarly, for *Ae. albopictus* very high larval densities were observed for NGK followed by NBU, HAS and APD. This points on the risk of dengue transmission in and around this sites during disease outbreak. To minimise the disease risk, proper control measures should be taken ahead of the disease outbreak season by destroying the mosquito breeding habitats and treatment with larvicide in water storage containers.

5.2. Insecticide susceptibility status and underlying mechanism in Aedes aegypti mosquitoes:

5.2.1 Insecticide susceptibility status of Aedes aegypti mosquitoes:

In this study, insecticide susceptibility was tested against few commonly used insecticides (adulticides and larvicide) belonging to four different groups of insecticides. For larvicides, an organophosphate insecticide, *i.e.* temephos was chosen owing to the frequent usage of it by both governmental and non-governmental organisation in mosquito larvae control. Amongst the adulticides, one Organochlorine- DDT, one Organophospahate- malathion, three synthetic Pyrethroids- type I, permethrin; type II, deltamethrin and lambdacyhalothrin and one Carbamate, propoxur were tested.

5.2.1.1 Insecticide susceptibility status against organophosphates:

- *Temephos:*

Temephos or O,O,O’,O’- tetramethyl O,O’-thiodi-p-phenylenebis (Phosphorothioate), is widely used throughout the world for mosquito larvae control owing to its cost effectiveness as well as efficiency (Grisales *et al.*, 2013). However the wide usage has resulted in widespread resistance against this insecticide in majority of the mosquito vectors particularly dengue vectors. Majority of the sites from our sampling region have shown low resistance levels but higher resistance ratios. Moreover, one population NDP^{ae} was found to possess incipient resistance against 0.02ppm temephos, *i.e.* WHO recommended diagnostic dose and resistance against 0.0125ppm, *i.e.* NVBDCP recommended diagnostic dose . The increased RR_{50} values for most of the sites along with the presence of one resistant *Ae. aegypti* population from this dengue endemic region indicates that in near future temephos may become ineffective in dengue control throughout the region. For dengue vector, temephos is generally recommended for water storing sources and vessels at a dose not exceeding 1mg/L. Four of the sites were noted to possess trend towards resistance development with an RR_{50} value above 2 for APD^{ae}, DAR^{ae}, JPG^{ae} and NDP^{ae} (Table 14).

In India, moderately resistant *Ae. aegypti* have been noted to be widespread throughout the Indian capital, New Delhi with mortality percentages ranging from 66.22 to 95.11 (Singh *et al.*, 2014). Similarly, high level of resistance against temephos with mortality percentages ranging from 4% to 10% at 0.02 ppm were noted in Andaman and Nicobar islands (Sivan e*t al.*, 2015). Severe level of resistance to temephos have also been recorded in wild *Ae. aegypti* population from Assam (Yadav *et al.*, 2015) with RR_{99} values of 2.5-4.5. Even higher resistance ratio levels were also exhibited by mosquito population from Karnataka with LC_{90} values 1.00 ppm to 17.03 ppm (Shetty *et al.,* 2013). Throughout the world, the prime mosquito control organisations of most of the countries recommend and rely on the use of temephos for disease prevention such as America, Carribean, India, Srilanka *etc* (Rawlins, 1998; Karunaratne *et al.,* 2013; NVBDCP, 2019c). So resistance against temephos was noted in *Aedes aegypti* successively after a long period of its use worldwide, Carribean (Georghiou, 1987; Rawlins and Wan, 1995), French Polynesia (Failloux *et al.,* 1994) and other parts of the world (Vontas *et al.,* 2012). Field caught population of *Ae. aegypti* from Thailand have been reported to exhibit mortality percentages ranging from 50.5 to 96.0 (Jirakanjanakit *et al.,* 2014). Similarly *Ae. aegypti* have been recorded to possess severe to moderate resistance with mortality percent as low as 16% (Chen *et al.,* 2005) to moderate resistance with RR_{50} value ~ 2 (Ishak *et al.,* 2015; Rohani *et al.,* 2001). Severe resistance against this oraganophosphate has also been recorded in another Southeast Asian country *i.e.,* Indonesia with mortality percent of 22% to 605 and LT_{50} in the range of 2.2 to 8.5 (Mulyatno *et al.,* 2012). In Cambodia, incipiently resistant population of temephos have been recorded with LC_{95} value of 0.03mg/l (Polson *et al.,* 2001). From the neighbouring country of Pakistan, incipiently resistance *Aedes aegypti* mosquitoes have been reported, mortality percent 81.25 to 86.67% (Arslan *et al.,* 2016). Similar instance of temephos resistant *Ae.aegypti* mosquitoes have also been recorded, highly resistant; El salvador (Lazcano *et al.,* 2009) and incipiently resistant from Argentina (Llinas *et al.* 2010).

The LC_{50} value of the tested mosquitoes against temephos were found to be low for majority of the population ranging from 0.000057 to 0.002 ppm (Table 14). Similar trend of LC_{50} value of Temephos were also noted for mosquitoes collected from Assam region, (LC_{50}: 0.002 to 0.0029 ppm) (Yadav *et al.* 2015), Mumbai (LC_{50}: 0.0012- 0.010) and Delhi (LC_{50}:0.0014- 0.0125) population (Tikar *et al.* 2008).

Similarly the L_{C99} dosages of different field population were found to be ranging from 0.26 to 0.00011 ppm. In nearby state of Assam, similar LC_{99} values were observed for one population collected from Sotia *i.e.* LC_{99}-0.12, whereas higher for rest of the population (Yadav *et al.*, 2015). In our study, the recommended dosage for only one population was found to be above the WHO diagonostic dose *i.e.* for NDP^{ae}, 0.52 ppm and one population above NVBDCP range, JPG^{ae} 0.016ppm.Such a scenario indicates the silent mode of resistance development throughout these sites which may result in the failure of vector control approach.

Temephos is recommended not only for *Aedes* control but also for the control of other disease causing mosquitoes such as *Anophele*s sp., *Culex* sp., *etc* (WHO, 2009*)*. Apart from mosquito control, it is also used in control of blackfly, midge, flea and other insects of public health as well as on agricultural fields for cutworms, thrips, *Lygus etc* (WHO, 2009). Owing to the wide usage of temephos in different mosquito species, resistance against it is also common (Chakraborti and Tandon, 2000; Shetty *et al.*, 2013; Soltani *et al.*, 2015). Resistance has also been noted against temephos in African population of blackfly (Hemingway *et al.*, 1991). Prevalence of resistance in all these vector species may have even more drastic outcome to the human public health disrupting the efficiency of all the vector control approaches in action.

There is an immediate need of assessing the insecticide resistance levels in other mosquito vectors against temephos and if resistance is noted then successive replacement of temephos by other effective insecticide.

- *Malathion:*

After mosquito population were found to possess resistance against DDT, it was replaced by hexachlorocyclohexane which was also effective for a very short time span and then was launched an organophosphate insecticide “Malathion” (Raghavendra *et al.*, 2010). However, initially providing efficient results, vector mosquito started developing resistance against this insecticide also. In our study, only one out of the five tested population was found to possess moderate resistance, *i.e.* mortality percentage 72.5 against malathion, APD population (Table 15). Another population, DAR^{ae} was reported to exhibit incipient resistance against it with mortality percentage 92.6. Rest of the population were completely susceptible to malathion as was the lab reared susceptible population. Malathion is being used in Alipurduar district (Personal communication) under which APD^{ae} site is located which explains the reason behind the resistance possessed by this population. However, the presence of insecticide resistance in DAR^{ae} site may be because of the cross exposure of insecticides sprayed on agricultural lands contaminating the mosquito breeding habitats as run offs containing the toxic moiety as have been reported in other mosquito vectors (Philbert *et al.*, 2014). Darjeeling district is known for tea cultivation and malathion being one of the key insecticide sprayed on tea fields apart from synthetic pyrethroids justifies involvement of above scenario and implying that similar might be the case for DAR^{ae} population (Saha and Mukhopadhyay, 2013). Malathion when used for mosquito control is used in 5% solution and similar or higher dosages are recommended for insect pests of agricultural importance (Anon., 2002a). The toxicity of this OP to non-target organisms has led to the withdrawal of its usage in majority of the agricultural pest control as well as mosquito control programmes.

Three of the population, *i.e.* JPG^{ae}, COB^{ae} and NDP^{ae} were recorded to be fully susceptible to malathion, thereby indicating that this OP can still be used during intense dengue outbreaks in the districts from which these population were collected. Throughout the country, both malathion resistant as well as susceptible *Aedes aegypti* population have been reported. Malathion resistant strains have been reported from Jharkhand, Andaman and Nicobar islands and many major cities of India possessing low level of resistance *i.e.* mortality percentage between 90-98% (Singh *et al.*, 2011; Sivan *et al.*, 2015; Tikar *et al.*, 2008). Whereas susceptible populations were recorded from Delhi (Katyal, 2001), Goa (Thavaselvam *et al.*, 1993), Assam (Yadav *et al.*, 2015) and Jharkhand (Singh *et al.*, 2011). Resistance against malathion in *Anopheles* sp. have been observed since 1987 with mortality as low as 12%` (Raghavendra *et al.*, 2010), whereas amongst the northern states of India the mortality have been noted to range from 68.46 to 95.43 % (Tikar *et al.*, 2011). Mosquito population with similar level of resistance, incipient resistance against malathion have been reported from Malaysia, mortality 91% (Ishak *et al.*, 2015), Thailand with RR_{90} ranging 2.2-6.6 and also other regions of the world (Vontas *et al.*, 2012).

5.2.1.2 Insecticide susceptibility status against Organochlorine:

- *DDT:*

DDT has been used throughout the world in both agricultural and public health sector in both malaria control and dengue prevention tactics since the 20th century, as a result of it, majority of the regions of the world, intense DDT pressure exists (Vontas *et al.*, 2012). Throughout the studied region also, widespread resistance against DDT was noted with the mortality percentage noted to be as low as 47.9% for DAR^{ae} to 72.0% for JPG^{ae}. Severe to moderate resistance levels against DDT brings into notice the dominance of DDT based mosquito control programmes for a large time duration throughout the area. Similar pattern of DDT resistance in wild population of *Aedes* mosquito have also been noted in nearby regions, i) Assam, with mortality percentage in the range of 65.0 to 70.5 (Yadav *et al.*, 2015), ii) Delhi, with 74% mortality percentage (Katyal *et al.*, 2011), iii) Jharkhand with corrected mortality percentage 54.68 to 63.88 (Singh *et al.*, 2011), iv) Andaman and Nicobar island with 74% mortality implying moderate resistance in *Ae. aegypti* mosquitoes (Sivan *et al.*, 2015). Stoppage of DDT usage by most of the governmental and non-governmental mosquito control agencies (Raghavendra *et al.*, 2010) seems to be a good approach as evident through the level of resistance prevailing among the mosquitoes against this insecticide. Many species of malaria vector, *Anopheles spp.* are also resistant to DDT with corrected mortality percentage less than 10 since 1985 (Das *et al.*, 1986) with the trend of resistance still being followed in *Anopheles culicifacies* (Raghavendra *et al.*, 2010; Mishra *et al.*, 2012). The first report to record the resistance against DDT in *Ae. aegypti* dates back to 1967(Azeez, 1967).

Similar to the resistance levels, the KDT_{50} and KDT_{95} values of DDT were also higher for most of the tested populations. Amongst the field caught populations, the lowest KDT_{50} was 95.41 (JPG population) whereas the highest was recorded, 182.16 for DAR^{ae} population, the population possessing the greatest resistance amongst others (Figure 19). Similar values were obtained for KDT_{95} with the lowest time recorded for JPG^{ae} whereas the highest for DAR^{ae}. As compared to SP^{ae}, all the field population possessed higher values of both KDT_{50} and KDT_{95}, implying the pattern of resistance development in the populations. Similar pattern of KDT_{50} values were observed for JPG^{ae} and COB^{ae} population with that of the neighbouring state of Assam with KDT_{50} values of 99.5 min and 83.2 min for Serajuli and Kusumtola population respectively (Yadav *et al.,* 2015). In this study, even in the lab reared population the corrected mortality percentage against DDT was not equal to 100%, which again imparts light on the intensity of DDT resistance prevailing in the field population of dengue vectors.

5.2.1.3 Insecticide susceptibility status against synthetic Pyrethroids:

- *Deltamethrin, Lambdacyhalothrin and Permethrin:*

Synthetic pyrethroids are the most recent amongst the insecticide classes. In this study the mosquitoes were tested for their susceptibility against one type I pyrethroid *i.e.*, permethrin and two type II commonly used & recommended synthetic pyrethroid insecticide for mosquito control *i.e.*, deltamethrin and lambdacyhalothrin. The mosquitoes exhibited a higher resistance to the Type I pyrethroid than that of Type II. Against permethrin, three of the populations were resistant with mortality 60.0 to 76.5%, one incipiently resistant population, APD^{ae} (mortality percentage 83.3). The insecticide treated mosquito nets distributed throughout the districts by anganwadi centers as a part of national vector control programme contain either permethrin or deltamethrin (NVBDCP, 2019d) which maybe the reason behind the development of resistance against permethrin in these mosquitoes. Furthermore, none of the tested mosquitoes were found to be susceptible to this insecticide, this might have dreadful consequences in the near future for vector control programme using permethrin. The similarity between the resistance pattern between DDT and permethrin indicates the extent of cross-resistance occurring between the two insecticides (Table 15).

The KDT_{50} and KDT_{90} values against permethrin, though higher than other tested synthetic pyrethroids was significantly lower than that of DDT. The KDT_{50}

values were lower than 60 minutes for three of the populations *i.e.*, APD^{ae}, COB^{ae} and JPG^{ae} ranging from 37.40 to 52.76 min. Higher than 60 min KDT50 values were recorded for DAR^{ae} and NDP^{ae}, 67.66 to 78.98 min. Very few studies have been conducted to reveal the KDT_{50} and KDT_{90} values against permethrin in *Ae. aegypti* mosquitoes. Once such study from Nigeria reported very low KDT values with the KDT_{95} value ranging between 23.4 to 43.2 (Ayorinde *et al.*, 2015), however in another such study very low KDT_{50} and KDT_{95} values were recorded for susceptible population of *Ae. aegypti* from the same region (Ndams *et al.*, 2006), Senegal, KDT_{95} value 20.5 to 21.8 mins (Dia *et al.*, 2012).

Throughout the country resistant populations of *Ae. aegypti* against permethrin have been reported from Delhi-mortality percent 66.8-82.3 (Kushwah *et al.*, 2015), South Andaman district- mortality percent 84.76 (Sivan *et al.*, 2015), Southern India-RR- 5.1-6 (Muthusamy *et al.*, 2014). However susceptibility has also been noted against 0.75% permethrin (as used in this study) from Kerala (Sharma *et al.*, 2004), Jharkhand (Singh *et al.*, 2011); or against lower dose *i.e.*0.25% by Katyal *et al.*, 2001 with 100% mortality throughout the country. Mortality percentages have mostly been found greater than 60% for the tested field caught *Ae. aegypti* populations. Permethrin resistant wild population of *Ae. aegypti* have been reported in Malaysia (Othman-wan *et al.*, 2010; Ishak *et al.*, 2015), Columbia (Gonzalez *et al.*, 2011), Thailand (Somboon *et al.*, 2003) and other countries of the world (Moyes *et al.*, 2017). Altered susceptibility against permethrin have also been noted in *Anopheles* (Mittal *et al.*, 2004), *Culex* (Kumar *et al.*, 2011) or even in other insect vectors (Hemingway and Ranson, 2000).

Against other two synthetic pyrethroid insecticides, three population were incipiently resistant against both deltamethrin and lambdacyhalothrin. These insecticide resistant populations recorded the mortality percentage ranging between 80.9 to 85.0% for lambdacyhalothrin and 89.2 to 91.9 for deltamethrin (Table 15). Rest of the two tested population, *i.e.* DAR^{ae} and COB^{ae} reported complete susceptibility against both the synthetic pyrethroid insecticides. As was the scenario in case of permethrin resistance, same seems to hold true since LLITNs contain either permethrin or deltamethrin, thereby providing the exposure to deltamethrin in most of the population (Table 15). Also, these two synthetic pyrethroids are extensively used in agricultural fields for insect pest control (Gurusubramanian *et al.*, 2008). APD^{ae} and JPG^{ae} sites are located in tea belts of northern Bengal with dominance of intensive tea gardens, whereas NDP^{ae} has intense pineapple cultivations (Saha and Mukhhopdhyay, 2013; Das *et al.*, 2011). In both the tea and pineapple cultivating fields synthetic pyrethroids and Organophosphates are sprayed, which may again provide the cross resistance to the mosquito populations residing in the vicinity of the agricultural fields (Nkya *et al.*, 2013). The 100% mortality as recorded in COB^{ae} and DAR^{ae} leads to the recommendation that during intense dengue or chikungunya outbreaks in these districts usage of permethrin may help reduce the infection rates.

Ae. aegypti is an anthropophilic mosquito *i.e.* its habitat is placed near human dwellings, the altered susceptibilities against synthetic pyrethroid insecticides seem to be due to the direct exposure to household mosquitocidal tools such as mosquito repellent coils, fumigant spray and creams which chiefly contain pyrethroid derivatives (Yadav *et al.*, 2015). In India, a household spray sold under the brand name "Hit" mainly contains transallethrin whereas another commonly used tool, repellent coil sold under the brand name "Mortein" contains a cocktail of permethrin and transallethrin and fumigant sold under the brand name "all-out" contains allethrin.

Throughout India, majority of the studied population have been recorded to be susceptible to deltamethrin in Assam with mortality percentage 98.8-100 (Yadav *et al.,* 2015), Jharkhand with mortality percentage 98.26-100 (Singh *et al.,* 2011), Kerala (Sharma *et al.,* 2004), Southern Andaman, mortality percentage (Sivan *et al.,* 2015), Delhi with 100% mortality (Katyal *et al.,* 2001). Similar is the scenario for lambdacyhalothrin, however incipient resistance and resistance have been reported in *Ae. aegypti* population from Andaman island with 80.95% mortality (Sivan *et al.,* 2013), Jharkhand with onset of IR *i.e.* mortality percentage: 97.33-97.8 (Singh *et al.,* 2011). Rest of the studies have reported complete susceptibility of *Ae. aegypti* against lambdacyhalothrin (Katyal *et al.,* 2001; Sharma *et al.,* 2004)

Although few reports exist on the pyrethroid resistance in Indian *Ae. aegypti* populations, worldwide many such studies have been conducted reporting pyrethroid resistance such as in Thailand (resistant against deltamethrin with 61.2-73.9%) (Somboon *et al.,* 2003); South American countries, namely Cuba, Jamaica, Costarica, Peru and Venezuela (mortality percentages 56.3-96.4 against lambdacyhalothrin; 72.7-97.2% against deltamethrin (Rodriguez *et al.,* 2007); Senegal with mortality percentage 94.5 against deltamethrin and 81.6% for lambdacyhalothrin (Dia *et al.,* 2012); Vietnam with mortality 19.33-97.00% against deltamethrin and 25.0-96.0% against lamdacyhalothrin (Huong *et al.,* 2004).

Amongst the mosquitoes that result in knockdown, through closure of voltage gated sodium channel, the lowest KDT_{50} and KDT_{95} values were noted for these two synthetic pyrethroids. The KDT_{50} value for deltamethrin was lowest for COB^{ae} (14.11) and highest for NDP^{ae} (58.77), and all the KDT_{50} values were less than 60 minutes (Figure 19). The pattern of KDT_{95} was also similar as the KDT_{50} with the values ranging between 52.19-108.21 mins for the field caught populations of *Ae.*

aegypti. Similarly for lambdacyhalothrin, the KDT_{50} values were lower than 60 min ranging between 9.41- 43.33, with the lowest and highest values exhibited by the same population as in case of deltamethrin. Apart from two populations, *i.e.* APD^{ae} and JPG^{ae}, the rest population reported very low KDT_{95} value *i.e.* below 60 mins (Figure 19). However, two populations exhibited higher values of KDT_{95}indicating the onset of a resistance phenomenon which may be revealed in near future by the failure of dengue control efforts using lambdacyhalothrin. Lower susceptibility and knockdown rates have been recorded in an Senegal population of *Ae.aegypti* with KDT95 value of 27-30.3 min for deltamethrin and 26.9-34.2 for lamdacyhalothrin (Dia *et al.*, 2012), Central Africa with KDT95 as 11.4 –24.6 min (Kamgang *et al.*, 2011), central African republic with KDT95 values 23.5-42.0 min (Ngoagauni *et al.*, 2016), *etc*. Most of the studies with reports of higher resistance against these two synthetic pyrethroids did not present the data of knockdown times as comparatively few studies have till date been conducted on the KDT_{50} and KDT_{95} values of synthetic pyrethroids.

5.2.1.4 Insecticide susceptibility status against carbamate

- *Propoxur*

In India, the only carbamate insecticide that is recommended for *Aedes* mosquito control is Bendiocarb. However, in this study, we have tested the susceptibility against other carbamate insecticide *i.e*, propoxur which is used throughout different countries of the world and also recommended by food and agriculture organisation of the United nations for both dengue and malaria vector control (FAO, 2002). This insecticide was tested to assess whether this insecticide serve as an alternate vector control in India by assessing the prevailing resistance or intermediate resistance among different field population of *Ae. aegypti*. Throughout the study sites, none of the *Aedes* population was found to possess susceptibility to propoxur. Three populations were found resistant against propoxur, of which one population DAR^{ae} was found to possess severe resistance with mortality 50.0% and two had moderate to low resistance with mortality percentage 77.2 and 75.4 for JPG^{ae} and NDP^{ae} (Table 15). Rest of the populations were found to possess incipient/unconfirmed resistance with mortality percentages of 91.3 to 97.2 for APD^{ae} and COB^{ae} respectively. The districts from where APD^{ae} and COB^{ae} were collected are neighbouring districts and the similar resistance phenomenon observed for propoxur and also other tested insecticides seems to be a result of similar mosquito control practices at both individual level or organization level in both the districts. As already stated, propoxur is not used or recommended for mosquito control in India, yet the observed resistance phenomenon operating in all the tested *Aedes aegypti* population may be pertained to the usage of household insect repellents containing propoxur in a very high dose for larger insects such as termites, bed bugs *i.e* in Baygon spray and many other repellent chalks manufactured by local companies.

Field populations of *Aedes aegypti* with resistance against 0.1% propoxur have been reported in Delhi *i.e* mortality 85% (Katyal *et al.,* 2001), Madurai *i.e.* mortality 96% (Marriapan *et al.,* 2017), Karnataka in larval population (Shetty *et al.,* 2013). Throughout the country, propoxur susceptible population of *Aedes aegypti* were noted in Andaman island (Sivan *et al.,* 2015) and in Kerala (Sharma *et al.,* 2004). Around the globe, propoxur resistant *Ae. aegypti* population have been reported from Senegal (Dia *et al.*, 2012), Thailand (Pethuan *et al.,* 2007) Malaysia (Rong *et al.*, 2012), Cot D ivore (Konan *et al.*, 2012) *etc.*

Resistance have also been noted against another more frequently used carbamate, *i.e* bendiocarb (0.1%) in Andaman in India (Sivan *et al.*, 2015), Cameroon (Yougang *et al.*, 2017), Mexico (Deming *et al.*, 2006), Pakistan (Mohsin *et al.*, 2016; Arslan *et al.*, 2016), Malaysia (Rong *et al.*, 2012) *etc*.

5.2.1.5 Overall view on resistance in Aedes aegypti:

Amongst all the tested mosquito population namely APD^{ae} and JPG^{ae} showed resistance ranging from low to severe resistance against all the tested adulticides. Highest resistance data in case of APD^{ae} population was noted against DDT (mortality percentage 55.4), whereas that for JPG^{ae} population, it was recorded against permethrin. If we arrange the toxicity of the tested insecticides in order of descending potency/efficacy it would be

Deltamethrin>lambdacyhalothrin>temephos>malathion>propoxur>permethrin> DDT

Throughout the studied region, during intense dengue and chikungunya outbreak deltamethrin usage may help in efficient disease prevention. Except for two Alipurduar and Darjeeling, all other districts may also get good dengue prevention with the use of malathion (5%) amongst the adulticide. Moreover, temephos also seems to be the safest choice for dengue vector control throughout the studied region at WHO dose for NDP^{ae} and at NVBDCP recommended dose for all other sites.

5.2.2. Mechanisms of resistance:

5.2.2.1 Mechanism of insecticide resistance against Organophosphate:

- *Temephos:*

In mosquitoes, the CCE based resistance mechanism forms the primary mechanism of OP resistance and secondary mechanism of CB resistance (Hemingway and Karanaratne, 1998). It also has been found to confer resistance against synthetic pyrethroid in other insect species. Acetylcholine Esterases (AchEs) are the common target site for both OPs and CBs, so insensitive AchEs may also give rise to resistance against these insecticides.

In this study most of the studied mosquito populations were completely susceptible against temephos (Table 14). However, one population *i.e.*, NDP recorded incipient resistance against 0.02ppm (WHO dose) and moderate resistance against 0.0125ppm (NVBDCP dose). Significantly higher activities of both α-CCE and β-CCE compared to other tested population were noted in NDP^{ae} populations implying the role of CCEs based mechanisms behind resistance against temephos. The result of native PAGE also indicated similar involvement of CCEs in case of NDP^{ae} population. In this population, comparatively higher number of isozymes were noted *i.e.*, 5 in case of α-CCE and 3 in β-CCE. Moreover, the presence of intense bands in NDP^{ae} population indicates the elevated rate of temephos detoxification by over expression of CCE isozymes (Lima-catelani *et al.*, 2004). Similar involvement of CCEs isozymal variation conferring resistance against temephos in *Aedes aegypti* have been reported from India in Tamil Nadu (Muthusamy and Shivakumar, 2015) and beyond India (Bisset *et al.,* 2007; Grigoraki *et al.,* 2016). Mechanisms mediated by over-expression of CCEs activity have also been reported in *Aedes aegypti* populations resistant to temephos and other Organophosphate insecticide (Bellinato *et al.*, 2016). In an artificially selected population of *Aedes aegypti*, only after two generation of selection with temephos, CCE over-expression were noted governing the onset of resistance against it (Saavedra-Rodriguez *et al.,* 2014). Molecular investigation have identified the main CCE genes over-expressed in temephos resistant mosquito as *ccae3A* and *ccae6A* (Poupardin *et al.*, 2014; Grigoraki *et al.*, 2015).

From the result of the LC_{50} and LC_{99}, it was found that for one population that is JPG, the recommended dose of temephos was reported to be higher than the India Government recommended dose (NVBDCP, 2019a), thereby indicating the onset of undetected resistance which will be detected by the bioassays in near future.

Higher values of recommended dose (as calculated by 2 times of LC_{99} dose) serve as an indirect indicator of resistance development in mosquito population (Yadav *et al.,* 2015). For NDP^{ae} population the recommended dose was calculated to be higher than both the WHO and India Government doses. The extent of temephos resistance development could impart a significant negative effect on the dengue prevention strategies since this is the primary and preferable effort for dengue control (Bisset *et al.,* 2013). The rotation of larvicides with *Bacillus thuringiensis israleansis* or pyriproxyfen may help in minimizing the mosquito population as well as the resistance development as this have been approved for usage in drinking water (Bisset *et al.,* 2013).

The results of synergistic assays confirmed the role of CCEs based mechanisms behind the observed resistance against temephos in NDP populations as the exposure to TPP (CCEs inhibitor) was found to restore the susceptibility of the larvae to it (Table 16) whereas the role of CYP450 was ruled out as PBO could not increase the mortality percentages in the test mosquito populations. Different CCE genes have been pinpointed responsible for resistance against temephos (Poupardin *et al.*, 2014).

- *Malathion:*

Resistance against malathion have been associated with elevated activity levels of CCE enzymes of field population of *Aedes aegypti* (Hemingway and Karunaratne, 1998). Against malathion, only two populations, APD^{ae} and DAR^{ae} showed altered susceptibility. Same populations also reported significantly higher activities of α- and β-CCEs, indicating the role of CCEs behind the observed resistance. However, NDP^{ae} population was found to exhibit the highest activity of both the CCEs among the field population, yet there was complete susceptibility against malathion. This may be pertained to the fact that resistance against malathion are brought upon by some malathion specific esterases which is a separate group of esterase enzymes (Ziegler *et al.,* 1987; Hemingway and Karunaratne, 1998). Moreover, malathion is a OP insecticide with an open chain and CCEs mediated mechanisms governing resistance against such compound may involve a separate group of esterase with no relation to other organophosphates such as temephos (Rodriguez *et al.*, 2001; Gelasse *et al.*, 2017). Since in this study, activity level of CCEs as a whole was measured, it may be the scenario here that in NDP^{ae} the activities of other esterases are high but these of malathion specific ones are low thus resulting in no resistance against it (Table 17). Opposite occurs in case of APD^{ae} and DAR^{ae} population where specifically malathion specific CCEs may have elevated giving rise to moderate resistance and incipient resistance respectively. Similar instances of elevated levels of CCEs have been found to be linked to malathion resistance (Alvarez *et al.*, 2013; Francis *et al.*, 2017).

From the results of synergist assay, it was revealed that the use of TPP which inhibits CCEs could significantly restore the susceptibility partially in APD^{ae} population and to a lower extent in DAR^{ae} population. This confirms the involvement of CCEs linked pathways in providing resistance against malathion. However in no case was full susceptibility restoration recorded so mechanisms other than CCEs might also be associated with the observed resistance such as an insensitive AchE.

The results of native PAGE too gave a clearer insight into the observed resistance pattern against malathion. Single and darker bands with Rf values = 0.95-0.96 of α- and β- CCEs were observed in both APD^{ae} and DAR^{ae} with the band intensity more intense in the former (Figure 20-21). When equal amount of protein are loaded on to the gel, the intensity of bands in native PAGE gives an indication of the elevation/ overexpression of the particular isozyme (Lima-Catelani *et al.*, 2004). It may be stated that higher detoxification of malathion as a result of higher expression of α-ESTV and β- EST III may contribute to the levels of resistance occurring in APD^{ae} and DAR^{ae} populations.

The role of AchE cannot be ruled out behind the observed resistance against malathion in the field population of *Ae. aegypti* since the use of TPP could not increase the mortality to 100%. It seems that a combination of both elevated CCE levels and insensitive AchE might be governing the observed resistance pattern. However, the involvement of AchE insensitivity in malathion resistance is not common (Moyes *et al.*, 2017).

5.2.2.2 Mechanism of insecticide resistance against Organochlorine:

- *DDT:*

Resistance against DDT in mosquitoes can mainly be conferred by the elevated actions of Insecticide detoxifying enzymes (quantitatively or qualitatively), mainly GSTs (Hemingway *et al.,* 2004; Marcombe *et al.*, 2014), CYP450s (Ishak *et al.*, 2015) and CCEs (Ngoagauni *et al.,* 2016) or through the mutations in voltage gated sodium channel, *i.e.* knockdown resistance (Vontas *et al.,* 2012) or by a combination of both (Aponte *et al.*, 2013). From the results of enzyme activities it is seen that the population with higher resistance levels namely APD^{ae}, DAR^{ae} and NDP^{ae} possess significantly higher activities of both α and β-CCEs (Table 17). Similar association were also noted between the resistance levels and CYP450s monoxygenase level. Elevated activity levels of GSTs, *i.e.* the prime DDT detoxifying enzyme were recorded throughout different mosquito populations, however the elevation was statistically insignificant. The associations of elevated activities CCEs with DDT resistance have been recorded in a central Africa republican population of *Ae. aegypti* (Ngoagauni *et al.,* 2016). Some studies have also pinpointed on the partial role of CYP450 mediated pathways in DDT degradation (Ishak *et al.,* 2017; Prapanthadara *et al.,* 2002).

However the use of enzyme blockers throughout the mosquito populations presented a different scenario. Only in one population, PBO was found to restore the susceptibility against DDT, *i.e.* elevating the mortality percentage from 58.2 to 80%, thus implying that CYP450 might be involved partially behind the insecticide resistance against DDT in *Ae. aegypti* population (Table 16). Ability of CYP450S in restoring the susceptibilities in *Ae. aegypti* against DDT have also been recorded in Malaysia (Ishak *et al.*, 2015), in one population from Cameroon (Kamgang *et al.*, 2017), Thailand (Choovattanapakorn *et al.,* 2017).

Since, GSTs over-expression is regarded to be the prime DDT metabolising mechanism particularly, GSTe2 has been implicated in *Ae. aegypti* to confer DDT resistance (Lumjuan *et al.,* 2005). DDT hydrochlorinase activity has been implicated to be exhibited by GSTe2, GSTe5 and GSTe7 and the elevated level of epsilon class of GSTs have been shown to result in resistance against DDT (Lumjuan *et al.*, 2011; Marcombe *et al.,* 2009). But in this study, similar levels of GSTs activity was recorded throughout the mosquito population, so there may be the involvement of kdr mutations giving rise to DDT resistant populations.

In this study, the prevalence of two kdr mutations F1534C and V1016G was also tested, both of which though are mainly linked with synthetic pyrethroid resistance have also been shown to provide cross resistance against DDT (Davies *et al.,* 2007). The association between these mutations have been noted to be linked with DDT and synthetic pyrethroids resistance (Kushwah *et al.*, 2015). We have noted the presence of F1534c mutation in all the studied population (Table 18) which may be a factor behind the prevalence of DDT resistance throughout the studied *Ae. aegypti* population. The frequency of C allele was same throughout the test population, *i.e.* 50%, yet the variation between the DDT resistance levels may be because of the additive effect of another DDT resistance mechanism along with the kdr mutation (Aponte *et al.,* 2013). Out of the three populations recording the highest resistance against DDT, only one population NDP^{ae} was found to exhibit the mutated allele frequency of 70% along with presence of GG individuals, whereas the other two DAR^{ae} and APD^{ae} had the allele frequency of 33 and 25% respectively. So, no significant association could be made between the V1016G allele and DDT resistance which may be because V1016G is directly concerned with pyrethroid resistance (Du *et al.*, 2013). However, the presence of these kdr mutations might be the reason behind the elevated knockdown times recorded against DDT and a part of the observed resistance.

5.2.2.3 Mechanism of insecticide resistance against synthetic Pyrethroids:

- *Deltamethrin, Lambdacyhalothrin and Permethrin:*

Two mechanisms are generally linked to confer resistance against pyrethroid insecticides, *i.e.* increased activity of metabolic enzymes mainly CYP450s; partly CCEs and GSTs and the presence of kdr mutations contributing to target site insensitivity (Marcombe *et al.*, 2012; Vontas *et al.*, 2012). In this study we have found higher resistance against permethrin and lower levels for deltamethrin and lambdacyhalothrin. The CYP450s monoxygenases activity levels were higher than the SP^{ae} for most of the field population except COB^{ae} which was also recorded to possess 100% susceptibility to both the tested Type II pyrethroid. Two *Ae. aegypti* populations recording the highest resistance (though IR) against deltamethrin and lambdacyhalothrin were found to exhibit significantly higher activities of CYP450s than the SP^{ae}. Moreover, the same population were also found to possess elevated activity of CCEs too which leads to assume that both CYP450s and CCEs may be involved in degrading the pyrethroid molecules in the tested mosquitoes thus giving rise to resistance (Polson *et al.*, 2011).

The use of synergists revealed that in majority of the population PBO could restore the susceptibility towards deltamethrin and lambdacyhalothrin, thereby confirming the role of CYP450s associated pathways in conferring resistance observed in APD^{ae}, JPG^{ae} and NDP^{ae} population supplementing the results of enzyme activity levels. Moreover, the use of PBO was found to restore the mortality percentages to susceptible ranges in case of deltamethrin thereby confirming that detoxification by CYP450s enzymes alone conferred resistance against deltamethrin in these population. Whereas in case of lambdacyhalothrin, the use of PBO could only partially restore the susceptibility to an extent within incipient resistance range, thereby indicating that mechanisms other than CYP450s could be contributing towards the observed resistance. *Ae. aegypti* populations have been reported to possess resistance against synthetic pyrethroids mainly by the elevated activity of CYP9 family such as CYP9J26, CYP9J28 and CYP9M6 (Ishak *et al.,* 2017). The use of TPP, however was found to decrease the resistance levels (though slightly) in most of the tested population except JPG^{ae}, where a 2.2 % increase in mortality was noted against deltamethrin when used along with TPP. The reduced susceptibilities with the use of TPP may be explained by the fact that synergists have been found to sometimes suppress the insecticide entry into the mosquito body thereby increasing the resistance level (Kasai *et al.,* 2014). Many such populations of *Ae. aegypti* possessing CYP450s mediated resistance against deltamethrin and lamdacyhalothrin have been found throughout the world (Gonzalez *et al.,* 2011; Marcombe *et al.,* 2012).

Similarly involvement of metabolic enzymes behind permethrin resistance was also indicated by the results of enzyme bioassays. Involvement of CYP450s monoxygenases in permethrin resistance was noted in APD^{ae}, JPG^{ae}, DAR^{ae} and NDP^{ae} populations since they exhibited significantly higher activity of CYP450s. Whereas significantly elevated quantitative levels of α- and β- CCEs in NDP^{ae} and moderately in APD^{ae} were revealed implying the involvement of this family of insecticide detoxifying enzymes providing resistance against permethrin in these population. The detoxification of synthetic pyrethroids is generally linked with CYP450s family yet CCEs may play a minor role in providing a partial resistance against synthetic pyrethroids (Bisset *et al.,* 2013).

The results of synergist assays revealed that only one population, *i.e.* NDP^{ae} the susceptibility to permethrin increased partially with the use of PBO and TPP (8%

and 5% respectively), whereas in rest of the population negligible or negative differences were noted. It may however be stated that in NDP^{ae} population showing the highest resistance against permethrin among other tested population, CYP450s mediated reactions may provide a part of the observed resistance. Moreover a combination of both CYP450s and PBO may be involved and responsible for some of the observed resistance in NDP^{ae} population. But the prevalence of widespread resistance against DDT as well as permethrin amongst the tested mosquito populations implies the importance of underlying kdr mutation throughout the studied region.

The high frequency of F1534C allele in all the studied population brings into focus the role played by these enzymes behind the observed synthetic pyrethroid resistance. This mutation has been shown to be strongly correlated with pyrethroid resistance in *Ae. aegypti* (Kawada *et al.,* 2009; Chen *et al.,* 2019). Although this mutated allele was noted in all the population at same frequency yet widespread resistance was not noted against all the tested synthetic pyrethroids but only to permethrin. This may be pertained to the fact that F1534C mutation plays a key role in providing resistance against type I pyrethroids only and not type II pyrethroids (Li *et al.,* 2015). Similar scenario seems to be true in our study. This mutation has been shown to confer resistance against permethrin, NRD57 and biosemithrin whereas against cypermethrin and lambdacyhalothrin, this mutation has been revealed to reduce the resistance (Li *et al.,* 2015). So it may be stated that the observed permethrin resistance may be a result of the presence of F1534C kdr mutation. Whereas that of deltamethrin and lambdacyhalothrin are mainly because of elevated activity of insecticide detoxifying enzymes and other kdr mutation but not F1534C. Involvement of F1534C based resistance mechanisms against permethrin have been documented in Thailand (Yanola *et al.,* 2011). Kdr mutation, V1016G is generally revealed to confer resistance against many synthetic pyrethroids such as cypermethrin, lambdacyhalothrin, transfluthrin, d-allethrin, parallethrin *etc* (Li *et al.,*2015). Individuals with a V1016G mutation in their vgsc have been reported to have a lower susceptibility to permethrin than 1534C mutation (Maestre-Serrano *et al.,* 2019).This seems to holds true in some of the mosquito population in this study also. As the population with highest frequency of 1016G allele, *i.e.* NDP^{ae} (allele frequency= 70%) along with the presence of GG individuals was also found to record the highest resistance level (mortality 50%). The involvement of Kdr mutations behind IR is evident in COB^{ae} population with significantly lower enzyme activity levels but resistance against DDT and permethrin. Presence of V1016G providing resistance against permethrin have been reported in Indonesia (Brengues *et al.,* 2003) and Taiwan (Chung *et al.,* 2019).

There are also reports revealing the additive effect of two kdr mutations with V1016G increasing the resistance in individuals with F1534C (Plernsub *et al*., 2016). The same may be occurring in the tested population as both the mutated alleles have been found to be present in them. No individual with double homozygote mutation, *i.e.* GG and CC were found in this study which seems to be because of the fitness cost associated with such a haplotype as suggested in other such studies (Ishak *et al.*, 2015). This study goes well with reports suggesting the dominant role of metabolic detoxification in deltamethrin resistance and kdr mutations mainly F1534C in permethrin resistance (Ishak *et al.,* 2017). The variability in *Ae. aegypti* population against permethrin resistance may be imparted to the additive effect of both the mechanisms; insecticide detoxifying enzymes *i.e.* CYP450s and CCEs along with kdr mutation (Aponte *et al.*, 2013). Presence of both V1016G and F1534C in *Ae. aegypti* population with resistance against permethrin or DDT have been found in different regions of world (Harris *et al.,* 2010; Aponte *et al.,* 2013; Plernsub *et al.,* 2016).

5.2.2.4 Mechanism of insecticide resistance against Carbamate:

- *Propoxur:*

Generally detoxification of CBs have been studied to be conferred by the same mechanism as that of OP resistance (Karanaratne and Hemingway, 1998). However, involvement of CYP450 monooxygenases mediated pathway have also been inferred to provide partial resistance against CB (Ishak *et al.,* 2015). Here, severe resistance was found against propoxur in NDP^{ae} and DAR^{ae}, the same population with significantly higher CCEs activity. Elevated CCEs activity have been shown to provide resistance against Carbamates in *Ae. aegypti* (Seixas *et al.,* 2017). One population, JPG^{ae} was shown to possess moderate resistance with mortality 77.2%, with significantly higher activities of CCEs than SP^{ae} but lower than APD^{ae} which was found to possess IR against propoxur. Significantly higher activity of CYP450s monooxygenase seem to be also involved in providing resistance against propoxur in this population. Thus the combination of both CCEs and CYP450s might provide resistance against propoxur in the tested population of *Ae. aegypti.*

However, in most of the population except NDP^{ae}, the use of enzyme inhibition had negative impact on the mortality percentage. Synergistic assay confirm the partial role of CCEs in conferring resistance against propoxur. NDP^{ae} population has been reported to possess resistance against both temephos and propoxur, implying the possibility that detoxification via CCEs might provide cross resistance between temephos and propoxur in this population. Since in DAR^{ae} and APD^{ae} population partial resistance mechanism against malathion was recorded, it may be due to altered insensitive AchE, providing cross resistance between propoxur and malathion since it is the common target site for both the insecticide. Synergistic assay could not confirm the role of metabolic enzymes in JPG^{ae} population, it might be the case for JPG^{ae} that some different mechanism is operating in the population providing severe propoxur resistance which is yet to be explored.

The result of native PAGE also implies the involvement of CCEs isoforms behind propoxur resistance as maximum number of CCEs isoforms were observed in these populations. Isoforms other than α- Est-V and β- Est-III might be playing a key role in propoxur detoxification in NDP^{ae}. Since the involvement of CCEs in APD^{ae} and DAR^{ae} have been ruled out by synergist assay which exhibited high intense bands for the above mentioned two isoforms. However, the involvement of α- and β-CCEs isoforms in propoxur resistance is rare (Karunaratne and Hemingway,1998).

5.3. Insecticide susceptibility status and underlying mechanism in Aedes albopictus mosquitoes:

5.3.1 Insecticide susceptibility status of Aedes albopictus mosquitoes:

It was observed that throughout the study sites, there was an abundance of *Ae albopictus* (Table 11 and 13). This rural vector of dengue poses an immediate danger to region under study since majority of the studied district is composed of rural areas. Incidences of DENV and CHIKV infections are higher in areas where proper sanitation, waste disposal& drainage facilities are unavailable thereby increasing the disease statistics.

The demography of the region under study along with the common practices of livelihood, makes the assessment of insecticides susceptibility in this rural vector of Dengue and Chikungunya inevitable for effective dengue prevention. So, the susceptibility of eleven populations of *Ae. albopictus* were tested against one larvicide and six adulticides same as that of *Ae aegypti*.

5.3.1.1. *Insecticide susceptibility status against organophosphates:*

- *Temephos:*

Through the study of susceptibility against temephos, one of the field populations of *Ae. albopictus*, NGK^{al} was reported to be incipiently resistant against it through the WHO bioassay protocol using 0.02ppm of insecticide with the mortality 97%. Similar trend of incipient resistance were also noted for the NVBDCP dosage of temephos in two populations with the mortality percentage of 94 and 96 for SLG^{al} and NGK^{al} respectively. Rest of the nine populations were found to possess complete susceptibility against temephos with 100% mortality.

The LC_{50} values were lower for all the tested mosquitoes, however the LC_{99} values were calculated to be above WHO recommended dosage. The calculated RR_{99} value were low for majority of the populations however for two of the population the values were ≈2, the same population reporting incipient resistance (Table 21). The RR_{99} value gives an indication on the trend of insecticide resistance development that is not yet revealed by the bioassays. The population that recorded high RR_{99} values thus indicate that soon these may develop higher level of resistance against temephos, the prime larvicide. So, the authorities engaged in vector control should make prior strategy incorporating the knowledge of the resistance in progress against this larvicide in *Ae. albopictus*.

As compared to *Ae. aegypti*, very few reports exist on the insecticide susceptibility of *Ae. albopictus*. After the recent report proving the key role of this vector behind major Dengue and Chikungunya outbreaks, focus has been put on the control and thus insecticide resistance status of this species. Many Indian populations of *Aedes aegypti* have been reported to possess resistance against temephos from Andaman Island (Sivan *et al.,* 2015) with mortality percentages as low as 0% to 6%, Assam (RR_{99} values 2.5-5.4) (Yadav *et al.*, 2015) Whereas susceptible strain were reported from Karnataka (Shetty *et al.,* 2013), Kerala (Sharma *et al.,* 2004), Jharkhand (Singh *et al.,* 2011) and Assam (Dhiman *et al.,* 2014).

Similarly higher RR_{99} values have been reported in *Ae. albopictus* with values both greater and less than 2. Higher RR_{99} values *i.e.,* 2.5-5.4 were reported from Assam (Yadav *et al.*, 2015). The values of RR_{50}> 1 were noted in American strain of *Ae. albopictus* with the values ranging from 1.13-1.41 (Marcombe *et al.*, 2012).

Lower values of RR_{90} were 1.4-1.7 ppm, reported from military station of Assam (Dhiman *et al.,* 2014), Malaysia with RR_{95} values 1.15- 1.17 ppm (Mohiddin *et al.*, 2016) and 0.75-1.45 ppm (Chen *et al.*, 2013). From Orissa *Ae. albopictus* population with RR_{50} values as great as 15.3 ppm have also been reported (Rath *et al.,* 2018).

- *Malathion:*

All the tested mosquito populations resulted in susceptibility against malathion with the mortality rate ranging between 99-100%.Similar scenario was observed for *Ae. aegypti,* where three of the tested population were susceptible to malathion. This results go well with the other populations of *Ae. albopictus* tested against malathion throughout India.

In the neighbouring state of Assam also, field caught population of *Ae. albopictus* were found to be susceptible to 5% malathion (Yadav *et al.,* 2015; Dhiman *et al.,* 2016). Also from other parts of the country such as South Andaman district (Sivan *et al.,* 2015) and southern part of India (Sharma *et al.,* 2004), the wild population of *Ae. albopictus* were reported possess susceptibility to this adulticide. Many strains of this species have shown similar levels of susceptibility against malathion throughout the world (Duong *et al.*, 2016, Liu *et al.*, 2004; Srisawat *et al.*, 2011). The results suggest that malathion may be chosen during severe Dengue and Chikungunya outbreaks for *Ae. Albopictus* control in all the tested districts to minimize the transmission at the peak disease season.

5.3.1.2. Insecticide susceptibility status against Organochlorine

- *DDT:*

Amongst the adulticides tested, widespread resistance was noted against DDT (Table 22). Only one population was completely susceptible against DDT, *i.e.*, KHR^{al}, whereas six of the population possessed incipient resistance (mortality percentage 88.9-96.0) and three resistant to it. SLG^{al} with 40% mortality was severely resistant whereas other two JPG^{al} and NGK^{al} were moderately resistant to DDT with mortality percentage 75.4 and 63.1 respectively. Both JPG^{al} and NGK^{al} sites belong to same district and similar resistance pattern observed for both the sites imparts light on the similar mosquito control practice throughout the district. The high resistance observed in DAR seems to be a combined result of both the direct exposure to DDT as it was the chief control agent in the past and an indirect exposure through other vector control or pest control approaches of either agricultural or public health sector (Anon., 2002b). Altered susceptibilities against DDT in majority of the tested mosquitoes and also in *Aedes aegypti* confirm the level of DDT selection pressure prevailing in the studied region.

The complete susceptibility of KHR^{al} to DDT seems to be because of the absence of any government or non-government mosquito control organizations throughout the site and the conventional practices of the rural people for mosquito control rather than mass vector control using insecticides.

Most of the *Ae. albopictus* tested for DDT susceptibility have reported widespread resistance against it. In India, DDT resistance with mortality percentages as low as 35.76 has been reported from Jharkhand (Singh *et al.,* 2011), 78.09% in Andaman (Sivan *et al.*, 2015), 40.2% Northern Assam (Das and Dutta), 45.2% in Assam (Yadav *et al.,* 2015) Orissa (Rath *et al.*, 2018). Similarly, *Ae. albopictus* population from different countries have also exhibited resistance against this organochlorine such as in Pakistan (Arslan *et al.,* 2016), USA (Marcombe *et al.,* 2014), Central African Republic (Ngoagauni *et al.,* 2016), Malaysia (Ishak *et al.,* 2015), China (Li *et al.,* 2018)

The KDT_{50} and KDT_{95} values for DDT were found to be very high for most of the population (Figure 23). KDT_{50} values below 60 minutes was noted for a single population, KHR^{al} and for the rest of the field populations, range of KDT_{50} was 70.01- 230.21 mins. For KDT_{90} values, none of the population reported value less than 60 minutes. Even the most susceptible of all and the lab reared control reported a value of 42.64 and 169.13 mins respectively. Such a high value even for these two resistant population indicates the onset of inefficacy of DDT in the mosquito populations which as a result of the persisting condition and intense selection pressure may lean towards resistance in near future. Similar scenario of elevated KDT_{50} and

KDT_{95} values against 4% DDT have been reported throughout the world (Kamgang *et al.*, 2011; Marcombe *et al.*, 2014; Li *et al.*, 2018).

5.3.1.3 Insecticide susceptibility status against synthetic Pyrethroids:

- *Deltamethrin, Lambdacyhalothrin and Permethrin:*

Of the three synthetic pyrethroid insecticides, one showed variable pattern of resistance whereas the other two showed complete susceptibility in all the tested population. For permethrin, two resistant, four incipiently resistant and five susceptible population were recorded (Table 22). This variability in resistance pattern may be pertained to the variability in usage of personal mosquito protection tool, some of which contain formulations and cocktails of permethrin with other insecticide (Ponlawat *et al.*, 2005). Generally these tools are used inside human houses and in the vicinity of human dwellings but since *Ae. albopictus* is basically a forest dwelling mosquito, the exposure to such human tools might have occurred in only some of the population thereby causing variable pattern of susceptibility or resistance against permethrin throughout the eleven study sites ranging from susceptible to resistant.

Both permethrin resistant and susceptible population of *Ae. albopictus* have been reported throughout India. Many resistant or incipiently resistant field population of *Ae. albopictus* such as from Andaman with 73.3% mortality (Sivan *et al.*, 2013), Kerala and Delhi (Kushwah *et al.*, 2015) have been reported. Moreover permethrin resistance in *Ae. albopictus* is common throughout the world *i.e.* Srilanka (Karunaratne *et al.*, 2013; Pakistan (Mohsin *et al.*, 2016); China (Yiguan *et al.*, 2016), Malaysia (Ishak *et al.*, 2015; Rahim *et al.*, 2017).

Two of the tested population reported very high values of KDT_{50} and KDT_{90} which were also found to possess resistance against permethrin. However, some of the population that showed lower resistance levels reported comparatively higher values of KDT_{50} and KDT_{95}. Populations HAS^{al} and COB^{al} with mortality percentage 96.2 and 96.4 exhibited KDT_{50} value of >60 min *i.e.* 83.03 and 78.96 min respectively. Similarly NBU^{al} and COB^{al} with mortality percentage100 and 96.4 showed the KDT_{95} value to be 192.28 and 177.57 mins. Such high values of KDT_{50} and KDT_{95} associated with a low level of resistant or total susceptibility provides light on the inefficacy and delay in the manifestation of insecticide action and thus the scope of severe resistance development in the near future. Similar instance of high KDT_{95} values, *i.e.* 23-154 mins in Italian *Ae. albopictus* population (Pichler *et al.*, 2018) and higher values of KDT_{95} were also obtained in a Califiornian population of *Ae. albopictus* (Cornel *et al.,* 2016).

Complete susceptibility against deltamethrin and lambdacyhalothrin with mortality percentage 100 was recorded in the *Ae. albopictus* from northern districts of West Bengal. The populations were also found to possess KDT_{50} values below 60 min and KDT_{95} value around 60 mins. Similar susceptibility profile against deltamethrin has been noted in *Ae. albopictus* population from Assam (Dhiman *et al.,* 2014; Yadav *et al.,* 2015; Das and Dutta, 2014). Insecticides permethrin, deltamethrin and lambdacyhalothrin belong to the same insecticide group yet variability was noted in their susceptibility pattern. This may partly be explained by the fact that permethrin has been in use in mosquito control earlier than the other two which were brought into mosquito control programmes later.

As compared to *Ae. aegypti* resistance profile, against the synthetic pyrethroids, comparatively lower resistance was found in the sibling species *Ae.*

albopictus This may be because of the behaviour of these mosquitoes, *Ae. aegypti* being anthropophilic thus getting regular exposure to the personal protection measures against mosquito containing derivatives of synthetic pyrethroid while the zoophilic *Ae. albopictus* only gets occasional exposure to the pyrethroids targeted against mosquitoes. Populations of mosquito with higher resistance against permethrin but lower or none against other synthetic pyrethroids have also been reported in other studies (Sivan *et al.,* 2015; Ishak *et al.,* 2015; Arslan *et al.,* 2016). However, in other *Ae. albopictus* populations exhibiting resistance / incipient resistance against deltamethrin and/or lambdacyhalothrin have also been noted to occur (Kamgang *et al.*, 2011; Ishak *et al.*, 2015; Kushwah *et al.,* 2015; Ngoagauni *et al.*, 2016; Hasan *et al.*, 2016; Rahim *et al.*, 2017).

5.3.1.4 Insecticide susceptibility status against Carbamate:

- *Propoxur:*

Against the sole carbamate insecticide tested a pattern from susceptible to incipient resistant was noted. One severely resistant population, five incipient resistant and rest fully susceptible population was recorded. Such a pattern may be result of either direct exposure to propoxur through the household insect repellent tools containing this carbamate or through indirect exposure through agricultural sector. A severely propoxur resistant population, JPG^{al} with mortality 42.5% was reported for the first time from this study area. Reports on the prevalence of propoxur resistant *Ae. albopictus* population are few, some being noted in Central African republic, mortality 94% (Ngoagauni *et al*., 2016) and in China (Yiguan *et al.*, 2016; Li *et al.*, 2018). However, other studies conducted throughout India have reported, propoxur susceptible population of *Ae. albopictus* (Sharma *et al.*, 2014; Sivan *et al.*, 2015).

5.3.1.5 Overall view on resistance in Aedes albopictus:

Arranging the insecticides in the descending order of their toxicity in *Ae. albopictus* throughout the region will be in following order:

Deltamethrin and lambdacyhalothrin>malathion> temephos>propoxur>permethrin>DDT

Amongst the tested mosquito population, KHR^{al} was the only population that possessed susceptibility to all the tested insecticides followed by KMG^{al} (incipient resistance against DDT only) and then NBU^{al} (incipient resistance against DDT and propoxur). Rest of the population possessed variable pattern of resistance against different groups of insecticide.

5.3.2. Mechanisms of resistance:

5.3.2.1 Mechanism of insecticide resistance against Organophosphate:

- *Temephos:*

From the results of larval bioassay against temephos, most of the tested *Ae. albopictus* population was found to be resistant to it except NGK^{al} (which possessed incipient resistance against both WHO and NVBDCP doses) and SLG^{al} that possessed incipient resistance against NVBDCP dose. Similar results were also noted from the results of RR_{99} values, where these two population were found to possess significantly higher values ≈ 2 than other tested population. An RR_{95} value of 2 and above suggests the onset of resistance against insecticides in the mosquito population exhibiting it. Moreover a high variability was noted in the LC_{50} and LC_{99} values among different field populations of *Ae. albopictus*. This basically differs either due to the inherent variations occurring in geographically isolated populations or due to exposure to similar insecticide or allelochemicals (Wesson, 1990)

The site from where SLG^{al} population was collected is a site undergoing rapid urbanization and temephos is being used by different pest control agencies throughout the locality which may explain the altered susceptibility in this population (personal communication) whereas NGK^{al} site is located in a rural locality and no history of temephos usage yet the presence of incipient resistance against it seems to be the result of cross exposure to the other insecticides belonging to same group which is being used extensively in agricultural field such as tea, vegetable crops throughout the region (Gurusubramanian *et al.,* 2007). Similar instances of insecticide sprayed in agricultural sector *i.e.* cotton, vegetable have been found to contaminate mosquito breeding habitat (Kamgang *et al.*, 2011; Arslan *et al.*, 2016).

As already discussed, generally CCEs based mechanisms are involved in resistance against OPs in mosquitoes. Moreover, NGK^{al} and SLG^{al} population were found to exhibit significantly higher activity than SP for both α- and β-CCEs *i.e.* approximately 9.7, 5.1 and 2.3, 3.7 times respectively. The results of native PAGE also supplemented the above findings since more than one (two isozymes of α- CCEs in NGK^{al} and two isozymes of β-CCEs in SLG^{al}) and moderately intense bands of α- and β-CCEs were noted in these two populations implying that CCEs based mechanisms might be driving the detoxification of temephos in these populations (Lima-Catelani *et al.,* 2004). Significantly higher activities of CYP450s were also noted in ISL^{al} population indicating that a minor role might be played by this enzyme in conferring temephos resistance. CYP450s have been reported to provide resistance against temephos in *Aedes* mosquitoes (Grisales *et al.*, 2013).

However, the use of synergists provided a clearer insight into the scenario. Use of TPP along with temephos helped to restore susceptibility to temephos in NGK^{al} population at WHO dosage conferring the role of CCEs behind the observed resistance. Whereas in SLG^{al} population complete susceptibility could not be achieved with the use of TPP, though partial restoration in insecticide susceptibility in the mosquito population was noted. This brings into focus that apart from CCEs mediated mechanism, other detoxifying enzymes might also be involved in conferring a proportion of the observed resistance in SLG^{al} population. Detoxification mediated by GSTs may also provide resistance against temephos in SLG^{al} population as significantly higher activities of GSTs were noted in this population. Genes of GSTs mainly epsilon GSTs have been found to be over-expressed in temephos resistant colonies of *Aedes* mosquitoes (Saavedra-Rodriguez *et al.*, 2014). However, the role of insensitive AchE cannot be ruled out which also forms an important mechanism of resistance against temephos. Moreover tools addressing all these mosquitoes might help in the identification of exact mechanism of temephos resistance in SLG^{al}population. As in this study, in NGK^{al} population, similar involvement of CCEs behind temephos resistance in *Ae. albopictus* have been reported in (Ngoagauni *et al.*, 2016; Grigoraki *et al.*, 2016). Insensitive AchE providing such resistance has not yet been noted in *Ae. albopictus* mosquitoes throughout the world.

- *Malathion:*

As complete susceptibility was noted against malathion, it seems that malathion specific esterases which are generally noted behind resistance against malathion are very low or not present throughout the tested mosquito samples. Though malathion has been used for a large period of time in India for mosquito control yet 0% resistance against it seems to be because in India most of the mosquito control efforts target mosquito breeding habitats of other mosquito species such as drains but not that of *Ae. albopictus*. Moreover, since *Ae. albopictus* is a zoophilic mosquito and has recently as a result of deforestation migrated to human dwellings, the earlier interventions seem to have not provided malathion exposure to these mosquitoes. These mosquitoes also prefer to colonize breeding habitats outside human houses thereby again not getting exposure to household mosquito control tools in the past when it contained formulations of malathion (Anon., 2002b).

5.3.2.2 Mechanism of insecticide resistance against Organochlorine

- *DDT:*

Resistance against DDT is generally dependent on GSTs mediated mechanism and in few cases other detoxifying enzyme groups *i.e.* CCEs or CYP450s or kdr mutations providing cross resistance between DDT and synthetic pyrethroids (Vontas *et al.,* 2012). Throughout the mosquito population similar activities of GSTs were noted. However, the values were higher in case of SLG^{al} and NGK^{al} population, which were also found to possess severe resistance against DDT with mortality 40% and 63.1 % respectively. So, GSTs might be involved in these mosquito populations providing resistance against DDTs. Similar involvement of GSTs in DDT resistance have been reported in *Ae. albopictus* from India (Das and Dutta, 2014), U.S.A. (Marcombe *et al.*, 2014), China (Li *et al.*, 2018) *etc.*

These two populations were also recorded with higher activities of α- and β-CCEs which have also been implicated in conferring resistance against DDT in some mosquito colonies (Aponte *et al.,* 2013). Similarly, JPG^{al} and NGK^{al} were noted to express significantly higher activity of CYP450s which may also play a minor role behind DDT resistance (Ishak *et al.*, 2015).

The use of synergists also supplemented the above findings as use of CYP450s inhibitor was found to restore the susceptibilities against DDT moderately in SLG^{al}, JPG^{al}, NGK^{al}and to a lower extent in APD^{al}, HAS^{al}, KMG^{al}, ISL^{al} and COB^{al}; thereby affirming the role of CYP450S in a part of the observed resistance against DDT. Similarly use of CCEs inhibitor was found to moderately restore the susceptibility in SLG^{al} population and faintly in HAS^{al}, JPG^{al} and NGK^{al}. Thus similar partial involvement of CCEs can be confirmed in these mosquito population conferring DDT resistance. Or it may be stated that a combination of all the major insecticide detoxifying enzymes may be driving the detoxification of DDT in these *Ae. albopictus* population.

However, in no case complete susceptibility could be restored in any of the mosquito population, thereby putting into focus the role of target site insensitivity through kdr mutations in these mosquito population. In this study, presence of a major kdr mutation, *i.e.* F1534C was revealed in most of the mosquito population except SLG. From the results of kdr mutations genotyping it seems that in JPG^{al} and NGK^{al} population, these mutations might be playing a key role behind the altered susceptibilities of these population against DDT. So in JPG population, the combined predominant effect of GSTs, F1534C mutated allele along with minor role of CCE might be governing the observed resistance pattern. Similarly in ISL population, predominant role of all the detoxifying enzymes along with the kdr mutations might have resulted in severe resistance against DDT. Whereas in SLG population, only the role of CYP450s, CCEs and GSTs seem to be the driving factor of DDT resistance. Synergist assays with GST inhibitor and assessment of other kdr mutations would have helped in locating the exact mechanism of DDT resistance in SLG^{al} and other *Ae. albopictus* populations.

5.3.2.3 Mechanism of insecticide resistance against synthetic Pyrethroids:

- *Deltamethrin, Lambdacyhalothrin and Permethrin:*

Against permethrin also widespread resistance was observed with the most severe resistance observed in JPG^{al} and APD^{al}population. Both the population were recorded to express significantly higher activities of CYP450 monoxygenases than that of the SP^{al} and some other populations thereby indicating the involvement of this enzyme family behind the observed resistance against permethrin. In *Ae. albopictus* resistance to permethrin have been reported to be drawn through with elevated activities of CYP450s, particularly through the up-regulation of CYP6 and CYP9 subfamily (Ishak *et al.,* 2017). Similar detoxification mechanism may also be operating in these two field populations, however to confirm the involvement of specific subfamily, studies incorporating sophisticated molecular tools should be performed. CCEs may also detoxify synthetic pyrethroids in mosquitoes (Sahgal *et al.,* 1994; Aponte *et al.,* 2013; Chareonviriyaphap *et al.,* 2013). Similar associations have also been noted in JPG population where significantly higher activity of α-CCEs were noted and in APD population where significantly higher levels of β- CCEs activity were recorded implying the involvement CCEs based mechanisms behind the observed resistance.

The use of PBO before permethrin was found to increase the susceptibility percentages moderately in both APD^{al} and JPG^{al} population confirming the role of CYP450s in permethrin resistance. Lower levels of susceptibility restoration was also noted in NBU^{al}, HAS^{al}, NGK^{al}, NMZ^{al}, ISL^{al} and COB^{al} with complete susceptibilities in some of them, thus implying the similar inferences in this population behind the incipient resistance against permethrin. The partial association of CCEs was also confirmed in APD^{al}population through the result of synergistic assays. In NBU^{al}

population complete susceptibilities was restored with the use of both the enzyme inhibitors indicating the role of both the detoxifying enzymes in the resistance pattern.

The mutated allele 1534C have been linked with permethrin resistance in *Ae. aegypti*. Similar seems to hold true for *Ae. albopictus* too as evident in this study. Since 100% mortality against deltamethrin and lambdacyhalothrin amongst the tested mosquito population were recorded although the mutated allele was found to be present in most of the populations. Against permethrin, this allele seems to provide protection against it predominantly in JPG^{al} with C allele frequency of 54.1 APD^{al}, 41.6% and minorly in NGK^{al} and NMZ^{al} with very high allele frequencies.

Similar associations between pyrethroid resistance and F1534C mutation have been studied in *Ae. albopictus* (Li *et al.*, 2018; Auteri *et al.*, 2018). The low frequencies of these allele in HAS^{al}, NBU^{al}, ISL^{al} also correlate with the low level of resistance against permethrin. Moreover SLG^{al} population with complete susceptibility against all the tested synthetic pyrethroids insecticides, no mutant allele was recorded. Comparing the resistance against permethrin with DDT, the involvement of kdr mutation seem to govern DDT resistance in all the *Ae. albopictus* population except SLG^{al}. Interestingly, cross resistance between these two insecticides seem to be operating in JPG^{al} population with similar mortality % against both the insecticide.

5.3.2.4 Mechanism of insecticide resistance against Carbamate

- *Propoxur:*

Severe resistance in JPG^{al} population against carbamate insecticide in this study might be partially driven by the over-expression of CCEs as evident from the elevated levels of α- CCE activities. Similar involvement of CCEs also seem to hold true in NGK^{al} and APD^{al} population reporting significantly higher CCEs activity, both α- CCEs, β- CCEs and only β- CCEs respectively along with incipient resistance against propoxur. However role of CYP450s have also been noted to provide resistance against carbamates in larvae and adult of *Ae. albopictus* (Ishak *et al.,* 2015). Highest activities of CYP450s was recorded in the severely propoxur resistant mosquito population JPG indicating similar involvement of this detoxifying enzyme in detoxification of propoxur.

However, the results of synergist assay confirmed a partial role of CCEs behind propoxur resistance in JPG^{al}population restoring insecticide susceptibility to ≈8% only and even lower in case of SLG^{al}, NBU^{al}, NMZ^{al} and ISL^{al}. From the results of native page no clear association could be drawn between any CCE isoform and propoxur resistance. The incomplete restoration of susceptibility by the two enzyme blockers suggest the possible prevalence of insensitive AchE operating in the tested population behind propoxur resistance. However very few studies have reported the involvement of AchE alteration behind propoxur resistance (Ngoagauni *et al.,* 2016). Since few studies have been conducted to identify the specific and exact mechanism of resistance against propoxur in *Aedes* mosquitoes some different mechanisms other than that for other insecticides could also be involved. Studies concerned with mechanisms identification might help in filling the gaps for propoxur or other carbamate resistance in mosquitoes.