CHAPTER 6

<u>DISCUSSION</u>

<u>6. DISCUSSION</u>:

6.1. Survey of *Cx. quinquefasciatus*:

During the survey of *Cx. quinquefasciatus* larvae and pupae in northern region of West Bengal, this mosquito vector was mostly found occurring in high densities in cemented drains and muddy channels with high organic content. Throughout the study sites, *Cx. quinquefasciatus* was collected from polluted drains and was rarely found in clean water bodies. However, *Cx. quinquefasciatus* being an opportunistic breeder, may breed on any temporary or permanent water collection (Lopes *et al*., 2019). In the present study, BDN in Darjeeling district had the highest larval density among all other study sites followed by TFG in Coochbehar district (Table 9). Presence of such high larval densities may create a hurdle for vector management during disease outbreaks and also to check the biting nuisance of this mosquito vector.

Cx. quinquefasciatus are usually found in abundance in drains that are in close vicinity to human residential areas. Moreover, this mosquito vector is the most anthropophilic and abundantly found species of the genus *Culex* (Bhattacharya and Basu, 2016). As such, vector control strategy against *Cx. quinquefasciatus* should also focus on the elimination of probable breeding habitats along with improvement in community hygiene and waste water management (Lopes *et al*., 2019). Failure in the management of *Cx. quinquefasciatus* populations is a direct result of unplanned and rapid urbanization in different parts of West Bengal similar to other tropical and low-income countries of the world.

In the present study, *Cx. quinquefasciatus* was found in abundance after the rainy season *i.e*., from mid October to March in all sampling sites as compared to the monsoon period. Similar observance with high density of *Cx. quinquefasciatus* in the post-monsoon season as compared to the monsoon and pre-monsoon seasons was reported by Korgaonkar *et al*., 2012. Similar abundance of *Cx. quinquefasciatus* post monsoon is reported earlier from sub-Himalayan West Bengal (Rudra and Mukhopadhyay, 2010). Abundance of *Cx. quinquefasciatus* during the post-monsoon season is also thought to be one of the reasons behind higher resistance in this vector against synthetic pyrethroids like permethrin and deltamethrin as compared to other mosquito vectors (Uttah *et al*., 2013).

The mosquito vector *Cx. quinquefasciatus* is reported to exist with several other mosquito species such as *Cx. nigripalpus*, *Cx. australicus*, *Cx. pervigilans*, *Ae. polynesiensis*, *Ae. notoscriptus etc* in various regions (Bhattacharya and Basu, 2016). Association of *Ae. aegypti* and *Ae. albopictus* larvae in the domestic water containers and larvae of psychodid moth fly in sewages is also common (Hribar *et al*., 2004; Bhattacharya and Basu, 2016). However, in the present study, *Cx. quinquefasciatus* larvae were observed to co-exist with Anopheline mosquitoes, drain flies and chironomid larvae in few of the sampling sites (Table 9). Furthermore, the co-existence frequency of *Cx. quinquefasciatus* with drain flies was highest and that with Anopheline mosquitoes was least. Strong adaptation of *Cx. quinquefasciatus* to climate change and unplanned urbanization coupled with low sanitation may have led to co-existence of this vector with earlier unreported species in the larval habitat.

6.2. Insecticide resistance in *Cx. quinquefasciatus* populations from northern districts of West Bengal.

6.2.1. Resistance to Temephos – an organophosphate:

In the larval susceptibility assay, *Cx. quinquefasciatus* larvae of most of the studied populations showed susceptible status to temephos – an organophosphate in WHO recommended dose *i.e*., 0.02 ppm. SHM population in Darjeeling district was resistant to temephos as evident from only 80% mortality rate and TFG in Coochbehar district showed unconfirmed resistance with 92.31% mortality rate. However, when tested against NVBDCP recommended dose of temephos *i.e*., 0.0125 ppm, only 9 populations of *Cx. quinquefasciatus* was fully susceptible to temephos with 100% mortality. Three populations *i.e*., TFG, SHM and SLG showed resistance to NVBDCP recommended dose of temephos while other 4 populations COB, DPG, DLK and ISL were on the intermediate range of resistance with the possibility of either moving towards resistance or susceptible status. On the basis of RR (resistance ratio) calculated as RR_{50} in the study, only 3 populations *i.e*., APD in Alipurduar district, FLB and JPT in Jalpaiguri district were completely susceptible to temephos while COB in Coochbehar district, CPR and DLK in Uttar Dinajpur showed mild resistance to temephos (RR_{50}=2). Severe resistance to temephos was observed in SHM as evident from highest RR_{50} value of 11 among all of the studied sites followed by ISL (RR_{50}=7) and SAM in Malda district (RR_{50}=6). As such, the studied populations showed a higher resistance ratio against temephos.

The present findings of resistance development in *Cx. quinquefasciatus* in the northern districts of West Bengal suggest the ineffectiveness of this organophosphate in controlling the lymphatic filariasis vector in the near future. Ineffectiveness of temephos is alarming because of the fact that two of the 6 districts under study are endemic to lymphatic filariasis and disease control mainly relies on vector control approaches. Similar resistance development against temephos in the dengue vector *Aedes aegypti* from the northern districts of West Bengal is also reported (Bharati and Saha, 2018), posing a serious threat in terms of mosquito-borne diseases in West Bengal.

This observed resistance to temephos in many studied populations of *Cx. quinquefasciatus* from northern districts of West Bengal, India is an important issue and needs immediate attention as temephos is the most commonly used chemical larvicide in India owing to its cost effectiveness and easy availability in the market place (Grisales *et al*., 2013). Temephos or O,O,O'O'- tetramethyl O,O'- thiodi- p-phenylenebis (phosphorothiate) is also used to control mosquito larvae in different corners of the world because it is efficient in controlling mosquito larvae, is easily available and budget friendly (Grisales *et al*., 2013).

In India, temephos is usually applied to check the outbreak of dengue and malaria. However, the application of temephos in drains and water containers of the housing complexes, adjoining residential areas and commercial areas as a larvicide for dengue and malaria mosquito vectors also indirectly affects the lymphatic filariasis vector - *Cx. quinquefasciatus*. This is because drains are the most preferred habitat of *Culex* mosquitoes while dengue vector – *Aedes sp.* and malarial vector – *Anopheles sp.*

are found in other habitats like tree holes, tyres, plastic containers, shallow streams, channels along agricultural lands *etc*.

The non-targeted exposure of *Cx. quinquefasciatus* to temephos might be considered as the main reason behind the observed resistance in *Cx. quinquefasciatus* in the current study. The six districts incorporated in the present study from West Bengal, India is endemic to both dengue and malaria and as such, the region experiences heavy and regular spray of temephos for controlling the mosquito larvae and the spread of associated mosquito-borne diseases. Moderate to high levels of temephos resistance in the studied *Cx. quinquefasciatus* populations may therefore, be related to continuous application of temephos by the Government and other vector control agencies to keep in check the growth and proliferation of dengue and malaria.

Similar resistance to temephos in larval population of *Cx. quinquefasciatus* were reported from different parts of India. In Uttar Pradesh, *Cx. quinquefasciatus* larvae were reported to be resistant to temephos with a low mortality rate of 30% (Kumar *et al*., 2011). Likewise, with mortality rate ranging from 2.8% to 71%, temephos resistant populations of *Cx. quinquefasciatus* were reported from Delhi and from Haryana with 81.5% mortality (Thomas *et al*., 2013). However, in Andhra Pradesh, temephos susceptible population of *Cx. quinquefasciatus* was reported with 100% mortality when the mosquito population was exposed to 0.125 ppm temephos concentration (Mukhopadhyay *et al*., 2006). Apart from India, temephos resistance in *Cx. quinquefasciatus* is reported from Malaysia where the mosquito larval mortality was calculated to be 26.03% (Nazni *et al*., 2005). Severe resistance to temephos was recorded in larvae of *Cx. quinquefasciatus* in Japan with zero mortality (Kasai *et al*.,

2007) and resistance to temephos was also reported from Southern Louisiana with mortality percentage ranging from 24 – 77 (Delisi *et al*., 2017).

The LC_{50} values in the studied populations ranged from 0.001 – 0.011 ppm (Table 10). Similar LC_{50} values of *Cx. quinquefasciatus* larvae are reported from Rajasthan with 0.0056 ppm (Bansal and Singh, 2007), 0.0008 – 0.0083 ppm in Bengaluru (Shetty *et al*., 2013), 0.0162 ppm, 0.0086 ppm, 0.0048 ppm and 0.0136 ppm from four Indian cities *viz*., Jodhpur, Bikaner, Jamnagar and Bathinda respectively (Suman *et al*., 2010). *Cx. quinquefasciatus* larvae in Brazil were reported with LC_{50} value ranging from 0.002 – 0.036 ppm when tested with temephos (Amorim *et al*., 2013) while a comparatively lower LC_{50} value ranging from 0.0011 – 0.0068 ppm was reported by Skovmand and Sango (2018) from Burkino Faso. Similar low LC_{50} value of 0.0031 – 0.0044 ppm was recorded in *Cx. quinquefasciatus* from La Reunion (Tantely *et al*., 2010).

The LC_{99} values for temephos in larval populations of *Cx. quinquefasciatus* from 6 different districts of West Bengal was observed to be ≈34.5 times higher than the susceptible laboratory reared population (Table 10). A hundred fold higher LC_{99} value in *Cx. quinquefasciatus* larvae was reported from Japan as compared to the susceptible population resulting in severe resistance to temephos (Kasai *et al*., 2007). Likewise, there was similarity in the RR_{50} value obtained in the current study with RR_{50} values of *Cx. quinquefasciatus* populations from few other Indian cities *viz*., Bengaluru (RR_{50}=1-10.37), Jodhpur (RR_{50}=10.8), Bikaner (RR_{50}=5.73), Jamnagar (RR_{50}=3.2), Bathinda (RR_{50}=9.06) (Suman *et al*., 2010; Shetty *et al*., 2013). Resistance ratio (RR) is widely applied as an indirect method of resistance analysis in mosquito populations with RR>2 considered to be resistant for that particular insecticide.

The World Health Organization recommends temephos for control of other insect vectors like flea, midge and blackfly apart from mosquito vectors – *Aedes sp*., *Anopheles sp*. and *Culex sp.* (WHO, 2009b). This organophosphate insecticide is also applied in the agricultural sector to control thrips and cutworms (WHO, 2009b). As such, the wide application of temephos has resulted in the development of resistance in the target organisms over time (Shetty *et al*., 2013; Soltani *et al*., 2015). Resistance development to temephos in mosquito vectors and other insect vectors of public health is of prime concern as it may act as a hurdle in the efficient implementation of vector control strategies.

Prevalence of temephos resistance in *Cx. quinuefasciatus* in the current study along with reports on temephos resistance in dengue vector *Ae. aegypti* from the same region (Bharati and Saha, 2018) suggests the use of other insecticides that will be more effective against the mosquito larvae in the northern region of West Bengal, India.

6.2.2. Resistance to malathion:

The susceptibility assay of *Cx. quinquefasciatus* against another organophosphate – malathion showed that adult mosquitoes of all of the studied populations were resistant to malathion. Out of the 16 study sites, moderate resistance to malathion was observed in 3 populations *i.e*., SHM with 70.58% mortality, JPT with 75% mortality and DLK with 76.47 mortality percent having comparatively higher mortality rate than the other studied populations. However, rest of the 13 populations showed severe resistance to malathion with 100% survival rate in TFG, HCP and SAM. This severe resistance to malathion observed from different district of West Bengal as evident from very low mortality percentage may be because of malathion exposure in mosquitoes that accumulates in drains after being washed off from nearby agricultural fields. Moreover, development of insecticide resistance in mosquito vectors is reported to occur due to cross exposure of insecticides in the mosquito habitats from the agricultural run-offs (Philbert *et al*., 2014).

The NVBDCP, Government of India recommends the use of malathion in indoor residual spray (IRS) and this insecticide is also used in the agricultural sector to control insect pests of economic importance (NVBDCP, 2020). Out of the 6 districts of West Bengal under study, application of malathion against mosquito vectors is practiced only in Alipurduar district (personal communication). This application history may be the reason behind resistance development to malathion in *Cx. quinquefasciatus* from Alipurduar. However, in the other 5 districts, use of malathion to control mosquito vectors was not known. Therefore, malathion resistance in these districts may be linked to application of this insecticide in the agricultural sector. Four districts in the current study *i.e*., Alipurduar, Darjeeling, Jalpaiguri and Uttar Dinajpur depend largely on tea plantation for their economy (Saha and Mukhopadhyay, 2013). Moreover, Jalpaiguri district and Uttar Dinajpur also partially depends on jute cultivation and pineapple cultivation respectively (Das *et al*., 2011; Das *et al*., 2016). Coochbehar district mainly depends on the cultivation of paddy, tobacco and jute while Malda district relies on prime orchard crops like mango, banana and litchi for their economy (Das *et al*., 2013). As such, the use of malathion in huge amount for controlling insect pest in tea plantation and other agricultural practices (Saha and Mukhopadhyay, 2013) may be the reason of severe resistance development in *Cx. quinquefasciatus* from these districts. Moreover, excessive malathion spread in the tea gardens and other agricultural practices seeps into nearby drains and contaminates the habitat of *Cx. quinquefasciatus* and other drain dwelling insect species.

Low mortality rate against malathion observed in the present study correlates with similar report on resistance to malathion in *Cx. quinquefasciatus* from other regions of the country. *Cx. quinquefasciatus* adults from Andhra Pradesh were reported to be resistant to malathion with zero mortality (Mukhopadhyay *et al*., 2006) similar to the finding of this study. With a mortality percent of 9.75, 33.3 and 27, malathion resistance in adult *Cx. quinquefasciatus* populations were reported from Chhattisgarh, Gujarat (Raghavendra *et al.*, 2011) and Uttar Pradesh (Kumar *et al*., 2011) respectively. Malathion resistance in the lymphatic filariasis vector - *Cx. quinquefasciatus* is reported from neighbouring countries Sri Lanka with 22% mortality rate (Karunaratne and Hemingway, 2001) and Pakistan with only 30% mortality in the adult population (Tahir *et al*., 2013). Similar resistance to malathion in adult *Cx. quinquefasciatus* is reported from Malaysia with 100% survival rate (Nazni *et al*., 2005) and United States with 0-53% mortality (Richards *et al*., 2017). However, malathion susceptible population of *Cx. quinquefasciatus* with 100% mortality when exposed to malathion was also reported in Thailand (Sathantriphop *et al*., 2006) and Zambia (Norris and Norris, 2011).

Apart from the adults of *Cx. quinquefasciatus* resistance to malathion has also been observed in the larval population in Andhra Pradesh with 14% larval mortality (Mukhopadhyay *et al.*, 2006) and also in Rajasthan (Bansal and Singh, 2007).

Similar malathion resistance in *Cx. quinquefasciatus* larvae are reported from Malaysia with high LC_{50} value of 109.62 - 140.31 ppm (Nazni *et al.*, 2005; Low *et al.*, 2013), La Reunion with LC_{50} value of 0.0012 - 0.0028 ppm (Tantely *et al.*, 2010) and with 0.043 ppm LC_{50} in Bukina Faso (Skovmand and Sanago, 2018).

This observed resistance to malathion in the lymphatic filariasis vector - *Cx. quinquefasciatus* from all 6 districts under study implies the phenomenon of resistance development due to cross exposure of insecticides applied in the agricultural sector (Philbert *et al*., 2014). The ineffectiveness of this insecticide to control *Cx. quinquefasciatus* in the studied region is evident from the current resistance status of the mosquito vector. Similar resistance to malathion in dengue vector – *Ae. aegypti* was reported from Alipurduar and Darjeeling district (Bharati and Saha, 2018). Malathion is toxic not only to the mosquito vectors and agricultural pests but also to other non-target organisms and humans (Kesavachandran *et al*., 2009). Therefore, the use of this insecticide has been banned in few parts of the world (Kesavachandran *et al*., 2009).

6.2.3. Resistance to propoxur:

Propoxur – a carbamate insecticide is recommended for mosquito vector control in many countries of the world because of its rapid killing effect and lower toxicity on non-target organisms (Food and Agricultural Organization, 2002; WHO, 2016). Propoxur and bendiocarb are two prime carbamate insecticides to be used in vector control along with synthetic pyrethroids in indoor residual spray and outdoor spray activities like fogging (WHO, 2016b). However in India, only bendiocarb is recommended for mosquito control while propoxur is used in controlling other household pests like cockroaches, termites and bedbugs but not against mosquito vector (WHO, 2016b; NVBDCP, 2020).

In the present study, field collected population of *Cx. quinquefasciatus* were tested against 0.1% propoxur in order to assess the probability of future application of this insecticide to control the lymphatic filariasis vector in West Bengal. Though, the World Health Organization Pesticide Evaluation Scheme (WHOPES) recommends an exposure period of 2 hours to propoxur for *Cx. quinquefasciatus*, 1 hour exposure time was followed for all six insecticides in order to maintain the homogenity of the experiment. Insecticide susceptibility assay of adult *Cx. quinquefasciatus* from 6 districts of West Bengal against propoxur showed severe resistance to this carbamate insecticide with mortality percentage ranging from 0 – 62.5 (Table 12). SAM showed the highest resistance level among all of the studied population with zero mortality.

This result of resistance development against propoxur in *Cx. quinquefasciatus* complies with similar report of resistance development from other countries of the world. In Thailand, susceptibility test of adult *Cx. quinquefasciatus* against 0.1% propoxur resulted in resistance status with 77 – 93.3% mortality (Sathantriphop *et al.*, 2006), however, propoxur susceptible populations of *Cx. quinquefasciatus* were also reported (Thanispong *et al.*, 2008). With 3.24% – 68.89% mortality, adult *Cx. quinquefasciatus* population showed resistance to propoxur in Malaysia (Chen *et al*., 2013; Low *et al*., 2013b).

In India, there is no report on resistance to propoxur in *Cx. quinquefasciatus* adults. However, susceptibility assay against propoxur in the larval population was studied and reported from Mysore with 0.00013 ppm LC_{50} (Kumar *et al*., 2011), LC_{50} value ranging from 0.0001 – 0.1166 ppm from Bengaluru (Shetty *et al*., 2013)

Carbamate resistance in adult *Cx. quinquefasciatus* is reported in India against bendiocarb from Chhattisgarh with a low mortality rate of 5.7% thereby, indicating severe resistance (Raghavendra *et al.*, 2011). Similar resistance to bendiocarb was also reported from Gujarat where the adult *Cx. quinquefasciatus* showed 16.2% mortality when exposed to bendiocarb (Raghavendra *et al.*, 2011). Compared to other three classes of insecticide recommended for mosquito control, reports on carbamate resistance though few, are severe when present with less mortality percentage. Comparatively fewer reports on the susceptibility status of *Cx. quinquefasciatus* against propoxur suggest the less exploitation of this insecticide in vector control.

Severe resistance to propoxur in this study from all of the sites suggest cross resistance in *Cx. quinquefasciatus* to bendiocarb as there is no history of propoxur application in India against the mosquito vectors (NVBDCP, 2020). Second reason of resistance development might be the use of propoxur containing repellent sprays and chalks to keep in check cockroaches, termites and bedbugs in the household. *Cx. quinquefasciatus* being anthropophilic and indoor resting mosquitoes are at a higher risk of exposure to propoxur-containing insect repellents and as such develop secondary resistance to propoxur. These insect repellent chalks and sprays contain propoxur in a higher dose formulated for larger insects like termites and cockroaches. Therefore, the untargeted exposure of *Cx. quinquefasciatus* to such high dose of propoxur may be the reason behind severe resistance to propoxur observed in the present study with low mortality percent.

Low mortality rate in adult *Cx. quinquefasciatus* ranging from 0 – 18.18% in two lymphatic filariasis endemic districts *i.e.*, Coochbehar and Malda in the study is alarming with a negative probability of propoxur application for controlling mosquito vector in the near future. Ineffectiveness of carbamate insecticides against *Cx. quinquefasciatus* narrows the application of only three insecticide class for lymphatic filariasis control in the studied region.

6.2.4. Resistance to DDT:

The onset of DDT resistance was first reported in dengue vector – *Ae. aegypti* in the early 1950s (Liu, 2015) and thereafter, several reports on DDT resistance in mosquito vectors were recorded from different parts of the world. In India, first report of DDT resistance in *Cx. quinquefasciatus* dates back to the year 1952 from a small village in Delhi (Gopalakrishnan and Veer, 2018). With almost 70 years of resistance history to DDT in the lymphatic filariasis vector - *Cx. quinquefasciatus*, this insecticide is still being used for vector control in the country. Application of DDT in the agricultural sector is banned while this insecticide along with another organochlorine insecticide – dieldrin is recommended for mosquito control in India (NVBDCP, 2020). Worldwide use of DDT as an effective insecticide in both public health and agricultural sector since the 20^{th} century resulted in intense selection pressure on mosquito vectors resulting in resistance development across various regions of the world (Vontas *et al*., 2012).

Severe resistance to DDT was observed in the present study in adult *Cx. quinquefasciatus* populations from six districts of northern West Bengal. HCP and MLT populations in Malda district showed severe resistance to DDT with zero mortality (Table 12) (Figure 18). This low mortality rate may indicate the implication of DDT- based mosquito control strategies in the past years as these districts apart from being endemic to lymphatic filariasis are also endemic to dengue, malaria and Japanese Encephalitis (NVBDCP, 2020).

Similar resistance to DDT in adult *Cx. quinquefasciatus* populations are reported from the neighbouring state Assam with 11.9 – 50% mortality rate (Sarkar *et al*., 2009) and from Andhra Pradesh with zero mortality rate (Mukhopadhyay *et al*., 2006). In Uttar Pradesh, adult *Cx. quinquefasciatus* was observed to be resistant to DDT with 28.33% mortality (Kumar *et al*., 2011) and similar resistance status was reported by Raghavendra *et al*., (2011) from Chhattisgarh and Gujarat with zero mortality and 3.3% mortality in adult *Cx. quinquefasciatus* populations. Similar resistance was also reported from Maharashtra with a mortality percent of 12.3 when exposed to DDT (Karlekar *et al*., 2013).

With mortality rate of 1%, severe resistance to DDT was reported from Sri Lanka (Wondgi *et al*., 2008) and similar resistance was reported from Thailand, Benin, Tanzania and Ghana (Somboon *et al.*, 2003; Corbel *et al.*, 2007; Jones *et al.*, 2012; Kudom *et al.*, 2015; Yadouletan *et al.*, 2015). Susceptibility to DDT with 100% mortality was reported in *Cx. quinquefasciatus* population from Malaysia in the year 2005 (Nazni *et al*., 2005). However the *Cx. quinquefasciatus* adults were found to be resistant to DDT with only 33.33% mortality (Chen *et al*., 2013). This unfortunate change from susceptible to resistance in less than 10 years indicates the rapid onset of insecticide resistance in the mosquito vectors. In the current study mortality percentage of laboratory reared susceptible population (SP) was 98.92 and not 100% as the mortality of SP against other insecticides used. This finding highlights upon the heavy intensity of DDT resistance in *Cx. quinquefasciatus* from the study region.

KDT_{50} and KDT_{99} value of *Cx. quinquefasciatus* against DDT in the study was found to be many times higher than that of SP. Highest KDT_{50} value of 378.90 minutes was recorded in COB population in Coochbehar district among all of the studied populations with a mortality of only 4% thereby showing severe resistance to DDT (Table 13). Similarly, TFG in Coochbehar district and SHM in Darjeeling district showed highest KDT_{99} value of 2764.10 minutes. Higher KDT_{50} and KDT_{99} values indicate longer time taken by the insecticide to knockdown the mosquito vector and therefore inefficiency of the insecticide in controlling the vector population.

6.2.5. Resistance to Synthetic Pyrethroids:

In the present study, field collected populations of *Cx quinquefasciatus* were tested against 3 synthetic pyrethroid insecticides. One of the insecticide *i.e*., permethrin belongs to type I pyrethroid group and the other two insecticides – deltamethrin and lambdacyhalothrin are Type II pyrethriods. These 3 synthetic pyrethroids used in the study are recommended for mosquito control (NVBDCP, 2020) and also the most common pyrethroids used in both public health and agricultural practices. The mosquito populations exhibited similar resistance to all the 3 synthetic pyrethroids used in the study (Table 11). This finding highlights the use of these insecticides in the studied region for mosquito vector control.

Susceptibility assay of adult *Cx. quinquefasciatus* against Type I pyrethroid permethrin showed that all of the studied populations were resistant to permethrin except JPT with 95.65% mortality which was in the intermediate resistance or unconfirmed resistance state (Table 11). Apart from JPT in Jalpaiguri district, CPR and SLG populations showed moderate resistance to permethrin with a mortality rate of 80.55% and 73.91% respectively (Figure 17). Severe resistance to permethrin with 7.69% mortality was observed in SHM population in Darjeeling district. Distribution of permethrin and deltamethrin-impregnated mosquito nets by Government health workers including the Aganwadi workers in various districts of West Bengal might be linked to the observed permethrin resistance in this study. The distribution of synthetic pyrethroids-impregnated bed nets as a part of vector control strategy in India (NVBDCP, 2021c) in a continuous manner as such, may result in development of resistance in indoor mosquito vectors. The observed permethrin resistance in field collected *Cx. quinquefasciatus* populations may have a negative impact on vector control strategies, rendering the application of permethrin in the present dose ineffective against mosquito vectors in West Bengal. Permethrin resistance from this studied region in dengue vector – *Ae. aegypti* has also been reported earlier (Bharati and Saha, 2018). Moreover, the ineffectiveness of permethrin in checking the mosquito populations may have a serious consequence during disease outbreaks in West Bengal.

Incipient or intermediate resistance to permethrin in *Cx. quinquefasciatus* with 95.83% mortality is reported from Uttar Pradesh (Kumar *et al*., 2011). Resistance to permethrin in *Cx. quinquefasciatus* is spread worldwide with several reports from different parts of the world. However, in Thailand, *Cx. quinquefasciatus* was found to be susceptible to permethrin and also the presence of incipient resistance in the same population (Somboon *et al*., 2003). Likewise, incipient resistance against permethrin in *Cx. quinquefasciatus* was reported from Zambia (Norris *et al*., 2011) while the mosquito population was moderately resistant to permethrin in Malaysia with mortality percentage lower than 79.82 (Nazni *et al*., 2005; Chen *et al*., 2013). With mortality percent ranging from 35 – 93, *Cx. quinquefasciatus* populations in Benin showed both resistance and incipient resistance to a synthetic pyrethroid – permethrin (Corbel *et al*., 2007). Reports on severe resistance to permethrin in *Cx. quinquefasciatus* are also common with 1% mortality in Sri Lanka (Wondji *et al*., 2008), 14% mortality in Zanzibar (Jones *et al*., 2012), mortality percentage ranging from 4-24 in Benin (Yadouletan *et al*., 2015), 11.4% in Thailand (Yanola *et al*., 2015) and in Cameroon with 18% mortality (Nchoutponen *et al*., 2019). Few populations of *Cx. quinquefasciatus* exhibited all three status of resistance, susceptible and incipient resistance when tested against permethrin in Ghana, Malaysia and Thailand (Kudom *et al*., 2013; Low *et al*., 2013b, Boonyuan *et al*., 2016).

The KDT_{50} and KDT_{99} values of studied *Cx. quinquefasciatus* populations were lower than that of DDT (Table 13). BDN in Darjeeling district showed highest KDT_{50} value of 294.81 minutes. However none of the studied populations exhibited KDT_{50} values lower than 60 minutes in the present study. On the contrary, a low KDT_{50} value of 22.37 minutes was reported in *Cx. quinquefasciatus* from Selangor, Malaysia (Chen *et al*., 2013). This indicates a degradation of rapid knockdown effect of permethrin in the mosquito vectors. Highest KDT_{99} value of 1457.91 minutes was observed in TFG in Coochbehar district. This long time exposure required for knockdown of mosquito vectors in TFG is alarming as Coochbehar district is endemic to lymphatic filariasis and prolonged use of permethrin treated bed nets might be the cause of this very high KDT_{99} value in the study site. Permethrin resistance is not confined to *Cx. quinquefasciatus* but present in *Aedes sp.* (Ishak *et al*., 2015; Ishak *et al*., 2017), *Anopheles sp.* (Mittal *et al*., 2004) and other insect vectors as well (Hemingway *et al*., 2002).

Resistance to two Type II pyrethroids *i.e*., deltamethrin and lambdacyhalothrin is evident from low mortality rates in field collected populations of *Cx. quinquefasciatus* in the study (Table 11). Three of the studied populations *i.e*., BDN, JPT and CPR showed moderate resistance to deltamethrin with mortality rate of 82.86%, 83.33% and 76.92% respectively. Rest of the 13 study sites showed severe resistance to deltamethrin with mortality percentage as low as 7.69 in MEK of Coochbehar district. This observed resistance to deltamethrin in the present study may also be linked to the prolonged use of permethrin and deltamethrin-impregnated mosquito nets in northern districts of West Bengal.

Resistance to deltamethrin in *Cx. quinquefasciatus* is spread across the country with reports on resistance from Chhattisgarh and Gujarat with 44.9% and 75% mortality rate (Raghavendra *et al*., 2011). Similar resistance to deltamethrin in *Cx. quinquefasciatus* was also reported from Maharashtra showing 72.73% mortality (Karlekar *et al*., 2013). However, in the neighbouring state, Assam *Cx. quinquefasciatus* population was reported to have either incipient resistance or complete susceptibility to deltamethrin with 96.2 - 100% mortality (Sarkar *et al*., 2009). Deltamethrin resistance in India is reported not only in the adults of *Cx. quinquefasciatus* but also in their larval population with 0.0008 ppm LC_{50} value in Mysore (Fakoorziba *et al*., 2008) and Bengaluru with 0.0016 - 0.0065 ppm LC_{50} (Shetty *et al*., 2013). Resistance to deltamethrin was reported from Uttar Pradesh with 96.66% mortality (Kumar *et al*., 2011).

Deltamethrin resistance in *Cx. quinquefasciatus* is spread on the global scale and various reports from Zanzibar with only 19.4% mortality in *Cx. quinquefasciatus* when exposed to deltamethrin (Jones *et al*., 2012), Ghana with 23 - 58% mortality (Kudom *et al*., 2015), Benin with 24 - 48% mortality (Yadouletan *et al*., 2015) and Cameroon with 20% mortality (Nchoutponen *et al*., 2019) have come to the limelight. However, there are also reports on incipient resistance to deltamethrin from Zambia with mortality percentage of 97 - 98 (Norris *et al*., 2011), Thailand (Yanola *et al*., 2015) and United States (Richards *et al*., 2017).

In the present study, MLT and SAM population in Malda district and COB and MEK populations in Coochbehar district showed severe resistance to lambdacyhalothrin with mortality percentage below 12 for all 4 study sites (Table 11). This finding indicates serious problems related to mosquito vector control in these two districts using synthetic pyrethroids. Coochbehar and Malda are the only two districts to be endemic to lymphatic filariasis in the northern region of West Bengal, India (NVBDCP, 2020). As such, severe resistance to lambdacyhalothrin may act as a hurdle in combating the disease through vector control plans in these two lymphatic filariasis endemic districts.

Compared to all three synthetic pyrethroids used in this study, lowest mortality rate was observed against lambdacyhalothrin with maximum mortality percentage of 77.27 in JPT (Table 11). Moreover, least mortality percentage of 4 was also observed against lambdacyhalothrin among the three synthetic pyrethroids in MLT population from Malda district. On contrary to this finding, susceptible population of *Cx. quinquefasciatus* to lambdacyhalothrin was reported from Indian state of Uttar Pradesh where the mosquito vectors showed 98.33% mortality (Kumar *et al*., 2011). However, Shetty *et al*., (2013) reported lambdacyhalothrin resistant *Cx. quinquefasciatus* from Bengaluru with 0.0006 - 0.0041 ppm LC_{50} value.

Few reports on lambdacyhalothrin resistance in *Cx. quinquefasciatus* across different countries of the world complies with the current finding. Lambdacyhalothrin resistance was reported from Malaysia with 34.38 - 36.43% mortality (Nazni *et al*., 2005) and also incipient resistance with 80% mortality (Chen *et al*., 2013). Similar reports on lambdacyhalothrin resistance were recorded from Zanzibar with 24% mortality (Jones *et al*., 2012). However, with 100% mortality, *Cx. quinquefasciatus* from Thailand was reported to be susceptible to lambdacyhalothrin (Somboon *et al*., 2003).

The observed resistance to synthetic pyrethroids in *Cx. quinquefasciatus* population from all 6 districts of West Bengal is an important issue of concern because synthetic pyrethroids are the sole insecticide class to be used for impregnation of mosquito nets as recommended by WHO as a major control strategy against malaria in not only West Bengal but around the globe (WHO, 2007; WHO, 2009a; WHO, 2016b; NVBDCP, 2020). This insecticide class is also recommended for IRS (indoor residual spray) to control mosquito vectors due to their rapid action and minimal toxicity to humans (NVBDCP, 2020). Inefficiency of synthetic pyrethroid-impregnated mosquito nets to check the malarial vector *Anopheles gambiae* and lymphatic filariasis vector *Cx. quinquefasciatus* have already been reported (Guillet *et al*., 2001; Kudom *et al*., 2015).

In West Bengal, there are no reports on direct application of synthetic pyrethroids against *Cx. quinquefasciatus*. However, the use of synthetic pyrethroids in domestic household to control disease-causing mosquito vectors and also to counter the nuisance caused by mosquito biting may be the probable reason of resistance development in *Cx. quinquefasciatus* in this study. This statement holds true due to the anthropophilic and indoor resting behavior of *Cx. quinquefasciatus*. The indoor resting habit of *Cx. quinquefasciatus* prolongs the exposure time of this mosquito vector to various kinds of synthetic pyrethroids-containing anti-mosquito products like coils, mosquito repellent oils, sprays, fumigants and creams (Zinser *et al*., 2004). Direct contact of adult *Cx. quinquefasciatus* to such products in household activities results in the onset of resistance development in this vector to synthetic pyrethroids. In India, the most commonly used mosquito repellent coil which is available in the market place under the brand name 'Mortein' contains a mixture of permethrin and transallethrin. Similarly, the famous mosquito spray used in domestic household under the brand name 'Hit' contains transallethrin and the liquid fumigant sold under the brand name 'All-Out' usually contains allethrin.

Apart from the impregnation of long lasting insecticidal nets (LLINs) with synthetic pyrethroids and its presence in all mosquitocidal tools used in household, this insecticide class is also frequently used in the agricultural sector to combat insect pests of economic importance (Gurusubramanian *et al*., 2008). As stated earlier, 5 districts under study *i.e*., Alipurduar, Coochbehar, Darjeeling, Jalpaiguri and Uttar Dinajpur relies mainly on tea plantation for their economy as these districts are located in the tea belt region of northern part of West Bengal (Saha and Mukhopadhyay, 2013). Malda district however, mainly depends on orchard crops for its economy and Jalpaiguri district partly relies on jute cultivation while Uttar Dinajpur on pineapple cultivation (Das *et al*., 2011).

Synthetic pyrethroids along with organophosphate insecticides are mainly used to control insect pests in tea gardens and pineapple cultivation (Das *et al*., 2011). Use of synthetic pyrethroids in fruit orchards is also a common practice in West Bengal. Therefore, the accumulation of synthetic pyrethroids from the agricultural fields to nearby drains may provide cross resistance to *Cx. quinquefasciatus* that usually dwell in such drains and channels (Nkya *et al*., 2013). Moreover, the insecticide selection pressure created on *Cx. quinquefasciatus* due to intensive use of these two insecticide classes on the agricultural sector in the studied districts might be the reason behind severe resistance to synthetic pyrethroids in this study. Similar reports on secondary resistance in *Cx. quinquefasciatus* to synthetic pyrethroids used in the agricultural sector from different corners of the world is common (Nkya *et al*., 2013; Lopes *et al*., 2019). *Cx. quinquefasciatus* are opportunistic breeders and may habitat drains in the vicinity of agricultural fields thereby developing non-targeted resistance to synthetic pyrethroids and organophosphate insecticides applied in the tea gardens, pineapple fields and fruit orchards in West Bengal.

6.3. Mechanisms involved in the development of resistance against insecticides in *Cx. quinquefasciatus* populations from northern West Bengal:

6.3.1. Mechanisms involved in the development of resistance against Temephos:

Larval populations of *Cx. quinquefasciatus* from different districts of West Bengal was mostly found to be susceptible to WHO recommended dose of temephos except SHM which was resistant with 80% mortality (Table 10). Likewise, in the NVBDCP recommended temephos dose only three of the tested populations (TFG, SHM and SLG) were observed to be resistant to temephos (Table 10). In mosquitoes and other insect species elevated activity level of non-specific esterases are linked to the development of resistance to temephos and other organophosphate insecticides (Hemingway *et al*., 2004; Liu, 2015; Goindin *et al*., 2017). As such, ≈30 fold higher α-CCEs activity and ≈17 fold higher β-CCEs activity recorded in SHM population as compared to SP may positively correlate with the observed resistance to temephos in the same population. TFG and SLG populations also showed higher α-CCEs and β-CCEs activity compared to that of SP.

Though only few populations were resistant to temephos yet many populations had diagnostic dose greater than WHO recommended dose *i.e*., 0.02 ppm. Higher values of diagnostic dose also indirectly indicate development of resistance in mosquitoes (Yadav *et al*., 2015). Highest recommended dose of 0.138 ppm was calculated for SAM (Table 10). Out of the 16 populations under study, 11 populations had diagnostic dose that was greater than WHO recommended dose. The presence of higher elevated α-CCEs and β-CCEs activity in most of the tested populations therefore, may be taken as the reason behind diagnostic dose of temephos for most of the study sites. Involvement of elevated levels of non-specific CCEs to temephos resistance in *Cx. quinquefasciatus* has been reported from various corners of the world (Pietrantonio *et al*., 2000; Corbel *et al*., 2007; Tantley *et al.*, 2010; Delisi *et al*., 2017).

In mosquitoes, apart from enhanced esterase activity insensitive acetylcholinesterase (AChE) is also reported to be associated with organophosphate resistance (Weill *et al*., 2003; Low *et al*., 2013a; Liu, 2015). Highest diagnostic dose of 0.138 ppm temephos in SAM with significantly lower α-CCEs and β-CCEs activity level as compared to other tested populations (Table 10) might suggest the involvement of other mechanisms of resistance particularly insensitive AChE in the studied population. However, the amplification and over expression of non-specific CCEs is observed to be the most common mechanism of resistance in mosquito vectors against temephos and other organophosphate insecticides (Liu, 2015). Moreover, studies on the molecular level have revealed the over expression of 2 major CCEs genes *i.e*., *ccae3A* and *ccae6A* in mosquitoes showing resistance to temephos (Poupardin *et al*., 2014; Grigoraki *et al*., 2015).

Qualitative study of CCEs through native PAGE revealed the presence of varying number of α-CCEs and β-CCEs isozymes in the studied populations. Five of the studied populations *i.e*., SHM, MLT, SAM, FLB and CPR showed a maximum of 4 isozymes for α-CCEs (Table 17). Similarly, SHM, BDN and FLB showed 4 isozymes of β-CCEs in the present study while ISL showed five isozymes. Presence of maximum 4 bands indicating 4 different isozymes for both α-CCEs and β-CCEs in SHM population may be linked to the association of these esterase isozymes in resistance development to temephos in the same population. Furthermore, presence of darkly stained bands in gel electrophoresis is reported to indicate over expression of a particular CCE isozyme and its involvement in resistance development to temephos in *Ae. aegypti* (Lima *et al*., 2004). Similar involvement of elevated CCEs isozymes in organophosphate resistance development in field collected *Cx. quinquefasciatus* are reported by Pietrantonio *et al*., 2000; Gorden and Ottea, 2012. Presence of only 2 lightly stained bands for α-CCEs isozymes in JPT which is completely susceptible to temephos furthermore supports the association of CCEs with organophosphate resistance in *Cx. quinquefasciatus*.

Moreover, single isozyme of β-CCEs observed in HCP, MLT and SAM positively correlates to lower esterase activity of these study sites on quantitative analysis of enzyme activity (Table 18). Association of different isozymes of CCEs with resistance to temephos is also reported in the dengue vector *Ae. aegypti* worldwide (Bisset *et al*., 2013; Muthusamy *et al*., 2014; Grigoraki *et al*., 2015).

Though most of the studied populations of *Cx. quinquefas*ciatus are susceptible to temephos, higher values of site specific diagnostic dose of this insecticide calculated in the study might lead to problems in mosquito vector control in the studied districts with temephos. Therefore, rotational use of other larvicides such as Insect Growth Regulators (IGRs) and biolarvicides may be helpful in checking the mosquito population and prolonging the onset of temephos resistance in the studied area.

6.3.2. Mechanisms involved in the development of resistance against Malathion:

Development of resistance to malathion in the mosquito population is coupled by enhanced level of CCEs (Hemingway *et al*., 2002). The comparatively higher α-

CCEs and β-CCEs of the studied populations to that of SP might be linked to the observed resistance of *Cx. quinquefasciatus* populations against malathion (Table 12). HCP showed the highest value for both α-CCEs and β-CCEs activity among all of the studied populations (Table 16). Severe resistance to malathion with zero mortality rate in HCP may thus be correlated with higher esterase activity observed in that population. Similarly, higher mortality rate (>70%) in DLK and JPT with significantly lower esterase activity as compared to other populations might indicate the role of CCEs in resistance development against malathion in *Cx. quinquefasciatus* (Hemingway *et al*., 2004).

However, higher mortality rate against malathion (70.58%) in SHM, though with the presence of higher elevated levels of α-CCEs and β-CCEs hints upon the involvement of malathion-specific esterase in resistance development to malathion (Karunaratne and Hemingway, 2001). Malathion is an open chain organophosphate and resistance to malathion involves a separate esterase group known as malathion-specific esterases (Zeigler *et al*., 1987; Hemingway *et al*., 2004). Open chain organophosphate like malathion are usually reported to involve a separate esterase group different from those involved in other organophosphate insecticides like temephos (Gelasse *et al*., 2017). Present study focuses on measuring the quantitative activity of entire CCEs enzymes in the mosquito population. In SHM, therefore, other non-specific esterases may have elevated activity while the malathion-specific esterase may be present in lower amount thereby showing more than 70% mortality against malathion.

In this study, presence of malathion-specific esterases in study sites except SHM is evident from low mortality percent of *Cx. quinquefasciatus* adults when exposed to malathion (Table 12). Similar involvement of malathion-specific CCEs in resistance development to malathion in *Cx. quinquefasciatus* is reported by Karunaratne and Hemingway in 2001. Moreover, association of elevated esterase activity and resistance development to malathion in *Cx. quinquefasciatus* are reported from various countries (Corbel *et al*., 2007; Norris *et al*., 2011; Low *et al*., 2013b; Tahir *et al*., 2013).

Electrophoretic analysis of CCEs isozymes reveal the presence of a maximum 4 different isozymes of α-CCEs in a single population while 5 different isozymes of β-CCEs in ISL (Table 17, 18). Malathion resistance in this study may be linked to varying number of α-CCEs and β-CCEs isozymes observed with different band intensities (Figure 20, 21). CPR with complete susceptibility to temephos showed 4 isozymes of α- CCEs and one darkly stained isozyme of β-CCEs (Table 17, 18). This suggests the presence of malathion-specific esterases in the population as the population showed resistance to malathion with only 23.53% mortality (Table 12). The result obtained in qualitative analysis of esterase isozymes revealed involvement of mostly β-EST VII and β-EST VIII in resistance development to malathion with the presence of deeply stained bands. However, α-EST I, II and III were usually present in most of the studied populations indicating its role in resistance development to organophosphates.

Results of synergist assays using 10% TPP- an inhibitor of CCEs activity and 4% PBO- inhibitor of CYP_{450}s monooxygenases also provides hints on the mechanisms of resistance to malathion in *Cx. quinquefasciatus*. Though the exposure of adult mosquitoes to the synergists prior to exposure of malathion resulted in an increase in the mortality percentage, yet complete susceptibility of mosquito populations to malathion could not be obtained (Table 14). A significant increase in mortality percentage was observed in MEK but with no restoration of the susceptibility status to malathion.

This finding suggests the presence of other resistance mechanisms apart from elevated CCEs activity against malathion. As stated earlier, resistance to organophosphates occurs either by elevated activity of CCEs or through insensitive AChE (Weill *et al*., 2003; Hemingway *et al*., 2004; Liu, 2015). However, involvement of target-site insensitivity in resistance development to malathion in mosquito vectors is rarely reported (Moyes *et al*., 2017).

From the present observation, application of malathion in combination with synergists or enzyme blockers is not suggested in northern districts of West Bengal. However, new insecticide formulations with slightly different target sites might be the more probable choice of vector control. Furthermore, insensitive AChE in *Cx. quinquefasciatus* should also be screened and studied in this region for a better conclusive action to be proposed.

Higher values of GSTs as compared to SP in the present study, suggests the role of this enzyme group in resistance development to malathion (Table 16) as GSTs overproduction in mosquito vectors is associated with organophosphate resistance (Hayes and Wolf, 1988).

6.3.3. Mechanisms involved in the development of resistance against Propoxur:

Elevated esterase activity is not only associated with resistance to organophosphates but also confers resistance to carbamate insecticides (Karunaratne and Hemingway, 1998; Hemingway *et al*., 2004). In addition to CCEs, propoxur resistance in mosquito vectors is also reported to occur though in few cases through the elevation of CYP_{450}s and GSTs activity (Ishak *et al*., 2015). Therefore, the significantly higher CCEs activity observed in the study might be linked to propoxur resistance as well in the examined populations along with resistance to organophosphates. Furthermore, higher CYP_{450}s activity may also be associated with the observed resistance to propoxur in the studied populations. This suggests the combined action of both CCEs and CYP_{450}s in conferring resistance to propoxur in *Cx. quinquefasciatus* populations of northern West Bengal.

The synergist assays using 4% PBO and 10% TPP increased mortality percentage of *Cx. quinquefasciatus* when exposed to propoxur but only to some extent with no significant difference (Table 14, 15). As such, the major detoxifying enzymes are not the only mechanisms of resistance development to propoxur in the studied districts. Resistance to propoxur may also develop as a result of insensitive AChE as this insecticide share a common target site with organophosphates. Moreover, similar target site of two insecticide groups also suggest the cross resistance of mosquito population to propoxur and temephos.

Apart from these mechanisms of resistance, some other resistance mechanisms may also be involved in resistance development to propoxur in the studied populations as the elevated enzyme levels and mortality percentage could not be fully correlated. Any other resistance mechanism (if present) needs to be studied and examined for proper vector control in near future and in turn to combat the dreaded mosquito-borne diseases in West Bengal.

Though the isoforms of α-CCEs and β-CCEs are rarely reported to be associated with propoxur resistance (Karunaratne and Hemingway, 1998), presence of 4 α-CCEs isozymes in SAM may be correlated to some extent with observed severe resistance to propoxur in the population with zero mortality. However, further studies are needed for better understanding of CCEs isozymes and its association with propoxur resistance.

6.3.4. Mechanisms involved in the development of resistance against Pyrethroids:

Resistance to synthetic pyrethroids in mosquitoes and other insect vectors are brought about either by an increased activity of CYP_{450}s enzyme family (Amenya *et al*., 2008) or by mutations in the voltage-gated sodium channel (vgsc) gene resulting in insensitive target site (Liu, 2015; Scott, 2019). The combination of these two mechanisms is usually reported to confer resistance against synthetic pyrethroids in the mosquito vectors (Marcombe *et al*., 2012; Vontas *et al*., 2012).

The studied populations of field collected *Cx. quinquefasciatus* was found to be resistant to permethrin – a Type I pyrethroid. Low mortality rate observed against permethrin in all of the study sites except JPT, showed resistance against this insecticide (Table 11). JPT however, showed incipient resistance or unconfirmed resistance to permethrin with 95.65% mortality. Higher values of CYP_{450}s activity obtained in the study may be attributed to the observed permethrin resistance as comparatively higher CYP_{450}s activity in BDN, HCP and DLK do coincide with lower mortality rate in those populations (Table 11, 16).

Similarly, JPT with incipient resistance to permethrin showed a significantly lower CYP_{450}s activity thereby, indicating the involvement of CYP_{450}s in resistance development to permethrin. Similar instances of elevated CYP_{450}s activity linked to permethrin resistance in *Cx. quinquefasciatus* are reported from different regions around the globe (Corbel *et al*., 2007; Low *et al*., 2013b; Wan-Norafikah *et al*., 2013). However, Kudom *et al*., (2015) reported permethrin resistance in *Cx. quinquefasciatus* with lower CYP_{450}s activity which might be the case in COB, MEK and SAM populations (Table 16). Lower mortality rates in these populations with lower values of CYP_{450}s activity indicates the involvement of other mechanisms of resistance mainly target-site insensitivity (Liu, 2015; Scott, 2019).

Elevated activity of CCEs observed in this study could not be correlated with permethrin resistance thereby, indicating the CCEs to be ineffective against pyrethroid resistance in *Culex* mosquitoes. Similar observation on no correlation of elevated CCEs with pyrethroid resistance in *Cx. quinquefasciatus* was reported from USA (Gordon and Ottea, 2012). On the contrary, elevated activity of CCEs is linked to pyrethroid resistance in *Cx. quinquefasciatus* by Kudom *et al*., (2015).

Similar to permethrin resistance, resistance to two Type II pyrethroids - deltamethrin and lambdacyhalothrin in *Cx. quinquefasciatus* observed in this study may be linked to the many fold high CYP_{450}s activity in the field collected populations as compared to SP (Table 16). The presence of lower CYP_{450}s activity in JPT population with significantly highest mortality percentage against both deltamethrin and lambdacyhalothrin also suggest the role of CYP_{450}s in resistance development against these insecticides particularly and synthetic pyrethroids in general.

Moreover, higher CYP_{450}s activity observed in DLK, BDN and HCP also coincides with lower mortality rate of these three populations upon exposure to deltamethrin and lambdacyhalothrin.

Several studies have reported a positive correlation between overproduction of CYP_{450}s enzyme family and resistance to synthetic pyrethroids in *Cx. quinquefasciatus* (Komagata *et al*., 2010; Liu *et al*., 2011; David *et al*., 2013; Delannay *et al*., 2018; Fagbohun *et al*., 2019). The elevated CYP_{450}s activity in *Cx. quinquefasciatus* is attributed to overexpression of mainly 2 CYP_{450}s genes – *cypm10* and *cyph34* resulting in resistance to synthetic pyrethroids (Komagata *et al*., 2010). The study leading to this finding was conducted in permethrin selected strain of *Cx. quinquefasciatus* (JPal-per). However, elevated level of CYP_{450}s due to overexpression of CYP_{450}s genes is not always related to pyrethroid resistance as a single mutation in transcription factor of these genes may result in overproduction of CYP_{450}s enzyme family even in the absence of insecticide exposure (Komagata *et al*., 2010).

In the present study, COB with comparatively lowest value of CYP_{450}s activity was observed to have lowest mortality rate against deltamethrin among all other tested populations (Table 16). This indicates that deltamethrin resistance in COB is not due to elevated activity of CYP_{450}s but because of some other mechanisms of resistance.

The use of 2 synergists – PBO and TPP in the study was unable to restore susceptibility of *Cx. quinquefasciatus* populations to synthetic pyrethroids except in BDN, MEK and JPT, though increase in mortality rate was observed (Table 16) (Figure 19). Inefficiency of the synergists used to revert resistance status of studied populations of *Cx. quinquefasciatus* against synthetic pyrethroids indicates only a partial involvement of major detoxifying enzymes in the development of observed resistance to synthetic pyrethroids in the lymphatic filariasis vector from 6 northern districts of West Bengal.

Insecticide resistance in insects against synthetic pyrethroids occurs due to an elevated CYP_{450}s activity (Hemingway and Ranson, 2000; Amenya *et al*., 2008) and PBO is reported to inhibit CYP_{450}s enzyme family (Kudom *et al*., 2015). This indicated the involvement of other resistance mechanisms along with metabolic enzymes in the studied populations.

There are reports on resistance in *Cx. quinquefasciatus* even after prior exposure to PBO (Corbel *et al*., 2007; Skovmand *et al*., 2018). However, in BDN and MEK, though susceptibility status was not achieved with the use of PBO yet, these 2 populations showed incipient resistance to deltamethrin in the synergist assay (Table 14). Moreover, the use of PBO in JPT along with synthetic pyrethroids for an efficient vector control may be suggested as JPT showed susceptibility towards deltamethrin and permethrin while incipient resistance to lambdacyhalothrin when prior treated with PBO (Table 14). This finding do correlated with similar increase in mortality percentage of *Cx. quinquefasciatus* when exposed to PBO prior to the exposure of synthetic pyrethroids in Malaysia (Norafikah *et al*., 2013) and Nigeria (Fagbohun *et al*., 2019).

In rest of the 12 populations under study, combination of PBO with synthetic pyrethroids for impregnation of Long Lasting Insecticide Treated nets (LLINs) (Gleave *et al*., 2018) may not provide a positive outcome in checking the growth and proliferation of lymphatic filariasis vector and in turn the disease.

Higher KDT_{50} and KDT_{99} values obtained in the study indicate longer time taken by synthetic pyrethroids to knock down *Cx. quinquefasciatus* populations. None of the study sites was found to have <60 minutes KDT_{50} value (Table 13) suggesting the inefficiency of synthetic pyrethroids in these populations as KDT assay is also a good indicator of the onset of insecticide resistance (Elissa *et al*., 1993). Longer knock down time of synthetic pyrethroids in mosquito vector suggests insensitive receptor as these insecticides are well known for their rapid action (Vontas *et al*., 2018).

Allele-specific PCR (AS-PCR) of L1014F mutation in the voltage-gated sodium channel (vgsc) gene showed the presence of maximum 5 genotypes in the populations under study (Table 19). Mutated resistant homozygote (F/F) was found to be 30% in MLT from Malda district, the highest among all other study sites while TFG and FLB showed absence of F/F genotype (Table 19). Presence of the resistant homozygote (F/F) in all of the studied populations except TFG and FLB is of prime concern owing to inefficiency of synthetic pyrethroids in controlling the mosquito vectors. The occurrence of L1014F mutation in vgsc of *Cx. quinquefasciatus* is reported earlier from India (Sarkar *et al*., 2009) and also around the globe (Corbel *et al*., 2007; Wondji *et al*., 2008; Liu *et al*., 2009; Norris *et al*., 2011; Jones *et al*., 2012; Chen *et al*., 2013; Kudom *et al*., 2015; Yadouletan *et al*., 2015; Yanola *et al*., 2015; Chi-Chim *et al*., 2018; Delannay *et al*., 2018; Maestre-Serrano *et al*., 2020). However, resistance to pyrethroids and DDT due to mutations in vgsc occurs only in the presence of resistant homozygote genotypes. This is because of the fact that insecticide resistance due to mutations in vgsc of mosquito vectors through inheritance is incompletely recessive (Scott, 2019). However, recently novel mutations in the voltage-gated sodium channel *i.e*., L932F and I936V of *Cx. quinquefasciatus* are reported to also be associated with pyrethroid resistance (Sugiura *et al*., 2021).

Though kdr mutation is a recessive trait, presence of high heterozygote frequency (L/F) of 35% in TFG and CPR (Table 19) should not be neglected as the absence of susceptible mosquito or presence of lower wild homozygote frequency may result in the development of irreversible resistance to insecticides due to a heavy selection pressure (Soko *et al*., 2015). Apart from L1014F mutation, other mutations in vgsc *i.e.*, L1014H, L1014C and L1014S are also reported to occur in *An. gambiae* and *Cx. pipiens* (Scott *et al*., 2015). However, these mutations are not reported to occur in *Cx. quinquefasciatus*.

The present study observed occurrence of L1014S mutation (though in a less frequency) along with L1014F mutation in *Cx. quinquefasciatus* populations from northern districts of West Bengal (Table 19). Co-occurrence of the these 2 mutations might be correlated with observed resistance to synthetic pyrethroids as L1014F is reported to be associated with resistance to pyrethroids and DDT in the LF vector - *Cx. quinquefasciatus* (N'Guessan *et al*., 2009; Bkhache *et al*., 2016; Liu *et al*., 2019). However, reports with high mortality in mosquito vectors to synthetic pyrethroids even with the presence of kdr mutations (Asidi *et al*., 2005; Matambo *et al*., 2007) indicates inadequency of kdr mutations at DNA level alone in resistance development without RNA transcription leading to overproduction of detoxifying enzymes (Xu *et al*., 2006). Thus, resistance to synthetic pyrethroids in *Cx. quinquefasciatus* occurs mainly by the combination of increased CYP_{450}s activity and mutation in vgsc leading to kdr in the vector (Corbel *et al*., 2007; Kudom *et al*., 2015; Lopes *et al*., 2019).

6.3.5. Mechanisms involved in the development of resistance against DDT:

In mosquitoes and other insect vectors, DDT resistance is attributed to elevated activity of major detoxifying enzymes (Hemingway and Ranson, 2000; Hemingway *et al*., 2004) mainly GSTs (Hemingway *et al*., 2004; Marcombe *et al*., 2014) and due to mutation in vgsc leading to kdr in the vector (Vontas *et al*., 2012; Scott, 2019). The combination of both high activity of detoxifying enzymes and mutation in vgsc is also reported as the mechanisms behind DDT resistance (Aponte *et al*., 2013). Low mortality rate against DDT in the study depicting severe resistance to DDT may be linked to elevated detoxifying enzyme activity to some extent (Hemingway and Ranson, 2000). Highest esterase activity in HCP population from Malda district coupled with zero mortality against DDT may be attributed to the association of α-CCEs in DDT resistance (Table 16). Though, increase in GSTs activity is associated with DDT resistance (Marcombe *et al*., 2014), positive correlation between elevated GSTs activity with observed mortality percentage in the studied populations could not be obtained (Table 16). Failure to correlate GSTs activity with DDT resistance in this study hints upon the involvement of target-site insensitivity behind observed resistance to DDT. Results of synergist assays also provide similar conclusion as the use of PBO and TPP partially increased mortality percentage of *Cx. quinquefasciatus* populations against DDT (Table 14, 15). Furthermore, higher KDT_{50} and KDT_{99} values (Table 13) against DDT in the studied populations also indicate the presence of mutation in vgsc of the mosquito populations under study.

Two kdr mutations in vgsc observed in the study *i.e*., L1014F and L1014S in all of the studied populations may be linked to DDT resistance along with resistance to synthetic pyrethroids (N'Guessan *et al*., 2009; Bkhache *et al*., 2016; Liu *et al*., 2019). L1014S mutation in the vgsc of mosquito vectors confers comparatively higher resistance to DDT than to synthetic pyrethroids (Martinez-Torres *et al*., 1999; Hemingway *et al*., 2004; Liu *et al*., 2019). As such, severe resistance to DDT with lower mortality percentage compared to that of pyrethroids in the study and also higher KDT values for DDT may be due to the presence of L1014S mutation in the studied populations. The occurrence of both L1014F and L1014S mutations also imparts upon the phenomenon of cross resistance between synthetic pyrethroids and DDT (Fagbohun *et al*., 2019) due to their same target site *i.e*., vgsc. However, lower frequency of L1014S in the present study indicates the role of metabolic enzymes as well in resistance development (Hemingway and Ranson, 2000; Hemingway *et al*., 2004) though to a little extent. Therefore, DDT resistance in *Cx. quinquefasciatus* populations from northern districts of West Bengal may be due to the combination of both kdr mutations (mainly) and metabolic enzymes (partially). As such, use of enzyme blockers in combination with DDT might not yield positive result in controlling *Cx. quinquefasciatus* in the study region.

Mutations in vgsc of mosquito vectors leading to kdr are dependent on selection pressure of insecticides used and also on the environmental factors (Davies *et al*., 2008). However, the level of insecticide resistance brought about by mutations in 1014 codon of mosquito vgsc, increases with the presence of secondary cytoplasmic mutations in vgsc that occur simultaneously (Tan *et al*., 2002).