CHAPTER 2

REVIEW OF LITERATURE

2.1. TERMITES: BIOLOGY, ECOLOGY AND PHYLOGENY

2.1.1. Biology and Ecology:

Termites are polymorphic, eusocial invertebrates often referred to as white ants. In actuality they are little related to ants or any other social insect groups due to their diploid caste system with very distinctive morphologies (Eggleton, 2011). Among the social insects, termites were the first and oldest one to evolve into caste system at about 150 million years ago (Engel et al., 2009; Martínez-Delclòs and Martinell, 1995). The social structure in termites and other social insects is an attribution of convergent evolution (Thorne, 1997).

A true social structure or eusociality in an insect group comprised of a common habitation shared by overlapping brood of specialized individuals or castes assigned to perform different colony functions like reproduction, foraging, tending broods and colony defense etc. (Wilson, 1971). In a complex social system, a termite colony consists of live individuals and the non-living structure where these individuals live. Usually, these structures range from a simple network of few tunnels to extravagantly constructed marvelous termitariums (Eggleton, 2011). For living entity there are different castes such as the royal pair of King and Queen, and their subjects - reproductives, soldiers and workers (Figure 2.1) (Horwood and Eldridge, 2005). A queen mates with a single king for life and may survive for about 25-30 years or more (Korb, 2008). A queen except for few years of her active initial colonization period remains stagnant for rest of her life due to the physogastric condition. Physogastric females can be seen in many species of termites where a post-mating queens' abdomen swells dramatically to accommodate more and more number of ovarioles.

Life cycle of termites start from a fully matured queen which at times possess as much as 2,000 ovarioles in each ovary, producing almost 40,000 eggs a day (Kerkut and Gilbert, 1985; Kaib et al., 2001). These eggs hatch into different castes of the colony. Reproductive castes are the immature version of the king and the queen which develop wings before swarming and are known as alates. During the onset of monsoon they swarm out of the colony to find mate and reproduce. Once met their mate these alates shed off their wings (de-alates) and leave to burrows or crevices to start a new colony (Miller, 2010). The other castes consist of workers and soldiers. Workers are decisively the most important force in the colony as they forage food and water, tend the young ones, feed royal couple, reproductives, soldiers and nymphs, construct and mend the colony structures (Eggleton, 2011). They resemble the alates in general however, without wings, paler coloration and smaller in size (Roonwal and Chhotani, 1989). A soldier is tasked only to defend the colony and the royal couple. Therefore, a typical termite colony has workers about 90 per cent or more of the total population (Roonwal and Chhotani, 1989), however, it becomes difficult to document the societal system where either soldiers (*Anoplotermes* spp.) or workers (*Kalotermes* sp.) are missing (Eggleton, 2011).

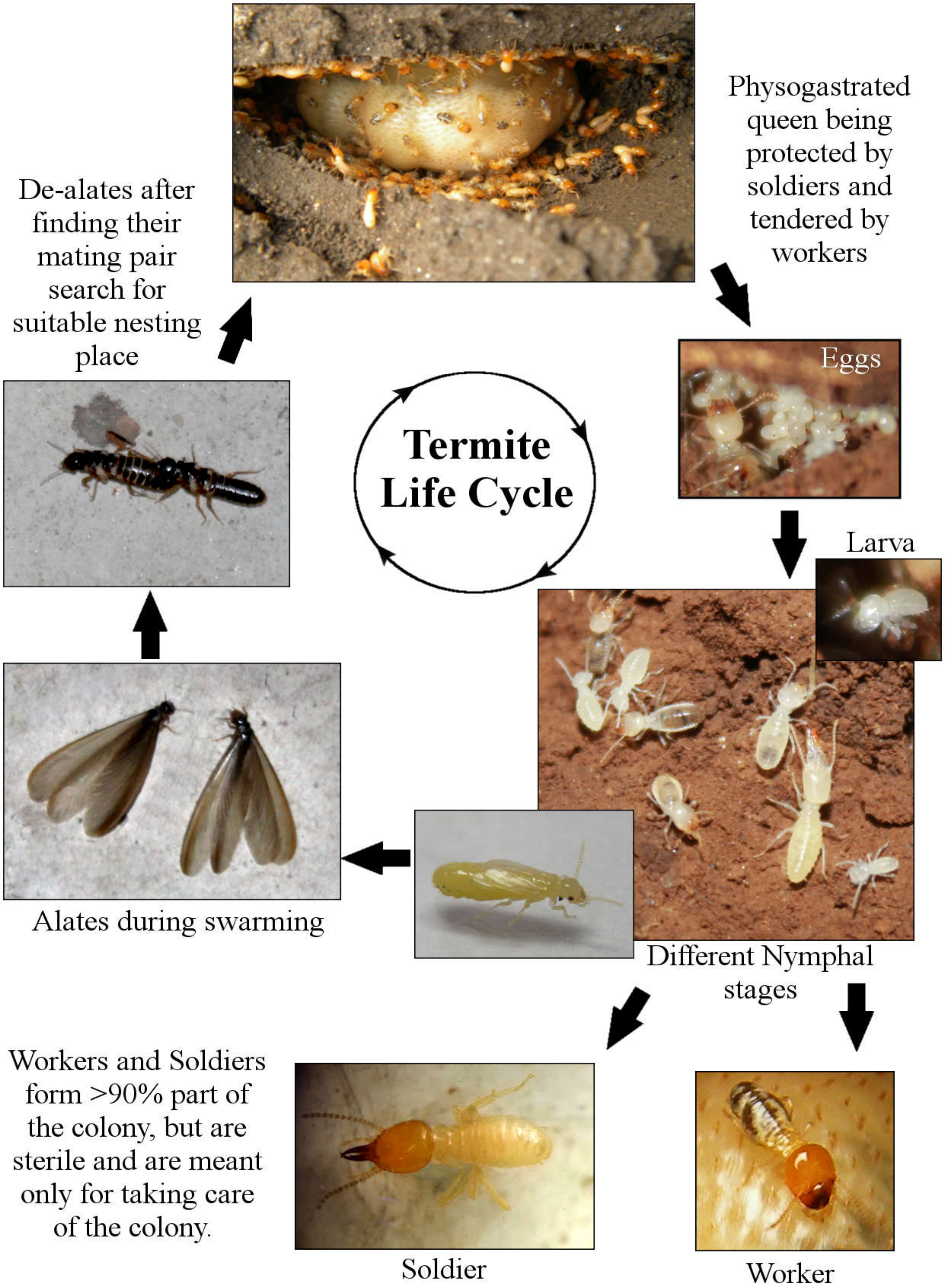

Figure 2.1: Termite life cycle illustrating different stages

A termite colony is often referred to as superorganism for its astounding coordinated and precise functioning (Eggleton, 2011). A spectacle produced out of a synchronous collective effort of different agents of a system is what known as superorganism (Hölldobler and Wilson, 2009). As organ system functions in concert with utmost fidelity to manifest various activities, in a similar fashion, a termite colony operates like a superorganism within which each caste commensurate to an organ system pursuing their assigned task. During the course of their eusocial evolution these insects have lost their solitary survival potency. A solitary insect essentially requires to feed, to defend, to reproduce and to migrate on its own. However, somehow a termite caste individually lacks many of these features and as such to compensate that they must live in colony system (Eggleton, 2011).

Termites chiefly feed on cellulose and lignocellulose (Engel et al., 2009). They get their food from varying stages of decomposing or dying plant materials (Donovan et al., 2000; Hyodo et al., 2008). This in turn also rejuvenates the ecosystem by recycling dead wood, humus and soil minerals (Eggleton, 2011; Freymann et al., 2008; de Souza and Brown, 1994). Cellulose and its inert form lignocellulose are very tough to break down in digestive system of organisms. In a tropical ecosystem termites are estimated to ingest 50%–100% of the dead plant biomass (Bignell and Eggleton, 2000). Termites belonging to lower group rely upon their symbiotic association with some flagellate protozoa and bacteria which reside in their guts, to break down the cellulose (Ikeda-Ohtsubo and Brune, 2009; Slaytor, 1992). However, this is not true for all group of termites as members of Termitidae family that chiefly feed upon wood and leaves are devoid of any flagellates, but have their own cellulase enzyme to digest their food (Lo et al., 2011; Bignell et al., 2011; Brune and Ohkuma, 2011; Eggleton, 2011; Watanabe et al., 1998; Tokuda and Watanabe, 2007; Li et al., 2013). Inside the hindgut of termite cellulose breaks down either with the help of symbiotic flagellates or their very own enzyme system in an anaerobic environment (Lo et al., 2011). This break down process releases an excess of gases from termites body and from the colony which is estimated to be 2%–5% of the world's total methane content (Sugimoto et al., 2000). This symbiosis between termite and its intestinal microbiota may have been the driving force behind the evolution of eusociality of termites, with their polymorphic caste system and intricate social life (Wilson, 1971; Grimaldi and Engel, 2005; Dietrich et al., 2014).

2.1.2. Phylogeny:

Since the inception of taxonomic identification of termite species, conventional use of morphological characters and character states have been the most favored technique (Roonwal and Chhotani, 1989; Chhotani, 1997; Donovan et al., 2000; Krishna et al., 2013). In fact morphometric character based identification is much more valid even in today's scenario. However, molecular taxonomy has revolutionized the whole concept of systematic due to its more precise and accurate results. Molecular techniques are commonly used for species/subspecies identification, colony characterization, and to address questions regarding the phylogeny and evolution of subterranean termites. These techniques include: restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), fingerprinting of variable number tandem repeats (VNTRs), simple sequence repeats (SSRs), and/or simple sequence length polymorphisms (SSLPs). RAPD, as a PCR based methodology uses decamer base pair primers without requiring any prior knowledge of specific genomic sequences (Williams et al., 1990). It has come up as a very useful method for identification and differentiation of closely related insect species and populations (Williams et al., 1990; Welsh and McClelland, 1990). RAPD may display a high degree of polymorphism and screening multiple primers against taxa of interest has proven to be a means of quickly identifying species-specific markers (Arnold et al., 1991). Additionally, RAPD markers derived from multiple loci have the potential to provide important information on insect population genetic structure that would not be available from a single locus marker. RAPD has been used in broader spectrum, like in applied and evolutionary aspects of insect biology (Harry et al., 2001). Though in case of termite phylogeny and evolution the use of RAPD technique is little limited, yet study on symbiotic bacterial or fungal evolutionary relationship with termite and within bacteria and fungus themselves have extensively elaborated with the help of RAPD (Harry et al., 2001).

Till few years back termites were classified at the taxonomic category of order Isoptera. However, inpouring of more and more molecular and evolutionary evidences have brought a dilemma about their phylogenetic stance (Kambhampati et al., 1996; Ware et al., 2008; Legendre et al., 2008; Engel et al., 2009). In fact, Cleveland et al. (1934) had already postulated termites' close relationship with wood feeding cockroaches (*Cryptocercus*) based on symbiotic gut flagellates that *Cryptocercus* and some species of lower termites commonly harbor in their gut. Recent advance studies on termite taxonomy have suggested termites to be placed in order Dictyoptera (with Cockroaches and Mantids) or more closely with cockroaches (Inward et al., 2007; Legendre et al., 2008; Ware et al., 2008). Inward et al. (2007) based on their extensive molecular investigation proposed termites to be placed as family Termitidae under cockroach order Blattodea within the superorder Dictyoptera. Which means Isoptera as an order or any other category will cease to exist. This hypothesis was supported by Eggleton et al. (2007), but some were of a view to keep it alive (Lo et al., 2007; Engel et al., 2009) by and large for nomenclatural stability. Now Isoptera as an Infraorder has been kept under a clade Xylophagodea along with its sister group Cryptocercidae (Engel, 2011).

In recent time termite taxonomy has gone through a major amendment (Inward et al., 2007; Engel et al., 2009; Krishna et al., 2013). As of 2013 there are 3106 recorded living and fossil species of termites out of which 363 species are considered as significant pests (Krishna et al., 2013). Thousands of scientific reports and research findings are published every year on termites and related topics, but they are mainly concentrated on control aspects. There has been very limited work on the phylogenetic probe of termites which has restrained studies of their macroevolution (Eggleton, 2001). Despite many stalwart workers working in this field the first ever comprehensive cataloging of termite group came only after 1949 when Snyder compiled his renowned "*Catalogue of the Termites (Isoptera) of the World*". It took another 60 plus years when Krishna et al. (2013) again recompiled works on termite phylogeny from over 5000 references and catalogued them into a seven volume masterpiece as "*Treatise on the Isoptera*". As of now infraorder Isoptera is sub-grouped into nine extant families namely Archotermopsidae, Hodotermitidae, Kalotermitidae, Mastotermitidae, Rhinotermitidae, Serritermitidae, Stolotermitidae, Stylotermitidae and Termitidae (Engel et al., 2009; Krishna et al., 2013). However, there has been debate over the authenticity of these families and whether to retain this classification as such (Eggleton, 2011). According to Eggleton (2011), family Indotermitidae is a specialised Apicotermitinae and family Serritermitidae is to be kept as subfamily of the Rhinotermitidae. The present family-level classification is still likely to change in future as two families, the Rhinotermitidae and the “Termopsidae” (split up by Engel into a number of families), are probably not natural groupings. He opines that the subfamily-level classification has been poorly worked out, hence, direly needs a thorough revision despite recent advancements.

2.2. DAMAGE POTENTIAL AND ECONOMICAL IMPACTS

Termites’ dependence on varied sources of cellulose as food is the major reason why some of them are considered as pests. Although out of 2933 living species of termites only about 12.4 per cent (371) pose threat as structural pests and even only 3.5 per cent (104) are considered as serious pest (Wood, 1996; Krishna et al., 2013). Termites feed upon various crops, furniture, wooden buildings and any form of wooden structures making them one of the prime troublemakers to manmade world (Mahapatro and Chatterjee, 2017). Termites are represented by a vast number of species and variable feeding strategies (Lee and Wood, 1971). These tiny creatures despite being dependent primarily on cellulose or its derivative, have their very own specific food resources like humus, grass, wood in various stages of decomposition and minerals from soil (Eggleton, 2011). Some workers like Paul and Rueben (2005) argued termites to be divided into three food preference type - damp wood, dry wood and subterranean. They reported heavy damage caused by subterranean termites which is also marked as a serious urban pest. Damp wood termites and dry wood termites are moderate to significant pests respectively and at a given situation can cause a serious damage.

Although, the number of pest species are meager compared to total species diversity of termites, yet the limited number are notoriously sufficient enough to cause havoc in many places resulting into loss in billions of dollars (Su and Scheffrahn, 1998). The urban setup attracts more of pest infestation due to the fact that unlike rural environment these pest species are devoid of additional food sources like litter, rotting woods, humus etc. in concrete jungles. Su and Scheffrahn (1998) were of the opinion that damages caused by these destructive pests in developed countries are much higher than the cost of their control. The annual economic loss caused by termite pests was estimated to be $40 billion worldwide (Rust and Su, 2012). In the USA alone value of damage inflicted by termite pests goes over $1.02 billion annually (Edwards and Mill, 1986). In New Orleans state, an estimated $300 million are annually invested to control and repair damage caused by Formosan subterranean termites (Suszkiw, 1998). About $1.5 billion of money is invested only in liquid termiticides to control termites (Su, 1994). Subterranean termites account for 80 per cent of these control expenditures. In Australia a whopping $ 4.0 billion is spent annually to control termite attack on buildings (Caulfield, 2002). Past records of the Building Research Institute, ACCRA shares that the annual cost of repairing buildings damage by termite in West Africa was 10% of their capital cost (Harris, 1961).

Apart from being a sever pest of buildings, termites has been infesting forests and crops with similar intensity (Howse, 1970). Many of these damaging species live in the soil facilitating them an easy access to their target food source (Ibrahim and Adebote, 2012). Gay and Galaby (1970) reported an astounding 92% of the total loss caused by a single species *Coptotermes acinaciformis* in Virgin *Eucalyptus pilularis* forest. *Macrotermes* reportedly found responsible for 5 - 18 per cent yield loss of plants from various locations in the northern states of Nigeria (Ohiagu, 1979). Termites cause denudation of grassland and compete with livestock in the removal of grass (Ibrahim and Adebote, 2012). Ohiagu (1979) in Nigeria reported a grass removal rate of about 81 kg per hectare per annum for *Trinervitermes germinatus* at Mokwa. Termite problem in forest plantations has been found to be of serious and recurring nature (Cowie et al., 1989; Tho and Kirton, 1990). Termites that belong to the families Hodotermitidae, Kalotermitidae, Rhinotermitidae and Termitidae cause great loss in agriculture (UNEP Report, 2000). In case of tea plantations, termite attack has been associated with at least 15 per cent of the total crop loss (Das, 1962). Sands (1977) reported about 50 per cent or more crop loss in agricultural fields in over a decade. Das et al. (1982) further reported more termites on poorly shaded hot slopes of Cachar tillahs (hillocks of Barak Valley). Termite infestation may be as high as 90% in old tea areas of Barak Valley (Choudhury, 1999). In one hand termites inflict considerable damage to newly planted tea which limits their growth and at the other hand they downsize the production by killing mature tea bushes. In a tea ecosystem some species of termites have been estimated to have active colonies for over a hundred years (TRIT, 2006).

2.3. TOLERANCE TO PESTICIDES

The World Health Organization defines *resistance* as "the development of an ability in a strain of an organism to tolerate doses of a toxicant which would prove lethal to the majority of individuals in a normal (susceptible) population of the species" (WHO, 1957). Resistance development in insects to acutely toxic insecticides generally occurs by selection of rare individuals in a population that can survive the insecticide; it is preadaptive and not a mutational effect (Georghiou, 1965; Oppenoorth and Welling, 1976; Matsumura, 1985; Oppenoorth, 1985). Genetics and intensive application of these insecticides become responsible for the rapid development of tolerance in insect pests. Selection by an insecticide allows some insects with resistance genes to survive and pass the resistance trait on to their offspring (Daly et al., 1998). The proportion of resistant insects in a population continues to increase as the susceptible insects are eliminated by the insecticide. Eventually, resistant insects outnumber susceptible insects and the insecticide is no longer effective. Planters use pesticides more in amount and in toxicity to tackle the more resistant population of insects with no feasible outcome. This situation sometimes is known as *Pesticide trap* as planters keep investing more for less benefits (Marten, 2005). Reports suggest that selective pressure created by the continuous and indiscriminant use of pesticide has lead to the change in pest status. Most commercial insecticides are for safety reasons designed to be poor mutagens, and their use results in an intense chemical selection (high dose, high lethality) which is not conducive to genetic alterations but allows survival of pre-adapted i.e. resistant individuals (Roush and McKenzie, 1987).

In 1908, the first documented case of insecticide resistance in arthropods was reported in Washington for the San Jose scale *Quadraspidiotus perniciosus* to lime sulfur (Melander, 1914). Incidence of resistance in the "field" has generally correlated with the length of time an insecticide has been used, hence the trend among insecticide classes is organochlorines > organophosphates > carbamates > pyrethroids > insect growth regulators, microbials etc. in number of documented cases (Oppenoorth and Welling, 1976). Resistances to bacterial pesticides (McGaughey, 1985, 1994) and to baculoviruses

(Briese, 1986) up to 800-fold have already been noted in insect populations. Virtually every chemical and microbial agent is prone to resistance development in arthropods.

Insecticide resistance is the major obstacle to control of agriculturally and medically important pests. This worldwide problem has been documented for over 500 arthropod species, particularly among flies, caterpillars, beetles and mites (Georghiou, 1990). Resistance results in increased pesticide application frequencies, increased dosages, decreased yields, environmental damage and outbreaks of arthropod-borne human and veterinary diseases. Resistances within or between whole classes of insecticides are an ever increasing problem for control of major crop pests. Given the tremendous difficulty and investment associated with development of new, safe and cost-effective insecticides (Szczepanski, 1990), there is a grave need to preserve the efficacy of current and future insecticides.

Quantitative and qualitative changes in acetylcholinesterase confer resistance to insecticides (Fournier et al., 1992). Chareonviriyaphap et al. (2002) established a deltamethrin resistant *Anopheles minimus* population through a careful series of laboratory selection procedures. This strain conferred 52% resistance to deltamethrin with a > 25-fold increase in the LD_{50} from the parent colony. The development of physiological resistance to deltamethrin in laboratory, resistant-selected generations of *An. minimus* is primarily associated with increased detoxification by over-expression of monooxygenases. The oxidases are the major contributors to pyrethroid resistance too (Chareonviriyaphap et al., 2003).

The problem of tolerant variety of pests has come up as a big challenge to the tea industries. It is indicated in the study that heptachlor and chlorpyrifos pesticides despite being banned, are still prevalent in made tea at higher concentrations than their respective MRLs (maximum residue levels). This may pose serious health hazards to consumers apart from losing export credentials (Bishnu et al., 2009). The higher degree of tolerance to the organosynthetic pesticides posses two serious problem to the planters- firstly, the loss of produce which can go up to 100% and secondly increased cost of maintenance. Studies showed the increased amount of tolerance developed in *Hyposidra talaca* and *H. infixaria* (a major pest of tea foliage in present scenario) against cypermethrin 10% EC (Das et al., 2010). Among the two looper species, *H. talaca* showed about three times higher LC_{50} value and about two times higher LC_{95} value than *H. infixaria.* In both species the LC_{95} value is much higher than the recommended field dose of cypermethrin for tea loopers which is 500 ppm (Das et al., 2010; Gurusubramanian et al., 2008). Variation in relative toxicity was observed between male and female populations of *Helopeltis theivora* from Jorhat and Darjeeling and from different sub districts of the Dooars (Grursubramanian and Bora, 2008). Populations of *Buzura suppressaria* caterpillars collected from Jorhat also showed similar trend against the tested insecticides due to selection pressure by insecticides (Rahman et al., 2005; Bora et al., 2007a,b). The presence of various oxido-reductase enzymes in the salivary and mid gut along with the basic hydrolyzing enzymes has enabled *H. theivora* to become one of the most destructive pests of tea by attacking the young leaves and growing shoots of tea (Sarker and Mukhopadhyay, 2006a; Saha et al., 2012; Basnet et al., 2015; Basnet et al., 2017). In addition, qualitative and quantitative changes were recorded in the enzymes pattern of the tea mosquito bug [general esterase (Sarker and Mukhopadhyay, 2003), glutathione *S*-transferase and acetylcholinesterase (Sarker and Mukhopadhyay, 2006b)], red spider mite [general esterase (Sarker and

Mukhopadhyay, 2006c)], and looper caterpillar [general esterase (Sarker and Mukhopadhyay, 2007)] indicated a higher tolerance/resistance status due to formation of greater amount of esterases, glutathione *S*-transferase and acetylcholinesterase. One of the main reasons for higher tolerance or resistance by tea mosquito bug and red spider mite to different pesticides was due to mixing of ineffective insecticides with acaricides to combat mixed infestation which, not only decreased the insecticide toxicity but also shifted the level of relative toxicity (Rahman et al., 2005). A comparison of expected effective dose of thirteen insecticides against tea mosquito bug based on their LC_{50} values with recommended dose revealed a pronounced shift in the level of susceptibility of *H. theivora* to all the chosen insecticides except acephate (Gurusubramanian et al., 2008). Study is in progress to estimate the tolerance status of some of the other tea pests which is otherwise neglected. This will give an index of insecticide use pattern and subsequent tolerance development in the pest population which is believed to be an added advantage to IPM strategies to combat pest problem in North Bengal tea plantation.

2.4. INSECTICIDE DETOXIFICATION: ROLE AND MECHANISM DETOXIFYING ENZYMES

Interestingly, only specific types of mutations appear to be associated with specific resistance mechanisms (Table 2.1). The two types of mechanisms that cause high levels of resistance are mainly metabolic resistance and target-site insensitivity. Detoxification of insecticides is an important mechanism for insect pests to tolerate regularly applied insecticide (Soderlund and Bloomquist, 1990; Yu, 1996). Continuous exposure of the pest

Table 2.1: The Mutations associated with enzymes and receptors that result in different types of resistance

Types of Resistance	**Associated Genetic Mutations**		
	Gene Amplification	**Altered Expression**	**Structural Change**
Metabolic			
P450 oxidases	ND[1]	+	ND
Esterases	+	ND	+
GSTs	ND	+	?
Target site insensitivity			
Acetylcholinesterase	ND	ND	+
GABA receptor	ND	ND	+
Sodium channel	ND	?	?
JH receptor	ND	?	?
Other			
Reduced penetration	—	—	—
Behavioral change	—	—	—

[1] **'ND'**: not detected; **'+'**: confirmed or strongly indicated; **'?'**: implied but not confirmed; **'—'**: no data available.

to insecticide makes them more tolerant by enhancing the activity of different defense enzymes. Despite the continuous use of insecticides there are repeated failures in controlling the pest species in recent years (Gurusubramanian et al., 2008; Roy et al., 2008) in different conventional tea plantations of North Bengal. Such failure occurs due to change in the susceptibility pattern of the pest species. Susceptibility level changes due to metabolic detoxification of the insecticides through higher activity of some detoxifying enzymes under stress of different management practices (Jensen, 1998; Maymo et al., 2002; Sarkar and Mukhopadhyay, 2003, 2007). Cytochrome P450-dependent monooxygenases, besides esterases and glutathione *S*-transferases, are the main enzymes involved in insecticide detoxification in arthropods (Matsumura, 1985; Omura, 1999;

Strange et al., 2001). Quantitative and qualitative changes in acetylcholinesterase confer resistance to insecticides (Fournier et al., 1992). Glutathione *S*-transferases (GSTs) in all organisms are known to protect insects from the toxic effects of plant chemicals and pesticides (Yu, 1992; Lagadic et al., 1993; Leszczynski et al., 1994).

Reports of termites resistance is meager, despite the fact that they are chiefly controlled by insecticides. Works on termites are mostly directed towards the phylogenetic, taxonomical and gut microbial field. Unlike other insects typical resistance development theory doesn't fit well with termites (Mahapatro, 2017). In a termite colony, queen lives for about 20-25 years, whereas workers and soldiers usually survive for 2–5 years and alates swarm out of colony to form one of their own. Therefore, in such a complicated caste system it is indeed difficult to assess resistance against xenobiotics. However, recent works on termites do show a development of relative amount of tolerance towards xenobiotic (Osbrink et al., 2001; Osbrink and Lax, 2002, 2003; Valles et al., 1998; Valles and Woodson, 2002). Though their eusocial structure plays major role in the tolerance against toxic and harmful chemicals (DeSouza et al., 2001), yet their physiological adaptability to toxic environment can't be denied (Roisin and Korb, 2011). Mahapatro (2017) also stresses that apart from genetic, phenotypic adaptation to xenobiotic environment can't be ruled out in termites. Resistance in termites is much more complex subject to study as it comes not only from their genotypic or phenotypic plasticity but most importantly because of their eusociality. Nevertheless their involvement in pesticide detoxification is very well evident (Davis et al., 1995). Reports suggest the role of enzymes in detoxification of xenobiotics in termites as similar to the cases in other insects. The activities and characteristics of termite GSTs were investigated in three Australian termite families. Termite GSTs exhibited a broad range of activities toward model substrates (Haritos et al., 1996). In some termite species, like *Mastotermes darwiniensis* and *Coptotermes acinaciformis*, multiple isoenzymes of cytochrome P450s, aldrin epoxidase, cytosolic esterase and microsomal esterases have been found to exhibit upregulated activity on exposure to cypermethrin, chlordane, chlorpyrifos and pyrethroid (Haritos et al., 1994; Valles et al., 2000). In recent times some more enzymes related to xenobiotic detoxification has been discovered in termite enzyme system, for example, an enzyme called Rhodanese has been discovered to be associated with the detoxification of cyanide (Okonji et al., 2010).

The second major resistance mechanism is target-site insensitivity, which refers to an alteration of the molecule(s) that directly interacts with the pesticide to reduce toxicity. Both acetylcholinesterase (AChE) and the gamma-aminobutyric acid (GABA) receptor are known targets of insecticides, and resistant alleles of each have been found (Zhang et al., 2016; Liu, 2015; Heong et al., 2013; Corbel and N'Guessan, 2013). Voltage-gated sodium channels and the juvenile hormone (JH) receptor are putative targets of insecticides. Their direct interaction with insecticides has not been confirmed, but it is clearly evident that they play a key role in the intoxication process (Scott, 1995).

2.5. CONTROL MEASURES AGAINST TERMITES

Termite attack has been a headache for home owners and farmers for centuries. At times controlling termite can be an expensive affair for them. There are many recommended methods of termite pest control, among which synthetic termiticides forms the major portion. Other methods are using of physical barrier, chemical barrier, heating or freezing soil, applying biological control agents, manual eradication of mounds etc. Toxic physical barriers include the use of chemical termiticides in the soil around the structure. Chlorfenapyr (BASF-Phantom) barrier treatment is very effective as it is non-repellent and has delayed toxicity (Rust and Saran, 2006). In Australia, protection of structures from subterranean termites has traditionally relied upon the creation of a zone of poisoned soil under and around the structure to prevent termites gaining access from the ground (Ewart, 2000). Choudhury et al. (2005) reported the use of inorganic insecticides like endosulfan and chlorpyriphos in different concentrations at termite infested areas. Fenvalerate and Phorate sprayed or applied at different concentration around the collar region of tea bushes.

The ambient humidity and temperature in the foraging galleries of subterranean termites are suited for the growth of biological agents. Laboratory studies have consistently demonstrated the pathogenicity of biological agents such as the entomopathogenic nematode *Neoaplectana carpocapsae* Weiser (Fujii, 1975) or the fungi *Metarhizium anisopliae* Sorokin and *Beauveria bassiana* (Balsamo) Vullemin (Lai et al., 1982). Field trials using these biological agents, however, have been generally unsuccessful (Lai, 1977; Mauldin and Beal, 1989; Chouvenc et al., 2011). To date, only Hanel and Watson (1983) were able to trigger *M. anisopliae* epizootic successfully in colonies of an Australian mound-building termite, *Nasutitermes exitiosus* (Hill). Because termites are known to be repelled by pathogenic microbes, the discovery of non-repellent strains of some fungi species may be the key to the successful control of the vast populations of subterranean termite colonies (Milner and Staples, 1996).