CHAPTER 5

RESULTS AND DISCUSSION

5.1. TERMITE ABUNDANCE AND IDENTIFICATION

5.1.1. Termite diversity of North Bengal tea plantations:

Termites from various parts of North Bengal tea plantations were collected for the present work (Figure 5.1). Termites belonging to two families, nine genera and ten species were recorded from different tea plantations of Darjeeling foothills (Table 5.1). Maiti (1983) had recorded 48 termite species from the state of West Bengal out of which 25 species have been reported to exist in Himalayan and Sub-Himalayan Faunal Zone. Das (1965) reported the existence of *Microtermes* sp., *Microtermes pakistanicus* Ahmed and *Microcerotermes* spp. among live wood termites and *Odontotermes assamensis* Holmgren [=*obesus* (Rambur)], *O. parvidens* Holmgren and Holmgren, *O. feae* (Wasmann), *Coptotermes heimi* (Wasmann), *Neotermes buxensis* Roonwal and Sen Sarma and *Capritermes* sp. among scavengers. Poovoli (2016) has reported 15 species of termite occurring in tea ecosystem of Kerala belonging to three families, four sub-families and ten genera. Total diversity of termite fauna so far recorded from tea ecosystem of India is listed in Table 5.1. The species recorded in this study and also from the previous studies, published by various authors (Das, 1965; Maiti, 1983; Chhotani, 1997; Singha et al., 2012, 2014; Biswa and Mukhopadhyay,

Figure 5.1: Soldier castes of termite species (a) *Microtermes obesi,* (b) *Odontotermes obesus*, (c) *Odontotermes parvidens,* (d) *Euhamitermes lighti,* (e) *Synhamitermes quadriceps,* (f) *Pericapritermes assamensis,* (g) *Procapritermes holmgreni,* (h) *Nasutitermes longviewensis*, (i) *Coptotermes heimi* and (j) *Heterotermes indicola*

Table 5.1: Termite pests of tea ecosystem reported from India

Family	Subfamily	Genera	Species
KALOTERMITIDAE	--	*Neotermes*	*N. buxensis* Roonwal and Sen Sarma
			N. greeni (Bugnion and Popoff)
		Postelectrotermes	*P. bhimii* Roonwal and Maiti
			P. militaris (Desneux)
RHINOTERMITIDAE	COPTOTERMITINAE	*Coptotermes*	*C. heimi* (Wasmann)*
			C. ceylonicus Holmgren
	HETEROTERMITINAE	*Heterotermes*	*H. indicola* (Wasmann)**
TERMITIDAE	AMITERMITINAE	*Euhamitermes*	*E. lighti* Snyder**
		Synhamitermes	*S. quadriceps* Wasmann**
		Microcerotermes	*M. beesoni* Snyder
	MACROTERMITINAE	*Macrotermes*	*M. hopini* Roonwal and Sen Sarma
		Odontotermes	*O. assmuthi* Holmgren
			O. ceylonicus (Wasmann)
			O. feae Wasmann
			O. horni Wasmann
			O. obesus (Rambur)*
			*O. parvidens** Holmgren and Holmgren
			O. redmanni (Wasmann)
			O. vaishno Bose
			O. yadevi Thakur
		Hypotermes	*H. obscuriceps* Wasmann
		Microtermes	*M. obesi* Holmgreen*

			M. pakistanicus Ahmed
		Ancistrotermes	*A. pakistanicus* (Ahmed)
	NASUTITERMITINAE	*Nasutitermes*	*N. sp*** Dudely
	APICOTERMITINAE	*Speculitermes*	*S. emersoni* Bose
	TERMITINAE	*Dicuspiditermes*	*D. incola* (Wasmann)
			D. hutsoni (Kemner)
		Labiocapritermes	*L. distortus* (Silvestri)
		Pericapritermes	*P. assamensis* (Mathur and Thapa)**
		Pericapritermes	*P. topslipensis* Thakur
		Procapritermes	*M. holmgreni* Snyder**
		Pseudocapritermes	*P. fletcheri* (Holmgren and Holmgren)
		Rinacapritermes	*R. abundans* Poovoli

**Reported for the first time based on present study; *Referred by earlier author as well as found in present study

(Source: Das, 1965; Maiti, 1983; Chhotani, 1997; Singha et al., 2012, 2014; Biswa and Mukhopadhyay, 2014; Poovoli, 2016)

Table 5.2: Table showing distribution pattern of termite species in different Tea growing regions of Darjeeling Terai and the Dooars

	O. obesus	***O. parvidens***	***M. obesi***	***M. holmgreni***	***S. quadriceps***	***E. lighti***	***P. assamensis***	***N.* sp**	***C. heimi***	***H. indicola***
WesternTerai	++	+	+++	-	+	-	-	-	-	-
EasternTerai	++	+	+++	-	-	+	+	+	+	+
Western Dooars	++	+	++	-	-	-	-	-	-	-
Central Dooars	+++	+	++	+	-	-	-	-	+	-
Eastern Dooars	+++	+	+	-	-	-	+	-	+	-

Symbols "+" and "-" symbolize positive occurrence and negative occurrence respectively of termite genera in different tea plantations

2014; Poovoli, 2016) are compiled here. A total of 34 species have been found to exist in Indian tea ecosystem belonging to three families, eight subfamilies and 21 genera. Rhinotermitidae with only three pest species constituted 8.80%, Kalotermitidae with 11.8% and family – Termitidae constituted a whopping 82.3% of total species count.

Based on available information from published works (Das, 1965; Singha et al., 2012, 2014) and species frequently record (Table 5.2) in the present study it was surmised that two species *viz. Odontotermes obesus* and *Microtermes obesi* have higher occurrence. Both had earlier been reported to be predominantly present in most of the tea plantations of North East as serious pests of tea (Choudhury, 1999; Singha et al. 2012, 2014). Findings of the present study also agree with the previous reports confirming them as the most common and serious pest of tea. Apart from these two, *O. parvidens* and *Coptotermes heimi* were also recognized as pests of tea (Ahmed, 2014), but the relative abundance of these species were negligible in Darjeeling Terai and the Dooars (Table 5.2). Results showed that *Odontotermes obesus* has highest distribution in eastern Dooars, whereas rest of North Bengal has moderate *Odontotermes* existence. Similar pattern of distribution was observed in case of *Microtermes* however, its distribution was more intense in eastern and western part of Terai region. Rest part of Darjeeling Terai and the Dooars had less to moderate distribution of these two species.

5.1.2. Systematic status and diagnostic characters of families Rhinotermitidae and Termitidae:

Present study mainly comprised of representative termite species from two families namely Rhinotermitidae and Termitidae. Diagnosis and keys to the families and subfamilies based on soldier and worker castes are given for these two families.

Keys to the family

Flat pronotum; mandibles without serration at inner margin............. **Rhinotermitidae**

Slight to distinct saddle-shaped pronotum; a faint to distinct serration present at inner margin of mandible ……………………..………............................. **Termitidae**

5.1.2.1. Family – RHINOTERMITIDAE Froggatt

As per the recent taxonomic updates from Krishna et al. (2013) family Rhinotermitidae has six subfamilies, 12 genera, 315 living species and 18 fossil species. Present study came across with two representative species of this family *viz.* *Coptotermes heimi* and *Heterotermes indicola.* Keys to subfamily and the diagnostic features species are given below.

Keys to subfamily:

Head pyriform to sub-circular in shape, with large, rounded fontanelle at base of clypeus; Mandible long, sabre-shaped, broad at base and narrowing distally to a sharply pointed, incurved apex ………………………………………***Coptotermitinae***

Head rectangular, much longer than broad. Mandibles fairly long with almost straight outer margin, faintly incurved at tip; labrum with needle-like hyaline tip ……..…………………………………………………………………***Heterotermitinae***

5.1.2.2. Subfamily Coptotermitinae Holmgren

Genus *Coptotermes* Wasmann

The genus *Coptotermes* has 67 recorded extant species worldwide out of which nine has been recorded from Indian subcontinent (Krishna et al., 2013; Roonwal and

Chhotani, 1989). Out of these nine species to this genus only one was recorded in the present study.

(1) ***Coptotermes heimi* (Wasmann)**

Coptotermes heimi (Wasmann, 1902), Holmgren (1911) in: K. Sv. vet. Akad. Handl, 46(6): 73.

Arrhinotermes heimi Wasmann (1902) in: Jb. (Syst.), 17(1): 104.

Coptotermes parvullus Holmgren (1913) in: J. Bombay nat. Hist. Soc., 22(1) : 102, 104.

Coptotermes travians (Haviland) Bugnion (1910) (Wrong determination) in: Ann. Soc. Ent. Fr., 79: 137.

Diagnostic characters – Soldier: head oval, longer than broad; sides weakly outcurved, converging anteriorly. Fontanelle large, opening with a brown chitinoid border. Antennae with 14-15 segments, 3 shortest. Labrum subtriangular, longer than broad, sides narrowing distally to a hyaline apex, distal tip with one long bristle on either side. Mandibles sabreshaped. Postmentum club-shaped, long, a few bristle like hairs present in anterior part. Pronotum, subreniform, much broader than long, anterior margin broadly convex with a median notch; posterior margin substraight, with a weak median depression (Figure 5.2).

Worker: it has drop shaped head, broader than long. Fontanelle, eyes and ocelli absent. Antennae with 12-15 segments; segment 3 shortest or subequal to either 2 or 4. Clypeus, labrum and mandibles as in imago; Pronotum pilose, much broader than long.

Distribution – This species is a widespread species being reported from all the countries under Indian subcontinent.

Remarks – In present study samples were collected from places like Longview T.E., Lakhipara T.E. and Kalchini T.E. Primarily found to infest shade trees and were collected from dead and rotting logs of trees.

5.1.2.3. Subfamily Heterotermitinae Holmgren

Genus *Heterotermes* Froggatt

The subfamily Heterotermitinae has two genera namely *Reticulitermes* and *Heterotermes*. Genus *Heterotermes* has 30 extant and two fossil species (Krishna et al., 2013) worldwide, and in Indian region five species have been reported (Roonwal and Chhotani, 1989). Out of these five species only one was recorded in present study and has been described as below.

(2) ***Heterotermes indicola*** (Wasmann)

Heterotermes indicola (Wasmann, 1902), Snyder (1933) in: Snyder, Proc. U.S. natnl. Mus., 82(Art. 16): 5.

Leucotermes indicola Wasmann (1902) in: Zool Jb. (Syst.), 17(1): II8-119.

Diagnostic characters – Soldier: rectangular head, much longer than broad. Antennae with 14-16 (generally 15) segments. Mandibles fairly long (0.73-0.93 mm) sabre-shaped, outer margin fairly straight with incurved apices, inner margin also fairly straight upto apices; left mandible with 3- crenulations in basal part of inner margin. Postmentum club-shaped, fairely long. Pronotum flat, subtrapezoidal; anterior margin substraight, medially weakly to distinctly notched. Legs long, pilose, apical tibial spurs 3:2:2, tarsi 4-segmented. Abdomen oblong, moderately pilose; Cerci 2-jointed; styli single-jointed (Figure 5.3).

Worker: trapezoidal, slightly wider than long head, widest at base of antennae. Fontanelle indistinct. Eyes and ocelli absent. Antennae with 14-15 segments. Postclypeus weakly swollen, length half of width. Labrum and mandibles as in genus. Pornotum flat, anterior margin substraight with a distinct median notch, posterior margin substraight to weakly emarginated. Legs and abdomen as in soldier.

Distribution – it is a very common wood-destroying termite in the Indian subcontinent and is found all over the India subcontinent extending up to Afghanistan.

Remarks – This species was sampled from only one tea plantation i.e. Longview T.E. during the study from an infested tea stem. Primarily a structural pest, it has never been reported from tea ecosystem before. Though not widely adapted to tea ecosystem yet can be a potential threat.

5.1.2.4. Family – TERMITIDAE Latreille

According to Krishna et al. (2013) family Termitidae has been recorded to have eight subfamilies, 238 genera, 2072 living species and 34 fossil species. Termitidae has the largest number of species of termites exclusively consisting of individuals without symbiotic flagellate protozoan. Present study came across with three subfamilies and eight representative species belonging to the family Termitidae. Keys to subfamily, genera and species, and the diagnostic features of species are given below.

Keys to subfamily

1. Head produced into a nasute or rostrum; mandibles functional in primitive genera; generally non-functional in others …………………................. **Nasutitermitinae**

Head not produced into rostrum, rather rectangular, squarish or oval; mandibles not degenerate, but well developed with or without teeth in the inner margins 2

2. Mandible either with or without tooth, left mandible with a prominent and right one with a smaller or minute tooth (*Odontotermes*) or with crenulations (*Hypotermes*, *Macrotermes*) .... **Macrotermitinae**

   Mandibles rod-like or twisted; as long as or a little longer than head length and shorter in some genera; fontanelle generally placed below frontal projection ……. ……………………………………………………………………… **Termitinae**

5.1.2.5. Subfamily Macrotermitinae Kemner

As of now, subfamily Macrotermitinae comprised of 12 genera, 373 extant and two fossil species (Krishna et al., 2013). This subfamily exclusively has all the fungus growing termites. Termite representatives of this subfamily are widespread to Africa to South-east Asian countries, but are not found in Australia and New world regions. In present study, two representative species from this subfamily has been recorded which also happen to be one of the most proliferated species in Darjeeling Terai and the Dooars region of West Bengal.

Keys to genera

1. Head oval, densely hairy. Antennae 12-15 segmented. Mandibles thin, delicate, weakly incurved distally. Left mandible either without or with a rudimentary tooth …………………………………………………………………….***Microtermes***

   Head oval to sub-rectangular, converging in front. Antennae 15-18 segmented. Left mandible with a small to prominent tooth ……………………***Odontotermes***

Genus *Microtermes* Wasmann

Genus *Microtermes* is reported to have 66 species which are distributed throughout Ethiopian, Palearctic and Oriental regions. Some of the species under this genus is considered to be serious pest of many crops, plantations and wooden structures. In the present study one species belonging to this genus was recorded.

(3) ***Microtermes obesi*** **Holmgren**

Microtermes obesi Holmgren (1911), in: J. Bombay nat. Hist. Soc., 21(1): 787-788.

Microtermes anandi Holmgren (1913), in: J Bombay nat. Hist. Soc., 22(1): 114.

Microtermes anandi f. curvignathus Holmgren (1911) in: J. Bombay nat. Hist. Soc., 22(1): 114.

Diagnostic characters – Soldier: with oval shaped head, a little longer than wide. 14 segmented antennae. Labrum long, lanceolate, reaching up to about 2/3 of mandibles. Mandibles thin, delicate and weakly incurved apically; a little longer than 1/2 of head-length. Postmentum a little longer than wide and slightly arched; sides weakly convex; Pronotum strongly saddle-shaped (Figure 4.3).

Worker: dimorphic. Major worker with sub-square head. Antennae 13-14 segmented; Postclypeus swollen; length almost half of width. Pronotum saddle-shaped. Minor worker is look like a major worker, except for its smaller size.

Distribution – Widespread throughout India and other parts of Indian subcontinent.

Remarks – This particular species is one of the serious pest of tea plantation and have been sampled from every part of Darjeeling Terai and the Dooars.

Genus *Odontotermes* Holmgren

Genus *Odontotermes* is another most widespread genus after *Microtermes* in the Indian subcontinent ranging from Pakistan, India, Sri Lanka, Nepal and Bhutan. Other than the Oriental region it is also distributed in Palearctic, Ethiopian and Papuan region. Total 199 species has been reported for this genus from these regions among which almost half is found in Oriental and half in Ethiopian region (Chhotani, 1997). Present study recorded two species of this genus for which a key to species and diagnostic characters are given as below.

Keys to species:

1. Tooth of left mandible minute and situated at basal third of mandible ………….
   ………………………………………………………………………… ***O. parvidens***

   Mandibles shorter with weakly incurved outer margin near the basal third. Labrum shorter and broadly rounded anteriorly ……………............................ ***O. obesus***

(4) ***Odontotermes obesus* Rambur**

Odontotermes obesus (Rambur, 1842), Krishna (1965) in: Am. Mus. Novit, No. 2210: 24-25.

Termes obesus Rambur (1842) in: Histoire nat. Insectes, Névroptéres: 304.

Termes obesus (Cyclotermes) orissae Snyder (1934) in: Indian For. Rec., 20(11): 10-11.

Diagnostic characters – Soldier**:** oval head, gradually converging to anterior. Antennae 16-17 segmented. Labrum tongue-shaped, with broadly rounded anterior margin. Mandibles long, slender, sabre-shaped. Left mandible with a sharp, prominent tooth at distal one third. Right mandible with a minute tooth a little below level of tooth on left mandible. Postmentum subrectangular. Pronotum saddle-shaped, anterior lobe semicircular.

Worker: dimorphic. Major with sub-square head, widest near antennae. Antennae 17-18 segmented. Postclypeus weakly swollen, length less than half its width. Pronotum strongly saddle shaped. Minor worker smaller than major worker with 16-17 segmented antennae (Figure 4.4).

Distribution – Widely distributed species and present in every countries of Indian subcontinent.

Remarks – *Odontotermes obesus* found to be one of the most serious termite pest in this region with abundance in every plantations where collections were made. Collected either directly from mounds in between tea bushes or from affected and dying tea plants.

(5) ***Odontotermes parvidens***

Odontotermes/*O*. (*Odontotermes*) *parvidens* Holmgren and Holmgren (1917) in: Mem. Dept. agric. Sci., 5(3): 154.

Termes (*Cyclotermes*)/*Odontotermes almorensis* Snyder (1933) in: Proc. U. S. natn. Mus., 84: 8-9.

Odontotermes distans Holmgren and Holmgren (Part) Thakur (1981) in: Indian For. Ree. (N.S.) Ent., 14 (2): 44-50.

Odontotermes microdens Holmgren (In Silvestri) Nom. Nude, Silvestri (1914) in: Rec. Indian Mus., 8(5): 428.

Diagnostic characters – Soldier: large head slightly rectangular to oval in shape, converging anteriorly. Antennae with 16-17 segments. Labrum tongue-like triangular shaped, with pointed tip. Mandibles strong, sized half or little more of the size of head with slightly incurved tips. Left mandible with a minute inner marginal tooth. Right mandible without or with a minute denticle near base. Postmentum subrectangular. Pronotum saddle-shaped [Figure 5.1(c)].

Workers: dimorphic; Major worker with almost square head. Antennae 17-19 segmented. Postclypeus weakly swollen; length less than half the width. Pronotum saddle-shaped. Minor worker, like major worker except for smaller size.

Distribution – In India it is distributed towards northern to north-eastern part, following the Himalayan belt. Also found in Bangladesh, Bhutan and Pakistan.

Remarks – This species has been widely reported from entire Himalayan region. During present work samples were collected from most of the tea plantations of Darjeeling Terai and the Dooars. Normally found making mud galleries over tea trunk.

5.1.2.6. Subfamily Termitinae

Subfamily Termitinae was erected by Latreille in 1802 as Termitines. At present this family comprises of 61 genera, 637 extant and 6 fossil species. Representatives of this subfamily are widespread to all the zoogeographic regions. In present study four species was recorded under this subfamily, hence, keys to genera and diagnosis for each species are given so forth.

Keys to the genera

1. Mandibles snapping type, slightly to strongly asymmetrical, rod-like, without a prominent tooth, little longer than head, shorter in some genera; Fontanelle generally placed below frontal projection 2

Mandibles not snapping type, symmetrical, sabre-shaped or hooked or curved at tips generally shorter than head, each with a prominent tooth or serration; Fontanelle open and distinct or indistinct ……………......................3

2. Head sub-rectangular, sparsely hairy; without frontal projection. Mandibles asymmetrical. Left strongly twisted at middle, without a point at tip. Right mandible blade-like, tip pointed and weakly outcurved apically .. ***Pericapritermes***

Head sub-rectangular, fairly hairy; median suture partly distinct. Antennae 14 segmented. Mandibles slightly asymmetrical, long, rod-like, weakly incurved at tip, not bent like a beak…..................... ***Procapriterrnes*** (=***Malaysiocapritermes*****)**

3. Smaller species, head sub-rectangular. Antennae 14 segmented. Mandibles short, sickle-shaped, thick, stout, very broad basally; each mandible with a small tooth lying in middle third …..***Euhamitermes***

Head sub-squarish, a little longer than wide. Antennae 13 segmented. Mandibles short, stout, strongly incurved near middle, and apical half of mandibles weakly incurved, inner margin not serrated but with a prominent triangular tooth at middle …..***Synhamitermes***

Genus *Pericapritermes* Silvestri

(6) ***Pericapritermes assamensis* (Mathur and Thapa)**

Pericapritermes assamensis (Mathur and Thapa, 1965), Krishna (1968) in: Bull. Am. Mus. nat. Hist., 138 (Art. 5): 293.

Capritermes assamensis Mathur and Thapa (1965) in: Bull. Ent., No.6: 10-12.

Pericapritermes ceylonicus (wrong determination), Maiti (1983) in: Occ. Pap. Rec. Zool. Surv. India, No. 42: 24, 87-89.

Diagnostic characters – Soldier: sub-rectangular head, sides slightly convex; in profile frons sloping in front and shallowly depressed medially; median suture present, not extending upto fontanelle. Fontanelle minute, circular; situated anteriorly. Antennae 14-segmented. Mandibles strongly asymmetrical; a little longer than half the length of head; left mandible strongly twisted at middle, without a point at tip; right blade-like, tip pointed and weakly out-curved apically. Postmentum long, club-shaped. Pronotum saddle-shaped (Figure 5.4).

Worker: with semi circular head. Fontanelle plate oval, whitish spot. Antennae 14-segmented. Postclypeus swollen, hairy. Labrum dome-shaped, hairy. Pronotum saddle-shaped.

Distribution – India: Assam, Sikkim and West Bengal.

Remarks – This species was collected from two tea estates namely Longview and Kalchini. In both tea estates *P. assamensis* were found to attack tea bushes and were collected from dying tea trunks. This species though commonly found in this part of country, yet was not reported earlier from tea plantations. This is the first time it is found to be associated with tea ecosystem.

Genus *Procapritermes* Ahmad and Akhtar

(7) ***Procapritermes holmgreni* (Akhtar)**

Procapritermes holmgreni (Akhtar, 1975) (102. transferred back to *Procapritermes*) Gathorne-Hardy (2004) in: Sarawak Mus. J. 60: 89–133.

Malaysiocapritermes holmgreni Akhtar (1975) in: Bull. Dept. zool. Univ. Punjab. (N.S.), Art 7: 179-183, 194.

Procapritermes holmgreni (Akhtar), Ahmad and Akhtar (1981) in: Pakistan J. Zool., 13(1-2): 10.

Diagnostic characters – Soldier: rectangular shaped head, weakly convex sides; longer than wide; frons sloping sharply towards anterior. Fontanelle transverse; situated anteriorly a little below base of slope of frons. Antennae with 14 segments. Labrum sub-rectangular. Mandibles weakly asymmetrical and pointed at tips; longer than head-length without mandibles. Postmentum short, club-like (Figure 5.5).

Worker with sub-circular, broader than long head. Fontanelle plate small, oval, translucent spot present in middle. Postclypeus hairy, swollen. Antennae 14 segmented. Mandibles each with an apical and two marginal teeth; apical teeth long finger like. Pronotum narrower than head; anterior margin slightly raised and with a weak median notch.

Distribution – India: Meghalaya and Arunachal Pradesh; Bangladesh.

Remarks – This species was collected from Grassmore T.E. These seem to be soil and humus feeder and were collected from surrounding soil of tea plantation. This is among the few rarely reported species, first ever record from West Bengal.

Genus *Euhamitermes* Holmgren

(8) ***Euhamitermes lighti* Snyder**

Euhamitermes lighti Snyder (1949) in: Smiths. misc. Colls., 112: 112.

Amitermes (*Euhamitermes*) *lighti* Snyder (1933) in: Proc. biol. Soc., 46: 93.

Diagnostic characters – Soldier: sub-rectangular head. Antennae 14 segmented. Postclypeus elliptical; anterior margin straight. Labrum broad, tongue shaped. Mandibles thick, stout, very broad basally; each with a small tooth at middle third of inner margin. Postmentum club-shaped. Pronotum strongly saddle-shaped (Figure 5.6).

Worker: sub-circular head, wider than long; Fontanelle plate indistinct. Antennae 14 segmented. Postclypeus pilose, swollen. Labrum broad, tongue shaped. Pronotum saddle-shaped.

Distribution – India: Uttar Pradesh: Dehra Dun.

Remarks – This species was collected from a hollowed root of tea bush at Ambootia T.E. near Kurseong. Present study is the first ever record of *E. lighti* from West Bengal.

Genus *Synhamitermes* Holmgren

(9) ***Synhamitermes quadriceps* (Wasmann)**

Synhamitermes quadriceps (Wasmann, 1902), Snyder (1949) in: Smiths. misc. Colis., 112; 129.

Amitermes quadriceps Wasmann (1902) in: Zool. Jb. Syst., 17(1): 123.

Hamitermes (*Synhamitermes*) *quadriceps* (Wasmann), Holmgren (1912) in: K. Svenska Vetensk.-Akad. Handl., 48 : 91.

Synhamitermes quadriceps (Wasmann), Snyder (1949) in: Smiths. misc. Colis., 112 ; 129.

Diagnostic characters – Soldier: sub-square head, a little longer than wide. Antennae 13 segmented. Postclypeus semicircular. Labrum broadly tongue-shaped. Mandibles short, stout, outer margin incurved medially; each with a triangular tooth on inner margin at about middle. Postmentum short, club-shaped. Pronotum saddle-shaped (Figure 5.7).

Worker: sub-square head having weakly rounded sides and posterior margin. Antennae 13 segmented. Postclypeus swollen, length less than half of width. Labrum sub-square. Pronotum saddle-shaped.

Distribution – India: Maharashtra, Goa, Daman, Rajasthan, Madhya Pradesh, West Bengal, Assam, Tripura and Kerala. Elsewhere - Bangladesh.

Remarks – This species was collected from soil at Atal T.E. The collection spot had cleared of tea bushes and was going through preparation for re-plantation. This species had never been reported from tea ecosystem before hence, it's a first report of this species being recorded in tea plantation.

5.1.2.7. Subfamily Nasutitermitinae

***Nasutitermes longviewensis* sp. nov.**

Diagnostic characters – Soldiers: head with pyriform to cylindrical rostrum tapering at tip, with spars long hairs and a few long hairs. Body sparsly to moderately pilosed. Head reddish brown in color, body creamish yellow to brownish in color. Antennae

13 segmented. Mandibles vestigial. Pronotum small, saddle-shaped, posterior margin strongly curved inward. Abdomen elongated (Figure 5.8). Nasute 0.4-0.5 mm long almost half of the length of head without nasus.

Worker: monomorphic. Head sub-square to broad and oval; epicranial suture distinct. Fontanelle plate elongated, narrow, translucent. Antennae 13-15 segmented. Postclypeus weakly swollen, length less than 1/2 of width. Pronotum and legs as in soldier. Abdomen elongate, hairy; cerci 2-jointed; styli absent.

Distribution – *Nasutitermes* is a cosmo-tropical genus and is known from the entire world except the Palaearctic region. In the Indian subcontinent, it is very widely distributed all over India (except north-western parts), Bhutan, Bangladesh, Sri Lanka and Burma.

Remarks – Samples of this genus was collected from Longview T.E. from a shade tree. It has made a nest inside the tree. Entrance to nest was through small openings made on the surface of tree trunk. From this part of West Bengal about three species of *Nasutitermes* has been reported earlier namely *N. jalpaiguriensis, N. thanensis* and *N. garoensis.* However, *N. longviewensis* sp. nov. seem to differ from them in characters like total body-length is about 3.5-4.0 mm. Length of nasute almost equal to the half of head length at base of mandibles nasute 0.4-05 mm long. head without rostrum 0.9-1.0, width 0.8-1.0 mm).

5.1.3. Discussion:

Present study recorded ten species of termites in tea ecosystem of Darjeeling Terai and the Dooars. An intensive and exclusive taxonomically oriented survey may further add to the total numbers of termite species from the tea plantations of these regions. In past few species like *Odontotermes obesus*, *O. parvidens*, *Microtermes obesi*, and *Coptotermes heimi* had already been reported to exist in tea ecosystem as common pest species (Das, 1965; Choudhury,1999; Singha et al., 2012, 2014; Biswa and Mukhopadhyay, 2014). *O. obesus* and *M. obesi* were among the most prevalent pest species. In fact, the abundance study also reciprocated the similar findings for this region where they were found to infest most of the tea plantations (Table 5.2). Rests of the species have never been reported from tea plantations of Darjeeling Terai and the Dooars. In fact one new species and five other species are being reported for the first time from tea plantations of North Bengal.

H. indicola is a well known pest of wooden structures and buildings (Biswa and Mukhopadhyay, 2010; Mahapatro and Kumar, 2013), however, had never been reported from tea environment before. Similarly other species of termites namely *Heterotermes indicola*, *Synhamitermes quadriceps* and *Pericapritermes assamensis* are some species which were previously been reported from this part of West Bengal but never from tea ecosystem as such. Based on present study they are being reported for the first time from tea ecosystem. *Procapritermes holmgreni* and *Euhamitermes lighti* had never been recorded in this part of country and are being reported for the first time from the state West Bengal.

The only survey report available on termites of tea ecosystem from North Bengal is by Das (1965). Although, there had been reports on termites fauna of West Bengal by Maiti (1983) and termites fauna of Himalaya by Mukherjee (2002) yet no substantial documentation is available on tea termites from Darjeeling foothills. After Das (1965) not much has been added to tea entomology of this area. In fact leading tea research agencies still rely on the conventional data available on termites (TRA, 2015; UPASI TRF, 2017). Reasons may be that termite pest does not apparently pose a serious threat to the tea plantations except for few places like Barak valley, Darang and Cachar districts of Assam and Darjeeling Terai where they have been recognised as serious pest (Choudhury, 1999; Choudhury et al, 2005; Singha et al., 2012, Biswa and Mukhopadhyay, 2014). There have been plenty of reports of termite pest instances in other crops like Coconut (Mariau et al., 1992; Tang et al., 2006; Mahapatro and Kumar, 2015), Eucalyptus (Constantino, 2002; Andersen et al., 2005; Werner et al., 2008), Coffee (Neves and Alves, 1999; Bhavana et al., 2015), Cocoa (Moura et al., 1998; Fernandes et al., 1998; Cruvinel et al., 1998), Wheat (Kranz et al., 1981; Cowie and Wood, 1989; Hashimi et al., 1983), Maize (Mill, 1992; Berti-Filho, 1993; Figueira et al., 1998; Fernandes and Alves, 1992), Rice (Sands, 1973; Czepak et al., 1993; Mill, 1992; Dario and Villela-Filho, 1998) and many more. However, there is still much work to be done on termite pests of tea ecosystem from this region. Therefore, this survey data would enable one to have a comprehensive idea and a comparative status of termite species in tea plantations of Darjeeling foothills, thereby adding to the knowledge to the total diversity of termites of West Bengal.

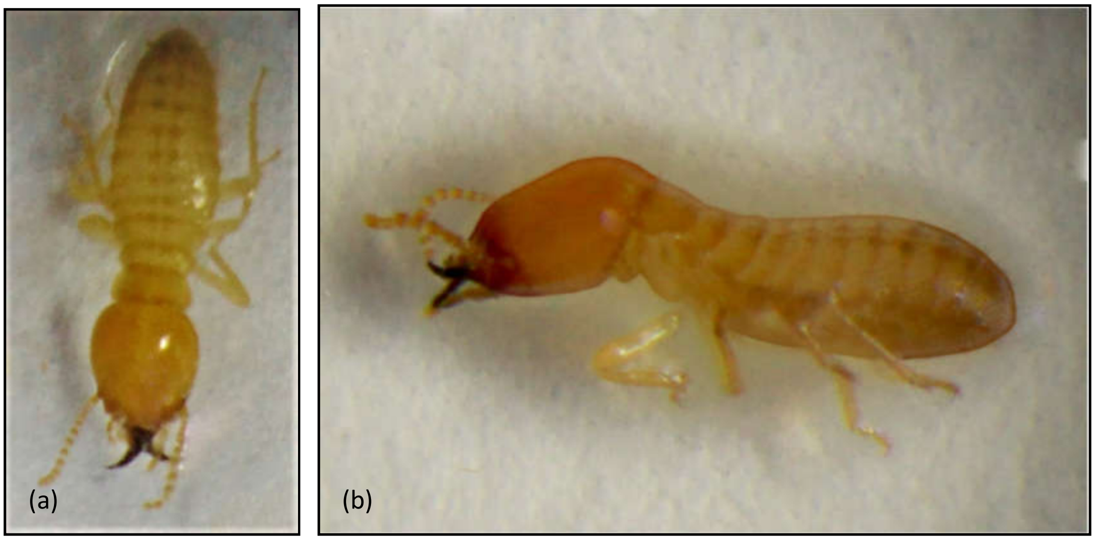


Figure 5.2: Soldier castes of *Coptotermes heimi* (a) planar view from top, (b) side view

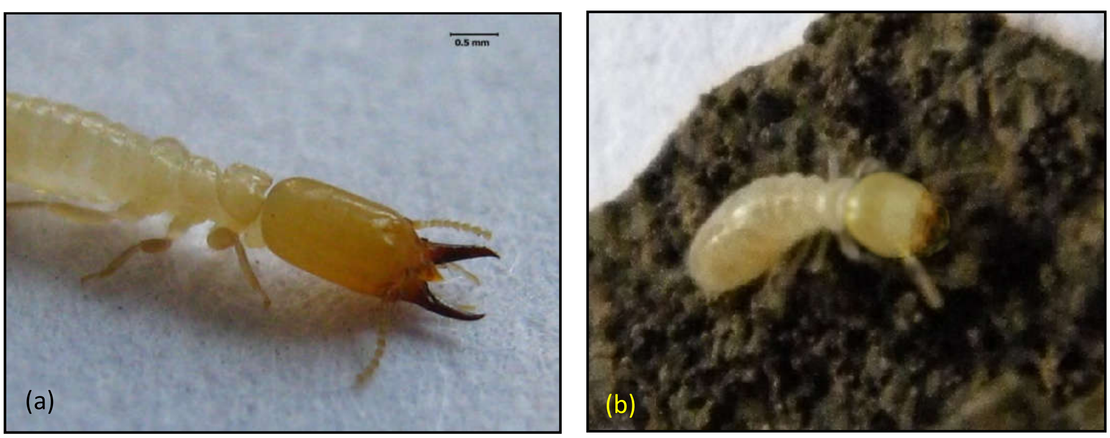


Figure 5.3: Termite castes of *Heterotermes indicola* (a) soldier and (b) worker

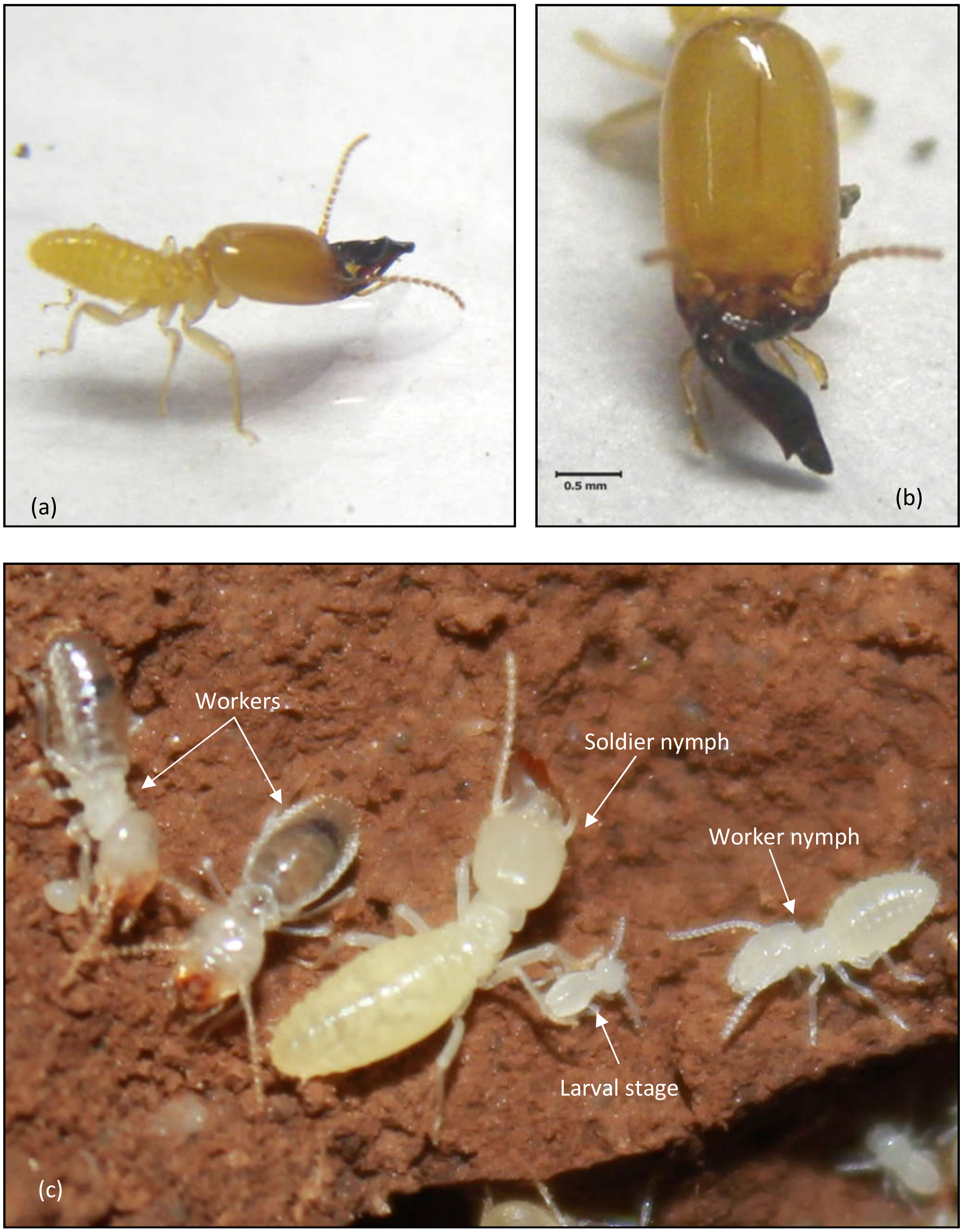


Figure 5.4: (a) Soldier caste of *Pericapritermes assamensis*. (b) The enlarged view of the soldier mandibles depicting the typical uneven shape, and (c) worker castes and other nymphal stages

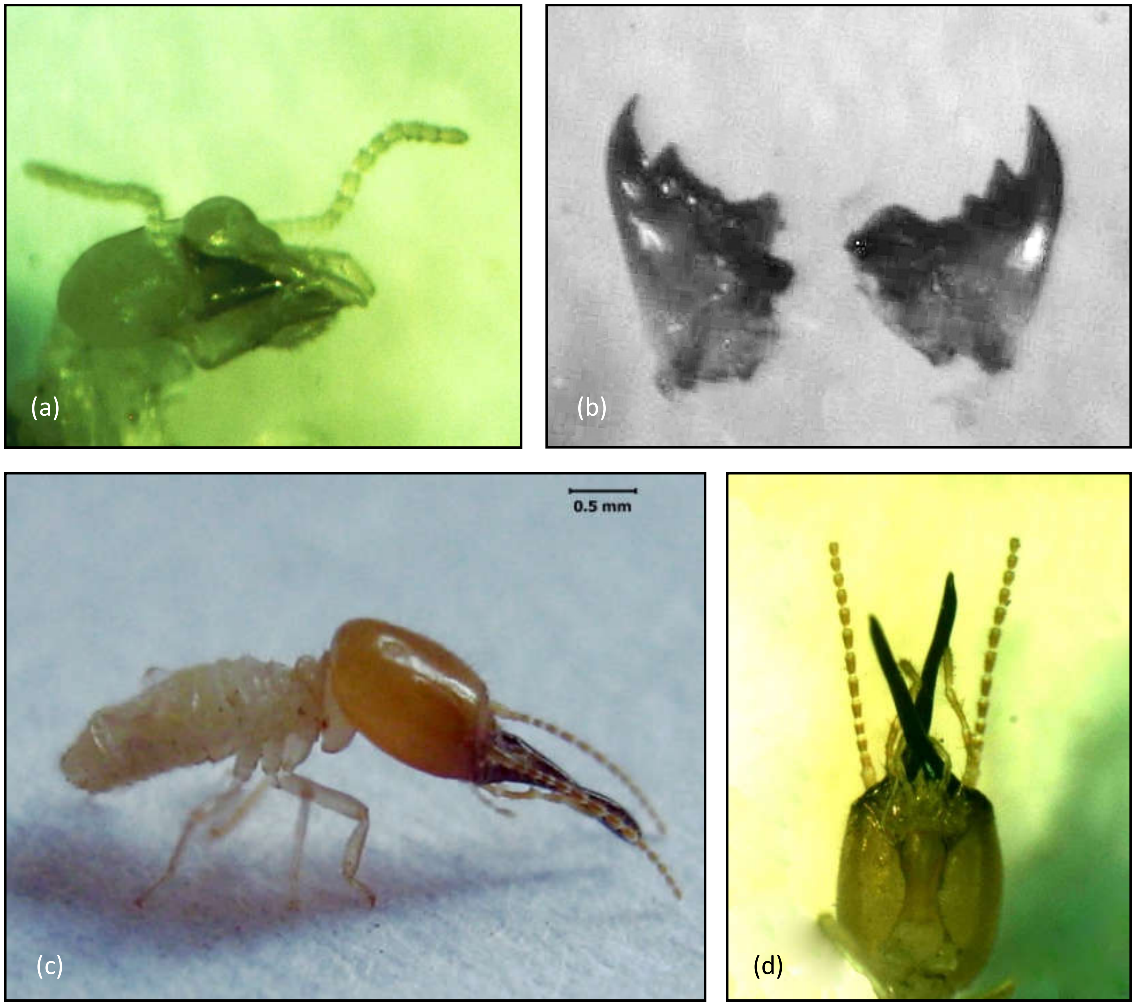


Figure 5.5: *Procapritermes holmgreni* (a) worker caste, (b) worker mandibles, (c) soldier caste and (d) soldier mandibles and antennae

Figure 5.6: (a) Soldier castes of *Euhamitermes lighti*, (b) The enlarged view showing underside of soldier mandibles clearly shows the marginal dentitions and curved beak like tips

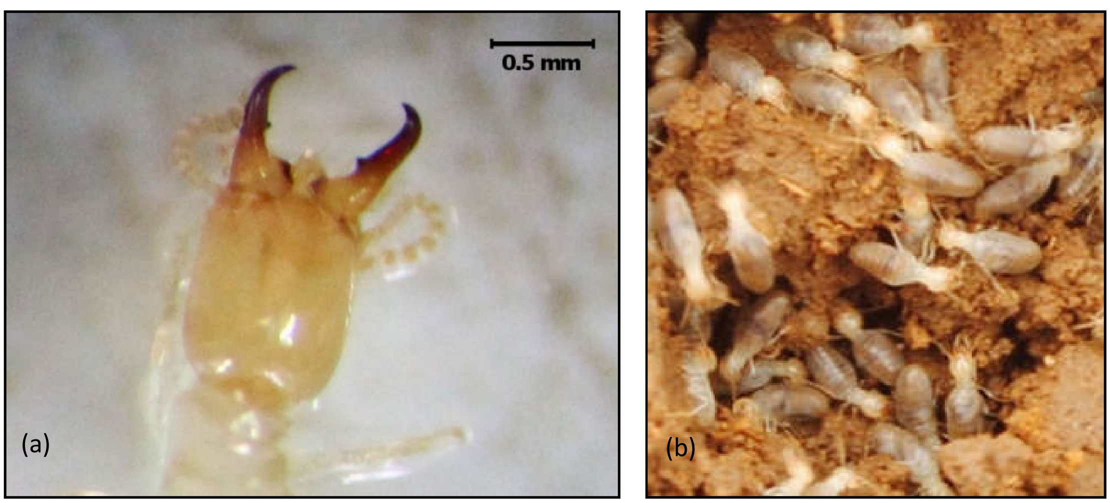


Figure 5.7: Castes of *Synhamitermes quadriceps* (a) Soldier and (b) worker

Figure 5.8: *Nasutitermes longviewensis* (c) soldier and (d) worker

5.2. TAXONOMY AND PHYLOGENETIC STUDY OF TERMITES

5.2.1. Morphometric analysis and Phenogram construction:

Twenty five morphological characters and character states were chosen (Table 4.2) based on the works of Donovan et al. (2000) and Engel et al. (2009). Characters which seemed homologous for both worker and soldiers were scored separately as they are believed to represent separate apomorphies (Donovan et al., 2000). The study involved two sets of data, i.e. for worker and soldier from all the representative termite species identified from tea plantations under present study. Data was processed using *PHYLIP v.3.695* to construct the dendogram. The dendogram obtained showed three primary clusters. In cluster one closest of relation was exhibited by *O. parvidens* and *P. holmgreni*, whereas *N. longviewensis* is distantly related to them (Figure 5.9). Second cluster also comprised of three species namely *P. assamensis, S. quadriceps* and *H. indicola* where first two species shared close similarity, whereas *H. indicola* exhibited distant relationship with *P. assamensis* and *S. quadriceps*. The species of third cluster *C. heimi, O. obesus and M. obesi* shared the close relationship. Among themselves *E. lighti* appeared to be the most distantly related species.

5.2.2. Random Amplified Polymorphic DNA (RAPD) based genetic diversity analysis:

In the present study PCR based RAPD marker system was used to probe the preliminary relationships among 10 termite species collected from the tea plantations of Darjeeling Terai and the Dooars. RAPD primers amplify random segments of genomic DNA by binding at unknown regions. Owing to this random binding of primers no prior

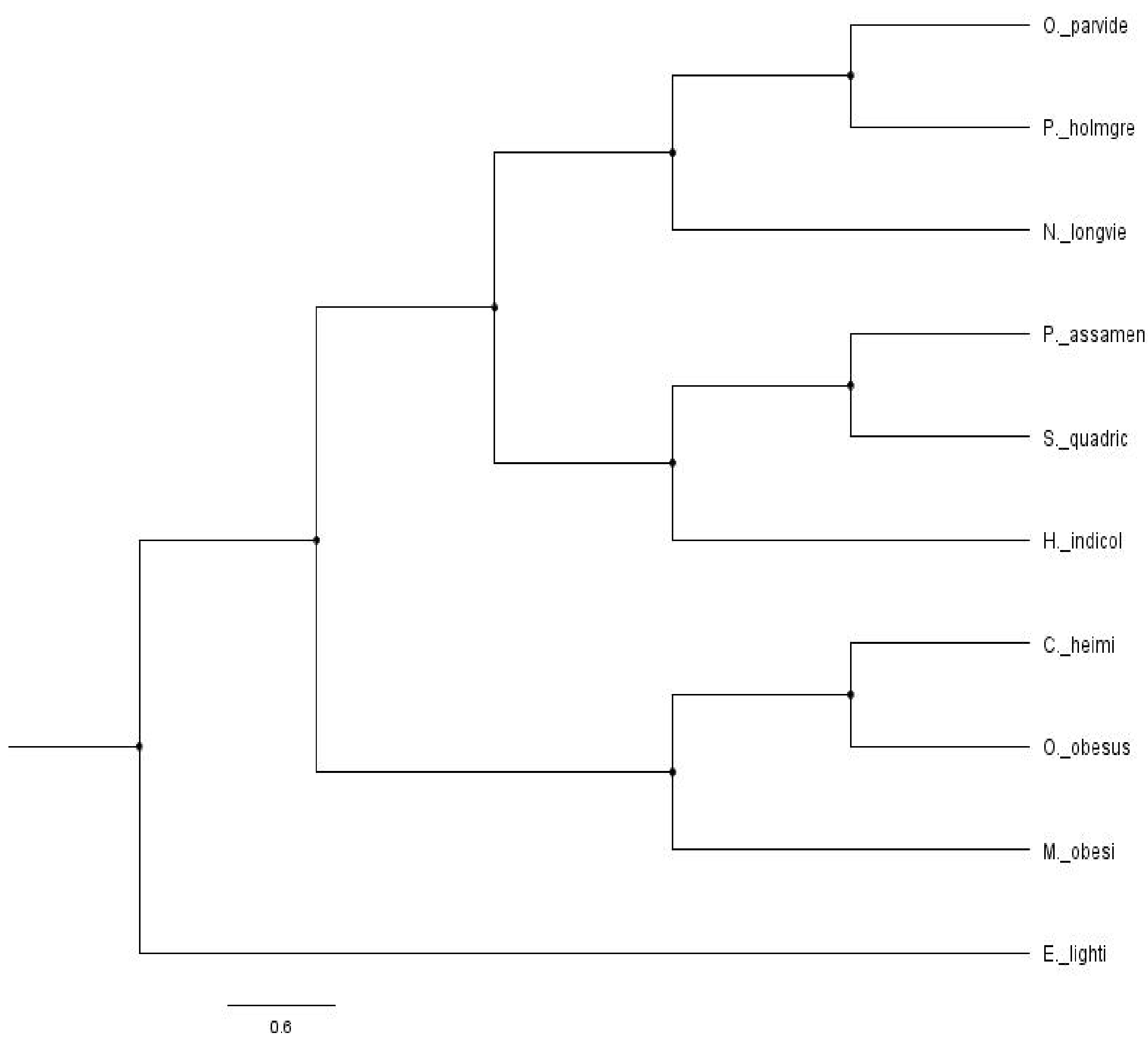


Figure 5.9: Genetic relatedness analysis among the termite species obtained on the basis of Morphometric characters and character state scoring patterns

knowledge of the sequence for the target DNA is necessary which makes this method popular for comparing the DNA of organisms (Prasad and Sethi, 2014).

5.2.3. RAPD based diversity analysis:

Genetic relatedness among the 10 selected termite species namely *O. obesus*, *O. parvidens*, *S. quadriceps*, *E. lighti*, *P. holmgreni*, *P. assamensis*, *N. longviewensis*, *H. indicola* and *C. heimi* were assessed using random decamer primers. Initially for optimization of DNA concentrations, 5 ng, 10 ng, 15 ng and 20 ng per 25 µl reaction mixture were used. Among these tested concentrations, 20 ng /25 µl reaction mixtures produced the best amplification. Lower or higher concentrations either reduced amplification of produced smearing, respectively. Therefore, in subsequent experiments 20 ng DNA per reaction was used in all PCR amplification (Figure 5.9).

Altogether 25 random decamer oligonucleotide sequences (primers) having 10 bp each were initially screened for determining polymorphism among the termite species to generate reproducible polymorphic bands out of which two (OPA-1 and OPA-2) showed good amplification. All amplified products with OPA-1 and OPA-2 primers showed polymorphic and distinguishable banding patterns. RAPD profiles showed that primer OPA-2 scored highest number of polymorphic bands which ranged between 200 bp to 2000 bp (Figure 5.10 A and B).

5.2.4. Analysis of polymorphism:

The size of the bands in both the cases varied from 200 to 2000 bp. However, both the primers differed in their capacity to detect polymorphism. Highest level of polymorphism was recorded in primer OPA-2 (51 %) followed by OPA-1 (38%) (Table 5.3).

Table 5.3: Determination of polymorphisms based on the RAPD banding patterns

Primers	Total No. of RAPD products	Total No. of polymorphic bands	% Polymorphism
OPA-1	36	14	38
OPA-2	31	16	51

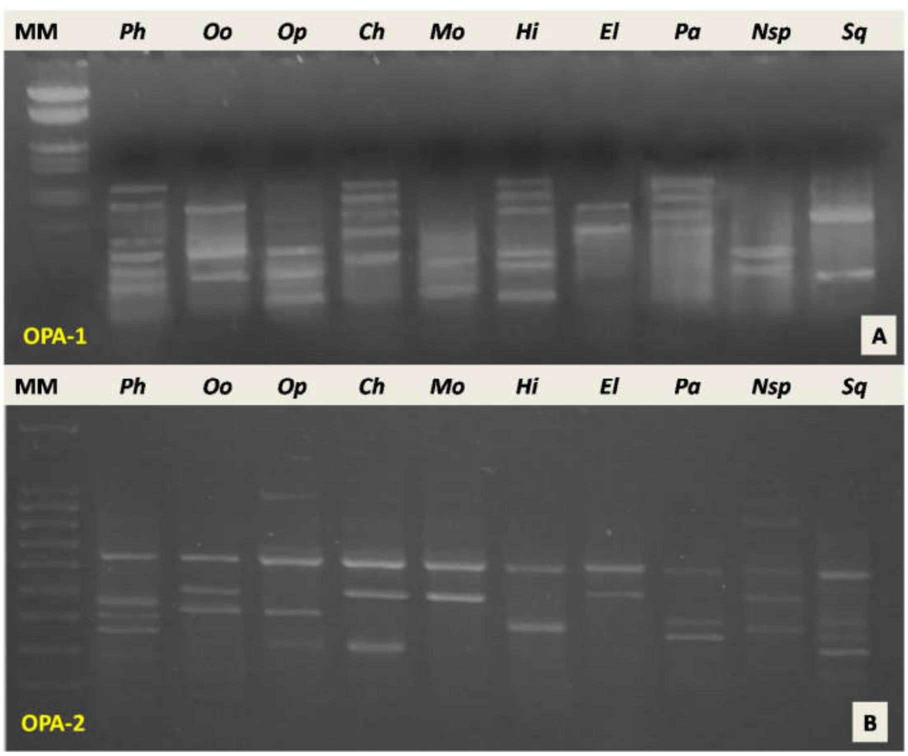
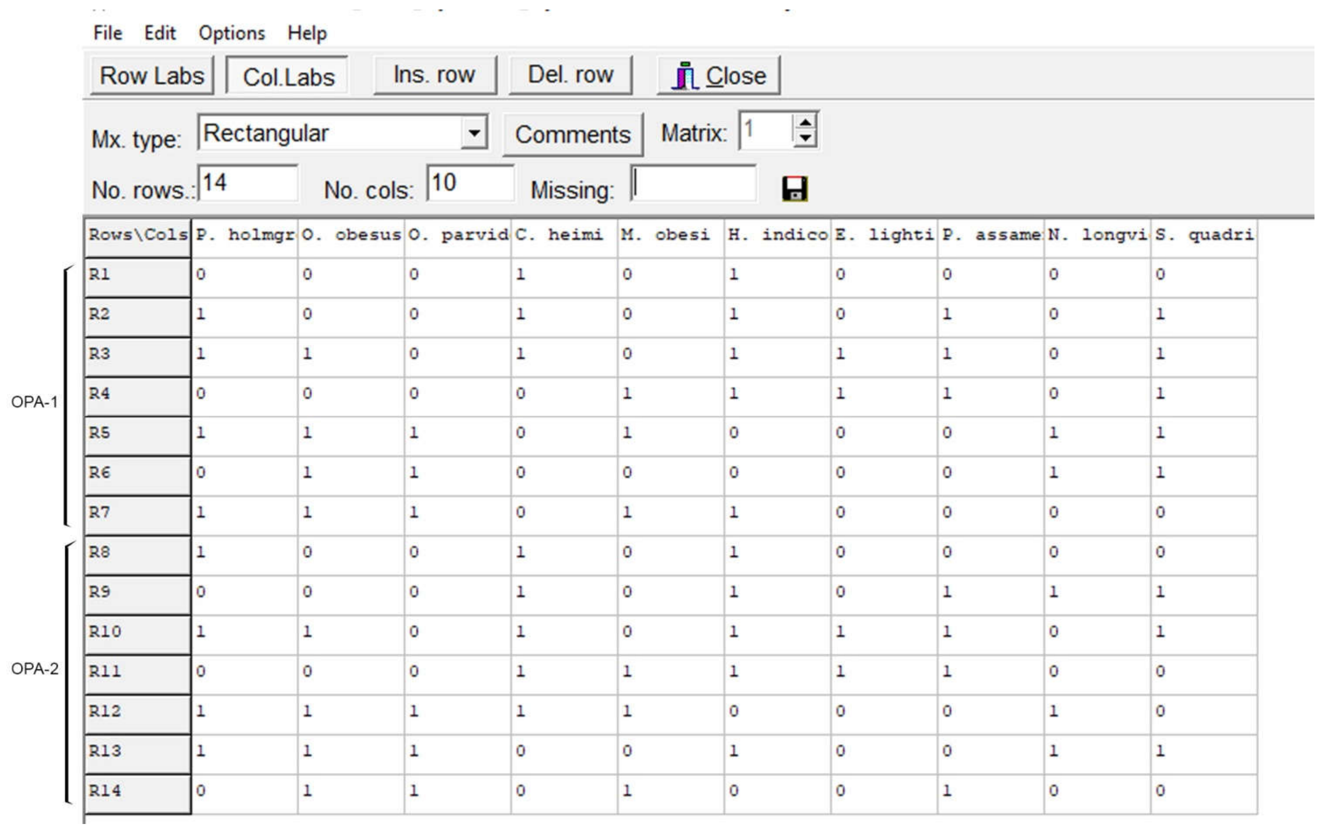


Figure 5.10: RAPD amplified products in different termite species. Lane-1, DNA wide range molecular marker.2 *P. holmgreni*, 3 *O. obesus*, 4 *O. parvidens*, 5 *C. heimi*, 6 *M. obesi*, 7 *H. indicola*, 8 *E. lighti*, 9 *P. assamensis*, 10 *N. longviewensis*, 11. *S. quadriceps*, obtained with the primers OPA-1 (A) and OPA-2 (B)



Rows\Cols	P. holmgr	O. obesus	O. parvid	C. heimi	M. obesi	H. indico	E. lighti	P. assame	N. longvi	S. quadri
R1	0	0	0	1	0	1	0	0	0	0
R2	1	0	0	1	0	1	0	1	0	1
R3	1	1	0	1	0	1	1	1	0	1
R4	0	0	0	0	1	1	1	1	0	1
R5	1	1	1	0	1	0	0	0	1	1
R6	0	1	1	0	0	0	0	0	1	1
R7	1	1	1	0	1	1	0	0	0	0
R8	1	0	0	1	0	1	0	0	0	0
R9	0	0	0	1	0	1	0	1	1	1
R10	1	1	0	1	0	1	1	1	0	1
R11	0	0	0	1	1	1	1	1	0	0
R12	1	1	1	1	1	0	0	0	1	0
R13	1	1	1	0	0	1	0	0	1	1
R14	0	1	1	0	1	0	0	1	0	0

Figure 5.11: A screenshot of data matrix sheet showing scores for different bands amplified out of two different primers OPA-1 and OPA-2 for different species of termites

5.2.5. Analysis of genetic similarity values:

Bands were scored by putting 1 (presence) and 0 (absence) at the homologous sites for both the primers for all the species to prepare a data matrix sheet (Figure 5.11). The analysis of similarity co-efficient was performed using NTSYS-PC software. Results of pairwise comparison showed the highest degree of similarity between *O. obesus* and *O. parvidens* (0.85714), whereas the lowest similarity was observed between *C. heimi* and *O. parvidens*, and *O. parvidens* and *S. quadriceps* with similarly coefficient of

Table 5.4: Genetic similarity matrix, obtained as the result of Simqual analysis of polymorphism obtained with OPA-1 and OPA 2 for RAPD

	Ph	***Oo***	***Op***	***Ch***	***Mo***	***Sq***	***El***	***Pa***	***Nl***	***Hi***
Ph	1.00000									
Oo	0.71428	1.00000								
Op	0.57142	0.85714	1.00000							
Ch	0.57142	0.28571	0.14285	1.00000						
Mo	0.42857	0.57142	0.71428	0.28571	1.00000					
Sq	0.57142	0.28571	0.14285	0.71428	0.28571	1.00000				
El	0.42857	0.42857	0.28571	0.57142	0.57142	0.57142	1.00000			
Pa	0.35714	0.35714	0.21428	0.64285	0.50000	0.64285	0.78571	1.00000		
Nl	0.50000	0.64285	0.78571	0.35714	0.50000	0.21428	0.35714	0.28571	1.00000	
Hi	0.57142	0.57142	0.42857	0.42857	0.28571	0.57142	0.57142	0.64285	0.64285	1.00000
" SIMQUAL; coeff=J; " by Cols, += 1.00000, = 0.00000: 3 10L 10 0										

0.14285 for both the comparisons. The overall result of the data based on 1/0 matrix showed that the similarity coefficient among all the termite species ranged from 0.14285 – 0.85714 suggesting considerable degree of genetic dissimilarity among all the tested termite species (Table 5.4).

5.2.6. Dendrogram Construction:

A dendrogram was constructed on the basis of shared fragments (Table 5.3) and the Similarity coefficients (Table 5.4). Due to the lack of an out group the dendogram is an unrooted tree. The presented dendrogram (Figure 5.12) shows broadly two major clusters (I and II) with coefficient value ranging from 0.38 to 0.86. The cluster I with coefficient values 0.55 to 0.86 is comprised of the termite species *P. holmgreni*, *O. obesus*, *O. parvidens*, *N. longviewensis* and *M. obesi* where *O. obesus* and *O. parvidens* appeared closest and were embedded in the same node. Both the species (*O. obesus* and

O. parvidens) are deeply joined with *N. longviewensis*. However, *P. holmgreni* and *M. obesi* appeared to be more distantly related to *O. obesus*, *O. parvidens* and *N. longviewensis*. The cluster II (coefficient ranging from 0.58 – 0.86) consists of *C. heimi*, *H. indicola*, *E. lighti*, *P. assamensis*, *S. quadriceps*. *E. lighti*, *P. assamensis*. *C. heimi* and *H. indicola* in cluster II showed highest relatedness between them. Both the clusters I and II are deeply joined with each other at coefficient 0.38.

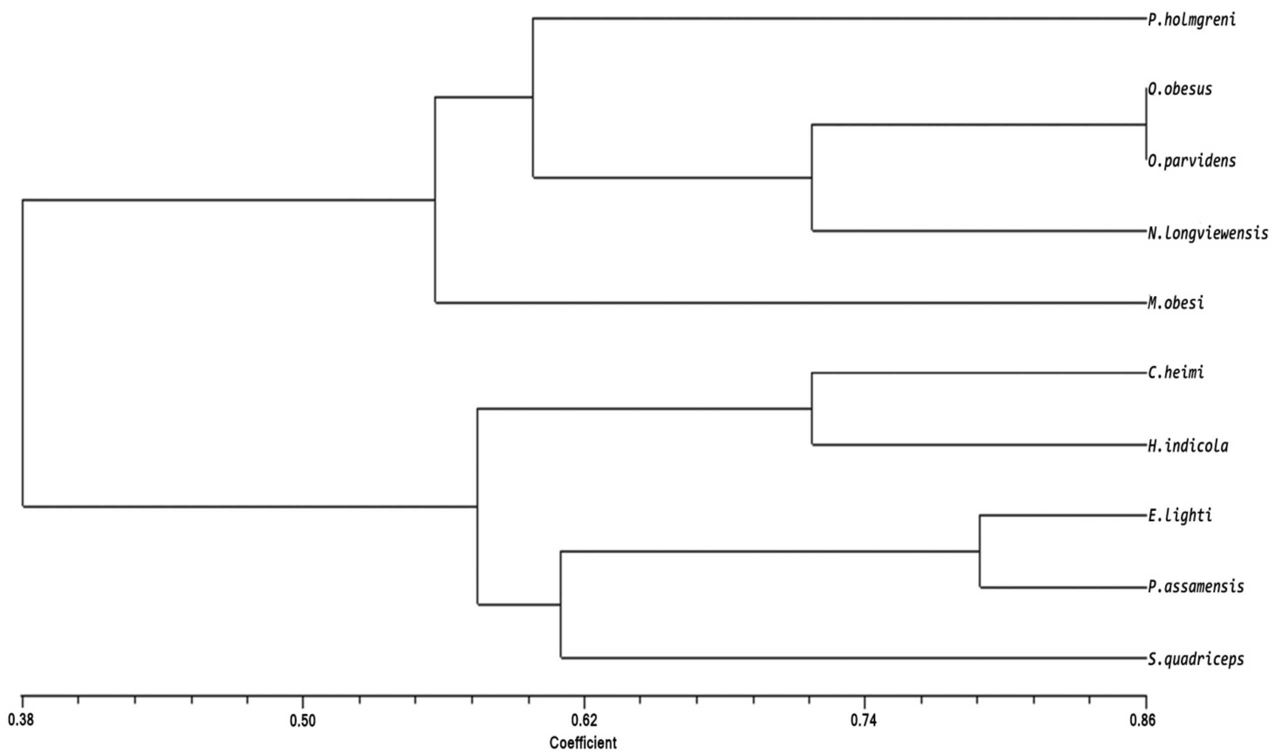


Figure 5.12: Genetic relatedness analysis among the different termite species obtained on the basis of RAPD banding patterns obtaned with the primer OPA-1 and OPA- 2. Dendogram showing all the termite species in different clades, similarity cofficient calculated on the basis of presence or absence of bands using NTSYS- PC software

The topology of the tentative phylogenetic relationship revealed by the RAPD analysis (Figure 5.12) is partially concurrent with the dendogram based on morphometrics (Figure 5.9), and both showed an overall similarity among the cluster I and II. RAPD markers were used to estimate overall variation/polymorphism among termite species and similar studies in different groups of insect have been conducted for preliminary variation study.

5.2.7. Discussion:

Termites (Blattodea) a diverse group of eusocial insects are phylogenetically closely related with cockroach and mantis (Kambhampati, 1995; Kambhampati et al., 1996; Krishna et al., 2013). The phylogenetic position of termites among cockroaches and mantids is a much discussed aspect of termite biology (Thorne and Carpenter, 1992; Kambhapati et al., 1996). Large numbers of studies on phylogeny of termites at family or subfamily level based on morphological characters are available, but there are differences in opinions (Krishna, 1970; Donovan et al., 2000; Engel et al., 2009; Bignell et al., 2011; Krishna et al., 2013). The dendograms based on morphometrics showing relationships among 10 species of termites belonging to two different families is partially congruent with the RAPD based dendogram which is consistent with the findings of Kambhapati et al. (1996). On different families of termites Wang et al. (2009) reported phylogenetic relatedness of different species of *Reticulitermes* in Indiana based on morphology and mtDNA 16 srRNA sequences and RFLP for species identification. In the present study 10 termite species under two families – Termitidae and Rhinotermitidae, collected from different tea plantations of Darjeeling foothills were studied for first time morphologically as well as RAPD based technologies to assess their phylogenetic relatedness and taxonomic status.

Singla et al. (2015) studied nine species of termites belonging to *Odontotermes*, *Microtermes* and *Microcerotermes* under family Termitidae from India using cytochrome oxidase-I (COI) sequences and showed that they are closely related, however, formed different clusters. Another study on termite species from the southern part of India comprised of four genera revealing similar result (Murthy et al., 2015). RAPD studies on 12 species of termites belonging to three genera (*Acanthotermes*, *Psammotermes* and *Microtermes*) resulting into two clusters is consistent with our finding. Our phylogenetic results based on morphometric and RAPD analysis are in agreement with the results of Singla et al. (2015) and Murthy et al. (2015).

The present study is a preliminary inventory to shed light on the phylogenetic status of the termites from this part of India. More extensive studies based on robust molecular markers and informative morphometric traits including an out group will be helpful in producing much closer and clearer picture of termite phylogeny.

5.3. EVALUATION OF PESTICIDE TOLERANCE

Relative toxicity of different insecticides (imidacloprid 17.8% SL, chlorpyrifos 20% EC, cypermethrin 35% EC and endosulfan 10% EC) was tested against two of the major termite pest species, namely *O. obesus* and *M. obesi*. Samples from different tea plantations having distinct management practices like organic (O) and conventional (C) were screened. An organically managed plantation has a distinction of using pest control strategies chiefly derived from biological sources, either in the form of plant extracts, herbal pesticides or bacterial formulations, whereas conventionally managed plantations primarily rely on synthetic chemicals for pest control and plant protection. The pesticides used for the bioassay belong to four different classes of synthetic chemicals viz Organochlorines (endosulfan), Organophosphate (chlorpyrifos), synthetic Pyrethroids (cypermethrine) and Neonicotinoids (imidacloprid). At present three of these chemicals (chlorpyrifos, cypermethrin and imidacloprid) are used in agriculture and are recommended by the Central Insecticides Board and Registration Committee (CIB), Government of India. Endosulfan being one of the most persistent organochlorine pesticides, a global ban was enforced in 2012. For the very same property of prolonged persistence, however, it was selected for bioassay as it was in use in tea industry from the time of its inception as a commercial formulation till recent past. Hence, their effect on termite is anticipated to be more than any other chemicals in this lot.

5.3.1. *Odontotermes obesus*

5.3.1.1. Chlorpyrifos 20% EC:

Chlorpyrifos is the most common organophosphate pesticide used in agricultural fields to control various insect pests and often used as termiticide to control termites. A series of bioassays of this pesticide against *O. obesus* populations from different tea plantations with either organic or conventional mode of management revealed that population from the organic plantations are the most susceptible ones (Table 5.5). Termite populations from the organically managed Terai plantations recorded the lowest LC_{50} value of 9.62 ppm at 24 hr of exposure followed by organically managed Dooars plantation with 10.48 ppm of LC_{50} value having no significant difference between them at $p \leq 0.05$. Among termite pests from conventionally managed plantations highest LC_{50} was recorded in the populations of Western Dooars (54.25 ppm) closely followed by populations of Eastern Terai (52.04 ppm) > Central Dooars (46.45 ppm) > Eastern Dooars (45.82 ppm) > Western Terai (43.50 ppm) (Table 5.5). These results can serve as an indicator of developing tolerance level against chlorpyrifos in the population of *O. obesus* from conventionally managed tea plantations which may be due to an excessive exposure to pesticides.

5.3.1.2. Cypermethrin 35% EC:

Cypermethrin, a synthetic pyrethroid pesticide is used to control a large array of pests. Considering its extensive use in tea plantations there is great chances that in conventionally maintained plantations termites come in contact to this pesticide in regular basis. Present findings showed that *O. obesus* populations from tea plantations which are heavily maintained on synthetic chemicals has higher amount of LC_{50} values, whereas the populations from organically maintained plantations retained their least tolerant status with very low LC_{50} values. Population from conventionally managed Eastern Dooars plantations recorded the highest LC_{50} value of 43.76 ppm followed by Central Dooars (35.90 ppm) > Western Dooars (27.25 ppm) > Eastern Terai (19.35 ppm) > Western Terai (19.00 ppm) (Table 5.6). Among Organically maintained plantations Terai population showed the lowest LC_{50} value with 4.76 ppm followed by Dooars population with 5.96 ppm. The LC_{50} values were significantly different at $p = 0.05$ level of probability based on Tukey's HSD analysis.

5.3.1.3. Endosulfan 10% EC:

Endosulfan is an organochlorine (cyclodiene) compound with a long half life having toxic effect to a wide range of organisms. The conventionally managed Central Dooars population of *O. obesus* was found to be highly tolerant with LC_{50} value of 51.09 ppm which is followed by such as Eastern Terai (49.84 ppm) > Western Terai (44.83 ppm) > Eastern Dooars (43.08 ppm) > Western Dooars (41.86 ppm) (Table 5.7). Among organically managed plantations Terai showed LC_{50} value of 9.46 ppm and least tolerant population was from the Dooars with 7.66 ppm. The difference in the LC_{50} values of organically managed plantations with that of conventionally managed plantations were found to be highly significant at $p = 0.05$ level of probability based on Tukey's HSD analysis.

5.3.1.4. Imidacloprid 17.8% SL:

Imidacloprid is a neonicotinoid pesticide, fairly a new compound often specifically used for controlling soil borne insect pests. The bioassay of this compound showed that the *O. obesus* population from conventional population has actually started developing tolerance against this pesticide as well. In contrast to low level of tolerance exhibited by the populations from organically managed plantations. (Terai – 7.56 ppm and Dooars – 9.74 ppm), population from conventionally managed plantations showed higher level of tolerance where highest was recorded for Central Dooars (65.84 ppm) followed by Eastern Terai

Table 5.5: Median lethal concentration (LC_{50}) values of Chlorpyrifos 20% EC against *Odontotermes obesus* populations collected from different tea plantations of northern part of West Bengal

Management Type	**Population**	***LC_{50} (Mean† ± SE)**	**95% FL of LC_{50}**		**RF**	**Regression value (Y)**		**X^2**	***LC_{95}**
			Lower limit	**Upper limit**		**bx**	**A**		
Organic	Terai	9.62^{a} ± 0.551	7.34	12.61	1.00	1.00	-2.45	1.98	73.77
	Dooars	10.48^{a} ± 0.643	7.70	14.25	1.09	1.09	-1.93	2.45	120.58
Conventional	Western Terai	43.50^{b} ± 1.217	29.29	53.61	4.52	4.52	-2.24	0.82	445.00
	Eastern Terai	52.04^{b} ± 5.748	39.19	69.18	5.41	5.41	-3.11	1.52	484.82
	Western Dooars	54.25^{b} ± 2.299	40.29	73.07	5.64	5.64	-2.63	1.67	588.48
	Central Dooars	46.45^{b} ± 5.111	33.33	62.71	4.83	4.83	-2.68	1.45	587.03
	Eastern Dooars	45.82^{b} ± 7.037	33.99	61.79	4.76	4.76	-2.61	2.15	486.33

*values are expressed in parts per million (ppm)

† Means followed by different superscripts denote significant difference in LC_{50} values at $p = 0.05$ based on Tukey's Honest Significant Difference (HSD) Post Hoc test

Table 5.6: Median lethal concentration (LC_{50}) values of Cypermethrin 35% EC against *Odontotermes obesus* populations collected from different tea plantations

Management Type	**Population**	**$^*LC_{50}$ (Mean† ± SE)**	**95% FL of LC_{50}**		**RF**	**Regression value (Y)**		**X^2**	**$^*LC_{95}$**
			Lower limit	**Upper limit**		**bx**	**A**		
Organic	Terai	$4.76^a \pm 0.520$	3.85	5.89	1.00	2.26	-3.30	10.51	25.78
	Dooars	$5.96^a \pm 0.553$	4.47	7.95	1.25	1.69	-1.37	2.40	57.47
Conventional	Western Terai	$19.00^b \pm 0.364$	13.96	25.86	3.99	1.54	-1.59	0.56	228.15
	Eastern Terai	$19.35^b \pm 2.933$	14.60	25.66	4.06	1.65	-2.06	0.91	197.43
	Western Dooars	$27.25^b \pm 3.506$	20.20	36.81	5.73	1.62	-2.16	3.76	315.54
	Central Dooars	$35.90^b \pm 5.685$	26.62	48.46	7.54	1.55	-2.06	1.40	410.80
	Eastern Dooars	$43.76^b \pm 5.836$	35.05	55.00	9.19	2.28	-5.52	8.71	302.45

*values are expressed in parts per million (ppm)

† Means followed by different superscripts denote significant difference in LC_{50} values at $p = 0.05$ based on Tukey's Honest Significant Difference (HSD) Post Hoc test

Table 5.7: Median lethal concentration (LC_{50}) values of Endosulfan 10% EC against *Odontotermes obesus* populations collected from different tea plantations

Management Type	Population	*LC_{50} (Mean† ± SE)	95% FL of LC_{50}		RF	Regression value (Y)		X^2	*LC_{95}
			Lower limit	Upper limit		bx	A		
Organic	Terai	$9.46^{a} \pm 1.091$	7.07	12.65	1.23	1.69	-1.72	3.85	90.08
	Dooars	$7.66^{a} \pm 0.931$	5.69	10.29	1.00	1.64	-1.35	2.55	77.99
Conventional	Western Terai	$44.83^{b} \pm 2.550$	33.90	59.30	5.85	1.76	-3.17	1.45	404.52
	Eastern Terai	$49.84^{b} \pm 1.664$	36.96	67.23	6.51	1.60	-2.52	1.77	555.99
	Western Dooars	$41.86^{b} \pm 3.149$	30.33	57.81	5.46	1.45	-1.71	0.79	585.68
	Central Dooars	$51.09^{b} \pm 5.392$	37.74	69.39	6.67	1.65	-2.77	2.08	658.34
	Eastern Dooars	$43.08^{b} \pm 3.183$	31.96	58.22	5.62	1.59	-2.39	1.48	489.53

*values are expressed in parts per million (ppm)

† Means followed by different superscripts denote significant difference in LC_{50} values at $p = 0.05$ based on Tukey's Honest Significant Difference (HSD) Post Hoc test

Table 5.8: Median lethal concentration (LC_{50}) values of Imidacloprid 17.8% SL against *Odontotermes obesus* populations collected from different tea plantations

Management Type	**Population**	***LC_{50} (Mean† ± SE)**	**95% FL of LC_{50}**		**RF**	**Regression value (Y)**		**X^2**	***LC_{95}**
			Lower limit	**Upper limit**		**bx**	**A**		
Organic	Terai	$7.56^a \pm 0.520$	5.66	10.12	1.00	1.69	-1.56	3.68	72.07
	Dooars	$9.74^a \pm 0.752$	7.31	12.99	1.29	1.69	-1.73	2.04	93.08
Conventional	Western Terai	$56.32^b \pm 15.632$	40.72	77.93	7.45	1.45	-1.83	1.23	799.74
	Eastern Terai	$57.13^b \pm 11.883$	42.67	76.59	7.55	1.66	-2.81	1.50	632.74
	Western Dooars	$56.40^b \pm 6.292$	41.62	76.51	7.46	1.67	-2.88	4.20	659.01
	Central Dooars	$65.84^b \pm 9.796$	49.16	88.31	8.70	1.60	-2.73	2.60	727.99
	Eastern Dooars	$54.91^b \pm 7.459$	40.12	75.16	7.26	1.44	-1.81	2.53	768.27

*values are expressed in parts per million (ppm)

† Means followed by different superscripts denote significant difference in LC_{50} values at $p = 0.05$ based on Tukey's Honest Significant Difference (HSD) Post Hoc test

(57.13 ppm) > Western Dooars (56.40 ppm) > Western Terai (56.32 ppm) > Eastern Dooars (54.91 ppm) (Table 5.8). The difference of LC_{50} values related to the tolerance level between the populations from organically and conventionally managed plantations was significant at $p = 0.05$ based on Tukey's HSD analysis.

5.3.2. *Microtermes obesi* –

5.3.2.1. Chlorpyrifos 20% EC:

Microtermes obesi populations from different plantations having either organic or conventional system of management were subjected to bioassay of chlorpyrifos pesticide. Like *O. obesus*, similar results were obtained for *M. obesi* populations from organically managed plantations displaying very low level of tolerance, compared to the high level of tolerance observed in the populations from conventional plantations. The highest value of LC_{50} was observed in case of populations of conventionally managed Eastern Terai plantations (20.14 ppm) which was followed by other plantations namely Western Terai (18.57 ppm), Western Dooars (18.46 ppm), Central Dooars (17.02 ppm) and Eastern Terai in a descending order (Table 5.9). The least tolerant populations were recorded from the organically managed plantation of Terai (3.92 ppm) and Dooars (4.47 ppm). The difference in the LC_{50} values between organically managed and conventionally managed plantations were significantly higher at $p = 0.05$ level of probability based on Tukey's HSD analysis.

5.3.2.2. Cypermethrin 35% EC:

In case of cypermethrin, *M. obesi* populations from conventionally managed plantation showed higher tolerance level in comparison to the organically manage plantations. The highest tolerance level has been observed for populations of conventional plantations from Western Terai with LC_{50} value of 31.99 ppm followed by Eastern Terai (25.07 ppm) > Western Dooars (24.73 ppm) > Central Dooars (24.20 ppm) > Eastern Dooars (20.08 ppm). The lowest LC_{50} was recorded for organic plantations of Terai (5.67 ppm) and the Dooars (6.43 ppm) (Table 5.10). The difference in the LC_{50} values between organically managed and conventionally managed plantations were significantly higher at $p = 0.05$ level of probability based on Tukey's HSD analysis.

5.3.2.3. Endosulfan 10% EC:

Among all four pesticides, Endosulfan seems to have least effect on *M. obesi* populations as they exhibited very high level of LC_{50} values. Among conventional plantations, populations from Central Dooars with LC_{50} value of 139.05 ppm was the most tolerant one followed by Western Terai (125.45 ppm), Eastern Dooars (117.32 ppm), Western Dooars (101.69 ppm) and Eastern Terai (97.43 ppm) in a descending order. For populations of organically managed plantations, the Dooars had LC_{50} value of 15.04 ppm, whereas the Terai populations were the least tolerant with LC_{50} of 6.86 ppm (Table 5.11). The difference in the LC_{50} values between organically managed and conventionally managed plantations were significantly higher ($p = 0.05$) based on Tukey's HSD analysis.

Table 5.9: Median lethal concentration (LC_{50}) values of Chlorpyrifos 20% EC against *Microtermes obesi* populations collected from different tea plantations

Management Type	Population	*LC_{50} (Mean† ± SE)	95% FL of LC_{50}		RF	Regression value (Y)		X^2	*LC_{95}
			Lower limit	Upper limit		bx	a		
Organic	Terai	$3.92^a \pm 0.058$	1.62	5.36	1.00	1.60	-0.74	0.14	42.59
	Dooars	$4.47^a \pm 0.201$	2.35	5.64	1.14	1.58	-0.71	2.46	46.41
Conventional	Western Terai	$18.57^b \pm 2.098$	13.96	24.75	4.74	1.61	-1.86	3.96	209.41
	Eastern Terai	$16.96^b \pm 1.843$	11.68	21.41	4.32	1.61	-1.77	0.89	168.30
	Western Dooars	$18.46^b \pm 3.639$	13.75	24.80	4.71	1.63	-1.89	1.27	196.45
	Central Dooars	$17.02^b \pm 3.223$	12.71	22.84	4.34	1.73	-2.24	1.01	172.17
	Eastern Dooars	$20.14^b \pm 1.946$	14.82	27.37	5.14	1.56	-1.62	1.01	240.46

*values are expressed in parts per million (ppm)

† Means followed by different superscripts denote significant difference in LC_{50} values at $p = 0.05$ based on Tukey's Honest Significant Difference (HSD) Post Hoc test

Table 5.10: Median lethal concentration (LC_{50}) values of Cypermethrin 35% EC against *Microtermes obesi* populations collected from different tea plantations

Management Type	Population	*LC_{50} (Mean† ± SE)	95% FL of LC_{50}		RF	Regression value (Y)		X^2	*LC_{95}
			Lower limit	Upper limit		Bx	A		
Organic	Terai	$5.67^{a} \pm 0.231$	4.24	7.59	1.00	1.69	-1.35	3.68	54.05
	Dooars	$6.43^{a} \pm 0.151$	4.62	8.78	1.13	1.49	-0.66	2.40	87.66
Conventional	Western Terai	$31.99^{b} \pm 7.183$	23.41	43.81	5.64	1.65	-2.36	1.55	450.58
	Eastern Terai	$25.07^{b} \pm 4.133$	18.60	33.80	4.42	1.60	-2.01	1.77	273.17
	Western Dooars	$24.73^{b} \pm 6.392$	17.69	34.59	4.36	1.38	-1.02	0.89	388.70
	Central Dooars	$24.20^{b} \pm 5.572$	17.73	33.08	4.27	1.48	-1.47	1.87	307.94
	Eastern Dooars	$20.08^{b} \pm 3.219$	14.82	27.24	3.54	1.55	-1.63	1.35	246.43

*values are expressed in parts per million (ppm)

† Means followed by different superscripts denote significant difference in LC_{50} values at $p = 0.05$ based on Tukey's Honest Significant Difference (HSD) Post Hoc test

Table 5.11: Median lethal concentration (LC_{50}) values of Endosulfan 10% EC against *Microtermes obesi* populations collected from different tea plantations

Management Type	Population	*LC_{50} (Mean† ± SE)	95% FL of LC_{50}		RF	Regression value (Y)		X^2	*LC_{95}
			Lower limit	Upper limit		bx	A		
Organic	Terai	$6.86^a \pm 0.115$	4.97	9.46	1.00	1.24	-1.07	1.24	76.28
	Dooars I	$15.04^a \pm 1.707$	11.87	19.05	2.19	1.98	-3.24	2.84	102.43
Conventional	Western Terai	$125.45^b \pm 45.730$	100.39	157.40	18.29	2.06	-5.44	3.15	856.84
	Eastern Terai	$97.43^b \pm 13.082$	72.85	130.41	14.21	1.71	-3.50	3.62	1048.52
	Western Dooars	$101.69^b \pm 18.329$	76.61	134.97	14.83	1.69	-3.42	3.75	991.75
	Central Dooars	$139.05^b \pm 33.557$	108.34	178.80	20.27	1.83	-4.38	3.78	1102.09
	Eastern Dooars	$117.32^b \pm 9.226$	88.77	155.10	17.11	1.69	-3.56	3.42	1140.66

*values are expressed in parts per million (ppm)

† Means followed by different superscripts denote significant difference in LC_{50} values at $p = 0.05$ based on Tukey's Honest Significant Difference (HSD) Post Hoc test

Table 5.12: Median lethal concentration (LC_{50}) values of Imidacloprid 17.8% SL against *Microtermes obesi* populations collected from different tea plantations

Management Type	**Population**	***LC_{50} (Mean[†] ± SE)**	**95% FL of LC_{50}**		**RF**	**Regression value (Y)**		**X^2**	***LC_{95}**
			Lower limit	**Upper limit**		**bx**	**a**		
Organic	Terai	$5.50^{a} \pm 0.346$	3.86	7.84	1.00	1.28	0.20	1.58	107.00
	Dooars	$8.68^{a} \pm 1.197$	6.66	11.32	1.58	1.74	-1.83	1.11	76.79
Conventional	Western Terai	$63.66^{c} \pm 26.129$	47.21	85.95	11.57	1.55	-2.37	3.18	693.65
	Eastern Terai	$29.30^{b} \pm 2.966$	21.01	40.90	5.33	1.45	-1.45	2.09	443.87
	Western Dooars	$49.44^{c} \pm 6.161$	36.38	67.22	8.99	1.59	-2.45	2.48	566.65
	Central Dooars	$48.79^{c} \pm 10.642$	35.51	67.11	8.87	1.46	-1.84	1.97	682.66
	Eastern Dooars	$41.81^{c} \pm 7.887$	30.32	57.71	7.60	1.53	-2.03	1.74	593.81

*values are expressed in parts per million (ppm)

[†] Means followed by different superscripts denote significant difference in LC_{50} values at $p = 0.05$ based on Tukey's Honest Significant Difference (HSD) Post Hoc test

5.3.1.1. Imidacloprid 17.8% SL:

Results of bioassay for imidacloprid 17.8% SL showed a similar trend like that of chlorpyrifos, cypermethrin and endosulfan. The *M. obesi* populations from conventional plantations exhibited the high level of LC_{50} values compared to the populations from organic plantations. The populations from conventionally managed Western Terai plantations recorded the highest LC_{50} value of 63.66 ppm which was followed by that of Western Dooars (49.44 ppm), Central Dooars (48.79 ppm), Eastern Dooars (41.81 ppm) and Eastern Terai (29.30 ppm) in a descending order. The LC_{50} value varied from 5.50 ppm (Terai) to 8.68 ppm (Dooars) among populations from organic plantations showing very low level of tolerance in comparison to the conventionally managed plantations (Table 5.12). The difference in the LC_{50} values between organically managed and conventionally managed plantations were significantly higher at $p = 0.05$ level of probability based on Tukey's HSD analysis.

5.3.3. Discussion:

Present study showed different levels of responses to different pesticides in the two species of termites in question. Dosage-mortality response data of both *O. obesus* and *M. obesi* acknowledged the good fit of probit responses in all bioassays based on the chi-square (χ^2) values, therefore confirming the heterogeneous nature between the observed and the expected responses. The findings of the present work strongly suggest a significant difference of tolerance level in both species when collected from various organic (without synthetic pesticides) and conventionally (with synthetic chemicals) managed tea plantations. A distinct pattern of the level of tolerance was evident from the mean value of LC_{50}s. Organic plantations showed very low level of tolerance whereas population from conventionally managed tea plantations remained more tolerant towards all the pesticides considered in the study. This varied response to the pesticides between the populations of conventionally or organically managed plantations may be due to their long exposure to various indiscriminately used pesticides as compared to their little or no-exposure to pesticides in the organic plantations. In recent time, due to various factors tea plantations were either abandoned (eg. Red Bank T.E., Surendranagar T.E.), or closed (eg. Mujnai T.E.), or changing management systems (eg. Samsing T.E., Kalchini T.E.) (CEC, 2007). Due to these reasons tea plantations often get sick and there is a resurgence of many pest species. This may also be one of the reasons for such a varied occurrence and response of termite populations to different pesticides.

The data presented here are lab based, however, for controlling termite in field, insecticides either need to be applied directly to the soil or at times directly to the opened up termitarium (Das, 1965). According to Harris (1972) for the effective control of a termite pest, the primary criterion was to understand the insecticide and its relation with soil structure. It was observed in abundance study that these two species were the only one which were found more frequently available throughout Darjeeling Terai and the Dooars region. Termites construct tunnels to reach out for food sources. In tea plantations when these insecticides are sprayed, either to control above ground pests or specifically to control soil borne pests, pesticides get leached into the soil system. This contaminated soil system is used by these termite species to make tunnels and even in the construction of colony. This leads to the maximum exposure of workers population which also carry food back to the colony and share with its unexposed inmates by the process of anal trophallaxis. These insecticide laced semi digested food then makes way to other healthy inmates and make them also sick (Myles, 1996; Kard, 2001). However, if we look into the abundance study, it seems like these two species have adapted to this dynamic environment of burgeoning variety of insecticides. Their increased LC_{50} value, LC_{95} value and RF value in conventionally managed tea plantations in comparison to that of organic plantations with least tolerance, clearly suggest a trend in developing tolerance towards the synthetic pesticides that are in use. Similar trends of developing tolerance by other pests against regularly used pesticides are not new to tea industry. In fact because of tolerant pest populations insecticide use pattern varies from place to place and through time (Roy et al., 2008). Similar trends of pesticide tolerance in pests like looper (*Hyposidra talaca* and *H. infixaria*) (Das et al., 2010), *Helopeltis theivora* (Roy et al., 2010), *Empoasca flavescens*, *Scirtothrips dorsalis* (Saha and Mukhopadhyay, 2013) and others have been reported from conventional tea plantations of Darjeeling Terai and the Dooars. Pests like termites have so far been little known for their tolerance status against pesticides, this may be due to the lack of investigation of this surreptitious and eusocial pest.

5.4. DETOXIFYING ENZYME ACTIVITY

5.4.1. *Odontotermes obesus*

5.4.1.1. General Esterase (GE):

The general esterase activity of *Odontotermes obesus* populations collected from different conventionally or organically managed tea plantations showed significant difference. The enzyme Activity Ratio (AR) was also estimated among the populations. An activity ratio (AR) is the ratio of the activity of defense enzyme of more tolerant population by the enzyme activity value of least tolerant population. *O. obesus* populations collected from organic plantations have low GE activity in comparison to the populations from tea plantations managed with pesticides (Table 5.13). Highest activity of 3.22 ± 0.31 was recorded for Western Terai population with an AR of 5.18 folds compared to the least tolerant organically managed Terai population. Populations from conventionally managed plantations expressed higher AR ranging between 4.43 – 5.18 folds than the populations from organic plantations (1.00 – 1.14 folds). The independent sample *t*-test further confirmed the significant difference in GE expression with t (46) = 9.267 and F-value= 19.11 at $p < 0.01$ between *O. obesus* populations of organic and conventional plantations. From this it could be inferred that the management practice type and the exposure to pesticide has direct effect on GE expression in *O. obesus* populations in these plantations.

In order to check if there is any possible relation between the Resistance Factor (RF) values of a pesticide (chlorpyrifos 20% EC) and the expressed activity of GE (AR), bivariate correlation between the GE activities of *O. obesus* population were run. The data yielded a very high Pearson's correlation coefficient of $r = 0.965$ at $p < 0.01$ (2 tailed). Further to confirm that this correlation is not random, a linear regression analysis was performed. The linear relationship between RF and AR was statistically significant with an $R^2 = 0.932$ and $F = 68.60$ at $p < 0.05$ which firmly attested that based on the management type of a plantation the expression of a detoxifying enzyme (GE) regulates the tolerance level of *O. obesus* [Figure 5.13 (A)].

The heterogeneity in the expression of GE in different populations of *O. obesus* collected from different conventionally managed plantations was also checked by pooling GE data to one-way analysis of varience (ANOVA). Results obtained from ANOVA confirmed the existence of strong variability among expression of GE of the tested population with $F = 2.82$ at $\alpha = 0.05$ and $df = 4, 34$.

5.4.1.2. Glutathione *S*-transferase (GST):

Similar results were observed for GST where its activity ranging from 113.59 – 244.52, was significantly high in the populations from conventionally managed plantations than that of the populations from organically managed ones (11.83 – 18.43) (Table 5.13). The least tolerant population was recorded from the organically maintained plantations of the Terai region. The AR values varied from 9.60 – 20.66 folds in the populations from conventionally managed plantations with a highest activity being recorded for Western Dooars population (20.66 folds). The Independent sample *t*-test analysis showed that the difference in GST expression between populations of organic and conventional plantations was significantly high with $t\ (46) = 6.33$ and F-value= 18.1 at $p < 0.01$.

The bivariate correlation coefficient analysis between RF and AR values for GST yielded a Pearson's correlation coefficient of $r = 0.862$ at $p < 0.01$ (2 tailed). The relation between hyperactivation of GST and subsequent ability of *O. obesus* to tolerate more toxic load was clearly evident with this correlation data. This also helped in predicting that elevated GST level can be used as an indicator of higher tolerance level in the population of *O. obesus*.

The dependence of RF on AR of GST was also cross-checked by pooling data to Simple linear regression analysis. The linear regression yielded highly significant $R^2 = 0.907$ and F-value of 48.77 at $p < 0.05$. The results showed the 90.7% dependence of RF values on AR, also indicating the significant linear relationship between them [Figure 5.13 (B)].

GST activities from conventionally managed plantations when subjected to one-way ANOVA showed the strong variation between the populations studied with $F = 3.574$ at $\alpha = 0.05$ having $df = 4, 34$. The variability showcased by these populations may be due to the amount, interval and intensity of pesticides used in different plantations to control pests.

5.4.1.3. Cytochrome P450 (CYP450):

Cytochrome P450 monoxygenase activity varied from 0.645 – 0.882 for *O. obesus* populations from conventionally managed plantation (Table 5.13), exhibiting highest activity of 0.882 for Eastern Terai population. Lowest activity of 0.239 was recorded for the organically managed Terai population. The AR value of CYP450 ranged between

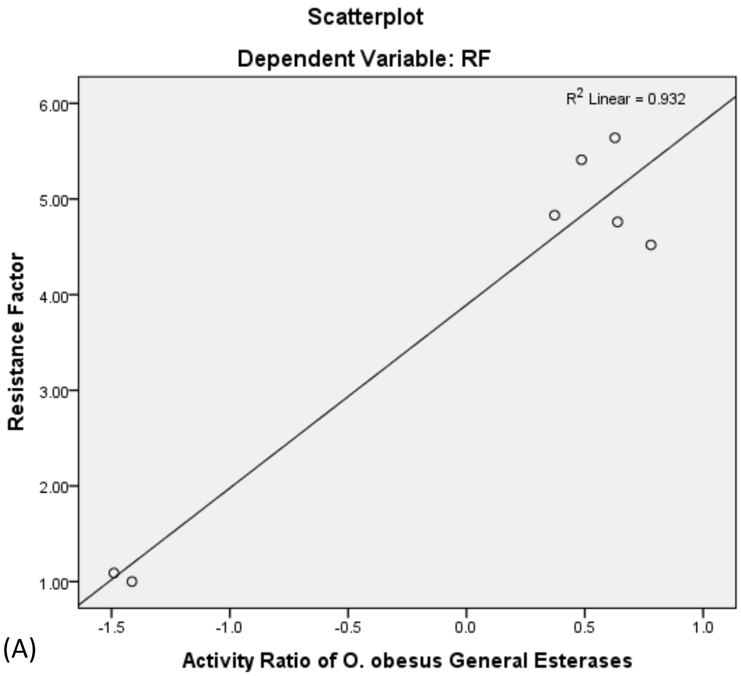
Table 5.13: Defense Enzymes (GE, GST and CYP450) activities in *Odontotermes obesus* population collected from organically and conventionally managed tea plantations of Terai and the Dooars regions

Management Type	Population	‡RF	GE (mM mg $^{-1}$ protein) (Mean* ± SE)	†AR	GST (µM min^{-1} mg^{-1} protein) (Mean ± SE)	AR	CYP450 (n mol min^{-1} mg protein^{-1}) (Mean ± SE)	AR
Organic	Terai	1.00	$0.62^{a} \pm 0.10$	1.00	$11.83^{a} \pm 1.05$	1.00	$0.239^{a} \pm 0.02$	1.00
	Dooars	1.09	$0.71^{a} \pm 0.16$	1.14	$18.43^{a} \pm 3.17$	1.56	$0.261^{a} \pm 0.01$	1.09
Conventional	Western Terai	4.52	$3.22^{b} \pm 0.31$	5.18	$113.59^{b} \pm 17.17$	9.60	$0.645^{b} \pm 0.03$	2.70
	Eastern Terai	5.41	$2.89^{b} \pm 0.15$	4.64	$174.12^{b} \pm 20.66$	14.71	$0.882^{b} \pm 0.09$	3.70
	Western Dooars	5.64	$3.05^{b} \pm 0.04$	4.90	$244.52^{c} \pm 20.50$	20.66	$0.706^{b} \pm 0.04$	2.96
	Central Dooars	4.83	$2.76^{b} \pm 0.16$	4.43	$154.20^{b} \pm 35.78$	13.03	$0.712^{b} \pm 0.06$	2.99
	Eastern Dooars	4.76	$3.07^{b} \pm 0.34$	4.92	$164.29^{b} \pm 20.04$	13.88	$0.744^{b} \pm 0.05$	3.12

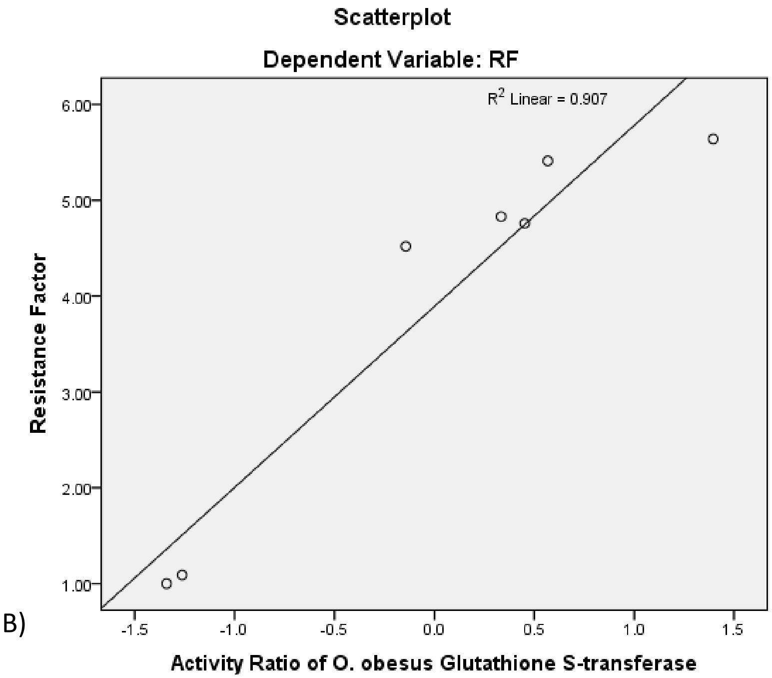
‡RF = Resistance factor, is the ratio of Median Lethal Concentration (LC_{50}) of more tolerant population to the least tolerant organic population

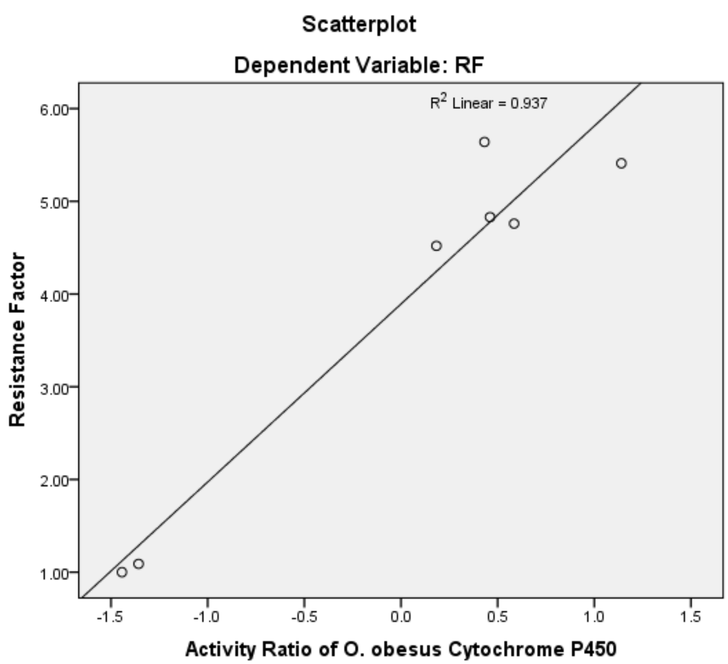
*mean values with different superscript alphabets in columns denote significant difference at 0.05% level of probability based on Tukey's HSD and Bonferroni multiple comparison tests

†AR = Activity Ratio, is the ratio of defence enzyme activity of a more tolerant population to the activity of the least tolerant organic population



(A)

(B)




(C)

Figure 5.13: Scatterplot of Linear Regression with R^2 values between Resistance Factor (RF) and Activity Ratios of (A) General Esterases; (B) Glutathione *S*-transferases and (C) Cytochrome P450 in *O. obesus*

2.70 – 3.70 folds for populations from conventional plantations whereas populations from organically managed plantations exhibited AR ranging from 1.00 – 1.09 folds only.

The difference in the expression of CYP450 activity in *O. obesus* populations from tea plantations of these two management types was highly significant with t-value of 8.058 at df = 45 and F value = 10.187 at P < 0.01. The bivariate correlation analysis between the RF values and the expressed activity of CYP450 (AR) a very high Pearson's correlation coefficient with $r = 0.968$ at $p < 0.01$ (2 tailed) was observed. The results validate the dependent relationship of RF to AR. This was further substantiated analyzing the data with linear regression which yielded a linear relationship between these two data set with a highly significant value of $R^2 = 0.937$ and $F = 74.14$ at $p < 0.05$ [Figure 5.13 (C)].

The variability in the expression of CYP450 activity of *O. obesus* collected from different conventionally managed plantations was analyzed by ANOVA. The results corroborated the strong variability in CYP450 expression of the tested populations with $F = 3.574$ at $\alpha = 0.05$ and $df = 4, 34$.

5.4.2. *Microtermes obesi*

5.4.2.1. General Esterases:

As like *O. obesus* commensurable results of GEs were obtained for *M. obesi* too (Table 5.14). Populations from organic plantations displayed a very low GE activity ranging from 0.23 to 0.33 with Terai being the least tolerant population. The highest activity of 2.26 was recorded for the population of conventional plantations of Eastern Dooars. The AR ranged from 5.78 – 9.73 folds in populations of conventionally managed plantations, whereas the populations from organically managed plantations ranged just from 1.00 – 1.40 folds.

The GE activities of *M. obesi* populations collected from two differently managed plantations yielded significant difference when analyzed by independent sample t-test with t (46) = 4.45 and F-value = 14.1 at $p < 0.01$ between populations of organic and conventional plantations. The bivariate correlation coefficient analysis between RF and AR values of GE produced a very high Pearson's correlation coefficient with $r = 0.937$ at $p < 0.01$ (2 tailed). The correlation between elevated GE activity and higher RF values was clearly evident with this analysis suggesting an elevated GST level can be used as an indicator of higher tolerance level in the population of *M. obesi*.

The dependence of RF on AR of GE was also checked by performing Simple linear regression analysis which furnished a significant R^2-value of 0.879 and F-value of 36.19 at $p < 0.05$. The result showed 87.9% dependence of RF values on AR indicating a significant linear relationship between them [Figure 5.14 (A)]. GE activities from conventionally managed plantations were also analyzed for one-way ANOVA which showed the strong variation between the populations studied with $F = 3.977$ at $\alpha = 0.05$ having $df = 4, 34$. The variability in the populations may have been due to the amount, interval and intensity of pesticide used in different plantations to control pests. Hence, variability and intensity of expression of the GE level correspond to the volume of pesticides used thereby channelizing tolerance to varied level.

5.4.2.2. Gluatathione *S*-transferase:

M. obesi populations from two differently managed tea plantations exhibited a significant difference in the level of expression of GST. Higher values of GST activity in the range from 100.19 to 198.96 were observed for the populations of conventionally managed tea plantations whereas organically managed populations of *M. obesi* recorded among the lowest (13.23–16.73) of all (Table 5.14). The activity ratio of GST for populations from organic plantation was between 1.00 - 1.25 folds while populations from conventionally managed plantations ranged between 7.53 – 15.04 folds. The populations of conventionally managed Central Dooars plantation recorded the highest GST activity of 198.96 with an AR of 15.04 fold.

The GST activities of *M. obesi* populations from two types of plantations were analyzed with Independent sample *t*-test which resulted to be significantly different with t (46) = 5.22 and F-value= 12.89 at $p < 0.01$. The bivariate correlation coefficient analysis between RF and AR values produced a significant Pearson's correlation coefficient with $r = 0.862$ at $p < 0.01$ (2 tailed). The correlation between RF on AR of GST was further examined by pooling data to linear regression analysis which yielded an $R^2 = 0.742$ and F-value of 14.4 at $p < 0.05$ signifying at least 74.2% dependency of RF on AR values [Figure 5.14 (B)]. This further suggests the significant linear relationship between these two factors.

The GST activities from conventionally managed plantations when subjected to one-way ANOVA, a strong variation between the populations was revealed with $F = 5.16$ at $\alpha = 0.05$ having $df = 4, 34$ indicating variability showcased must have been acquired under the influence of varied exposure to xenobiotics. In other words the variability in the

Table 5.14: Defense enzyme (GE, GST and CYP450) activities in *Microtermes obesi* population collected from organically and conventionally managed tea plantations of Terai and the Dooars regions

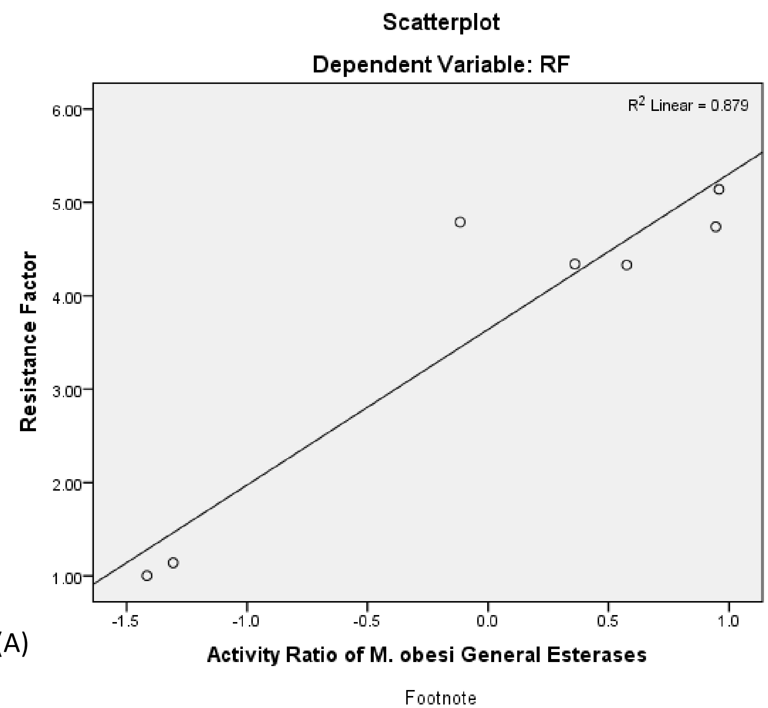
Management Type	Population	‡RF	GE (mM mg $^{-1}$ protein) (Mean*± SE)	†AR	GST (µM min^{-1} mg^{-1} protein) (Mean*± SE)	AR	CYP450 (n mol min^{-1} mg protein^{-1}) (Mean*± SE)	AR
Organic	Terai	1.00	$0.23^{a} \pm 0.03$	1.00	$16.73^{a} \pm 1.20$	1.26	$0.26^{a} \pm 0.01$	1.21
	Dooars	1.14	$0.33^{a} \pm 0.04$	1.40	$13.23^{a} \pm 1.37$	1.00	$0.21^{a} \pm 0.02$	1.00
Conventional	Western Terai	4.74	$2.25^{c} \pm 0.51$	9.68	$120.86^{c} \pm 21.93$	9.14	$0.75^{b} \pm 0.07$	3.55
	Eastern Terai	4.33	$1.93^{c} \pm 0.35$	8.32	$128.06^{c} \pm 10.81$	9.68	$0.73^{b} \pm 0.14$	3.47
	Western Dooars	4.79	$1.34^{b} \pm 0.30$	5.78	$100.19^{b} \pm 6.34$	7.57	$1.05^{b} \pm 0.18$	4.98
	Central Dooars	4.34	$1.75^{c} \pm 0.28$	7.53	$198.96^{c} \pm 52.99$	15.04	$0.95^{b} \pm 0.23$	4.47
	Eastern Dooars	5.14	$2.26^{c} \pm 0.36$	9.73	$156.93^{c} \pm 17.21$	11.86	$0.77^{b} \pm 0.06$	3.65

‡RF = Resistance factor, is the ratio of Median Lethal Concentration (LC_{50}) of more tolerant population to the least tolerant organic population

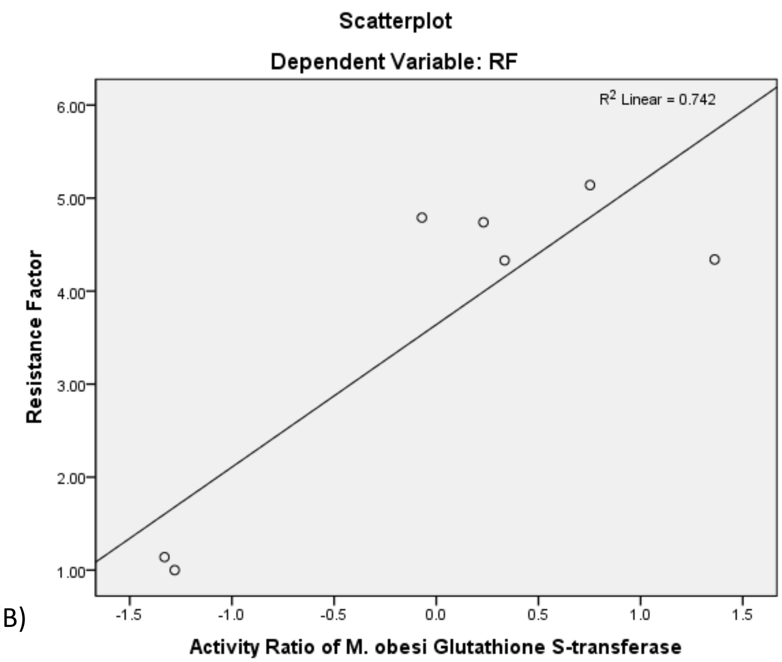
*mean values with different superscript alphabets in columns denote significant difference at 0.05% level of probability based on Tukey's HSD and Bonferroni multiple comparison tests

†AR = Activity Ratio, is the ratio of defence enzyme activity of a population to the activity of the most susceptible organic population (Dooars)

(A)



(B)



(C)

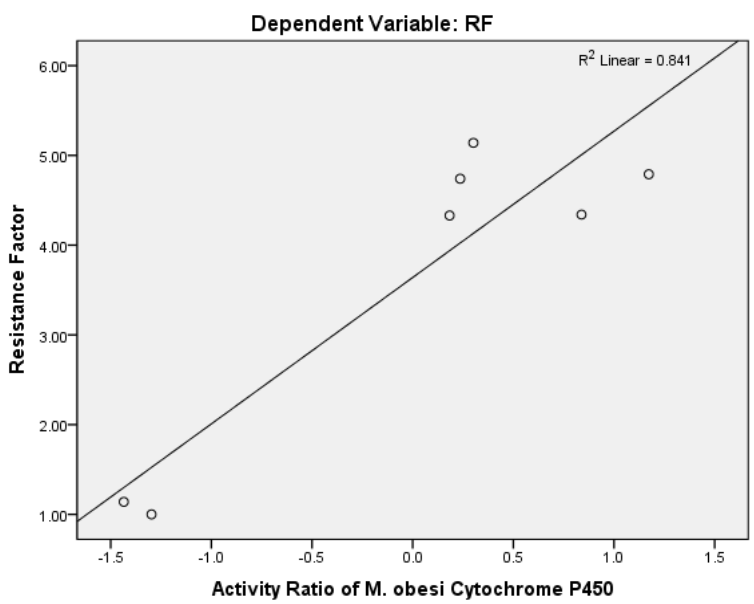


Figure 5.14: Scatterplot of Linear Regression with R^2 values between Resistance Factor (RF) and Activity Ratios of (A) General Esterases; (B) Glutathione *S*-transferases and (C) Cytochrome P450 in *Microtermes obesi*

activity of the detoxifying enzymes of *M. obesi* population suggests the type of management practice that is being followed in the plantations they have been sampled from. Basically a higher GST activity indicates higher level of pesticide use in the plantations which also means more pesticide use will lead to higher expression of the enzyme and higher level of tolerance.

5.4.2.3. Cytochrome P450:

Results similar to GE or GST were also obtained for CYP450, where the populations from conventionally managed plantations displayed significantly very high activity ranging from 0.73–0.95 than that of the populations from organically managed plantations with a very low activity range from 0.21–0.26 (Table 5.14). The least tolerant population was recorded from the organically maintained plantations of the Terai region with single fold of AR. The AR values varied from 3.47 – 4.98 folds in the populations from conventionally managed plantations with a highest activity being recorded for Western Dooars population with 4.98 folds.

The Statistical analysis of RF of chlorpyrifos and AR value of CYP450 yielded some significant results. Lavene's Test for equality of variance produced the significant difference in CYP450 expression with t (46) = 5.22 and F-value= 12.89 at $p < 0.01$ between populations of organic and conventional plantations. The bivariate correlation coefficient analysis run for RF and AR values of CYP450 yielded a Pearson's correlation coefficient of $r = 0.917$ at $p < 0.01$ (2 tailed). The relation between higher expression of CYP450 and subsequent ability of *M. obesi* to tolerate more toxic load was clearly evident with this correlation data. This also helped in predicting elevated CYP450 level can be used as an indicator of higher tolerance level in the population of *M. obesi*.

The dependence of RF on AR of CYP450 was also cross-checked by pooling data to simple linear regression analysis which produced a R^2 value of 0.841 and F = 26.51 at $p < 0.05$ [Figure 5.14 (C)]. Results showed very high dependency of RF values on AR indicating the significant linear relationship between them.

CYP450 activities from conventionally managed plantations when subjected to one-way ANOVA showed the strong variation between the populations from different conventionally maintained plantations with $F = 2.789$ at $\alpha = 0.05$ having $df = 4, 34$.

5.4.3. Electrophoretic analysis of General Esterases

5.4.3.1. ***Odontotermese obesus***:

Electrophoretic analysis of *O. obesus* populations from organically and conventionally managed plantations revealed a distinct difference in the levels of expression of GE enzymes. The analysis revealed three isozyme bands which were arbitrarily designated as – Est-1, Est-2 and Est-3 from cathode to anode. The bands were intensely stained and more expressive in the populations of conventional plantations. Populations from organic plantations expressed comparatively less intense bands. In both the populations Est-2 was the most prominent isozyme however, densitometric analysis revealed a high peak in the population from conventionally managed plantations than organic one. The major difference was in the expression of Est-3 where populations from conventionally managed plantations have a dark stained band showing prominent densitometric peak whereas organic counterpart had faint band with small peak. Est-1 was found to be least expressed with very faint or undetectable bands (Figure 5.15).

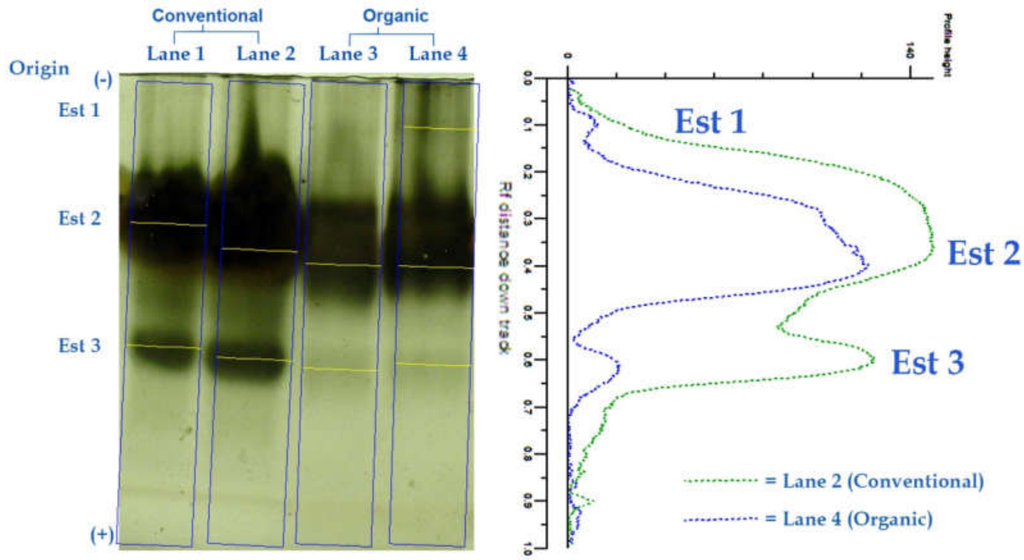


Figure 5.15: Densitometric analysis of electrophoregram of general esterases of *O. obesus* collected from organically and conventionally managed tea plantations

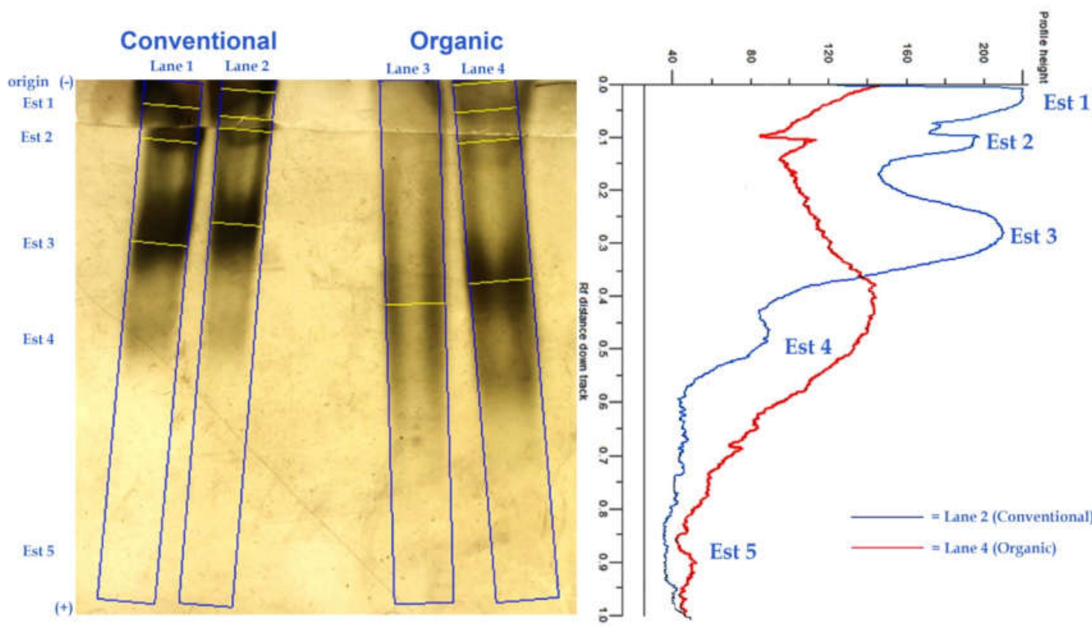


Figure 5.16: Densitometric analysis of electrophoregram of general esterases of *M. obesi* collected from organically and conventionally managed tea plantations

5.4.3.2. *Microtermes obesi*:

In case of *M. obesi* five bands of GE– Est-1, Est-2, Est-3, Est-4 and Est-5 were observed among the termite populations from different plantations. The intensity of staining of bands was higher and more expressive in the populations from conventional plantations. Est-1, 2, 3, and 4 were more intense and with comparatively high and detectable densitometric peaks. Populations from organic plantations expressed comparatively less intense bands, where only Est-3 was the most intensely stained and expressive one (Figure 5.16).

5.4.4. Caste level difference in detoxification enzyme activity

5.4.4.1. *Odontotermes obesus*:

General Esterase, Glutathione *S*-transferases and Cytochrome P450 were estimated in worker and soldier castes of *O. obesus* collected from the conventional tea plantations. Quantitative analysis of GE, GST and CYP450 showed significantly higher expression in worker termites compared to the soldiers. AR values of 3.46, 2.45 and 1.86 folds of GE, GST and CYP450 were observed respectively (Table 5.15). Densitometric analysis of the gels showed differential GE expression between worker and Soldier castes of *O. obesus*. In workers higher levels of expression of the GE was evident through densely stained gel bands whereas soldier castes tend to have lighter bands (Figure 5.17).

5.4.4.2. *Microtermes obesi*:

In case of *M. obesi* also similar trend was observed. The soldier and worker did show significant difference in the level of detoxifying enzyme expression. The AR values of 2.12, 4.78 and 2.59 folds indicating higher expression of GE, GST and

Table 5.15: Activities of different detoxifying enzymes (GE, GST and CYP450) in worker and soldier castes of *O. obesus* collected from conventionally managed tea plantations

	General Esterase ($mM\ mg^{-1}$ protein) (Mean*±SE)	AR	GST ($\mu M\ min^{-1}\ mg^{-1}$ protein) (Mean*±SE)	AR	CYP450 (nmol min^{-1} mg $protein^{-1}$) (Mean* ± SE)	AR
Soldier	$2.73^{a} \pm 2.41$	1.00	$122.34^{a} \pm 14.66$	1.00	$0.36^{a} \pm 0.05$	1.00
Worker	$9.44^{b} \pm 5.00$	3.46	$299.75^{b} \pm 80.28$	2.45	$0.67^{b} \pm 0.13$	1.86

*Different alphabets in the columns denote significant differences at 0.05% level of probability

Table 5.16: Activities of different detoxifying enzymes (GE, GST and CYP450) in worker and soldier castes of *M. obesi* collected from conventionally managed tea plantations

	General Esterase ($mM\ mg^{-1}$ protein) (Mean*±SE)	AR	GST ($\mu M\ min^{-1}\ mg^{-1}$ protein) (Mean*±SE)	AR	CYP450 (n mol min^{-1} mg $protein^{-1}$) (Mean* ± SE)	AR
Soldier	$2.09^{a} \pm 0.39$	1.00	$16.31^{a} \pm 2.48$	1.00	$0.39^{a} \pm 0.15$	1.00
Worker	$4.44^{b} \pm 0.91$	2.12	$77.93^{b} \pm 15.33$	4.78	$1.01^{b} \pm 0.06$	2.59

*Different alphabets in the columns denote significant differences at 0.05% level of probability

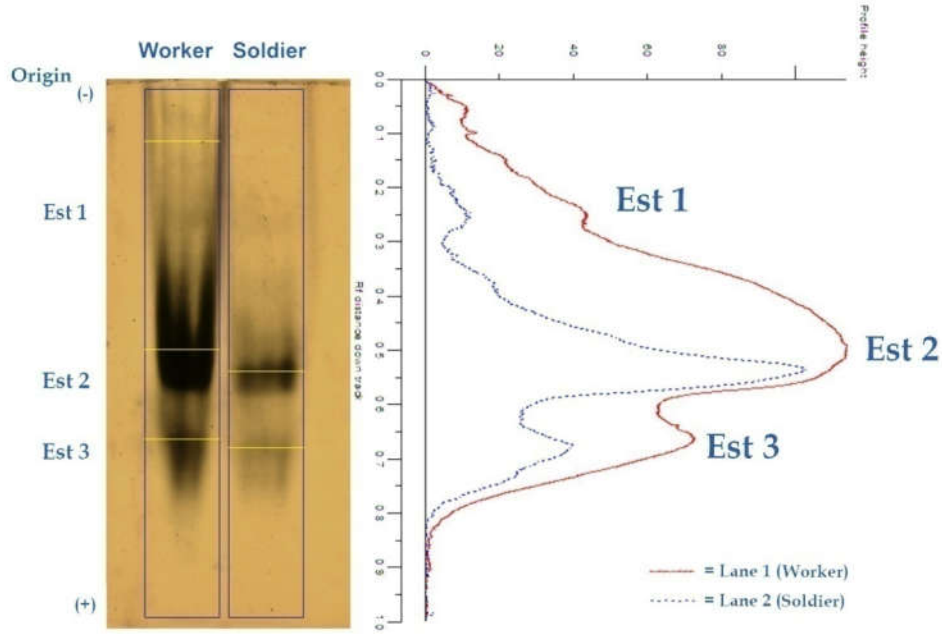


Figure 5.17: Comparative densitometric electrophoregram of general esterases between worker and soldier castes of *O. obesus*

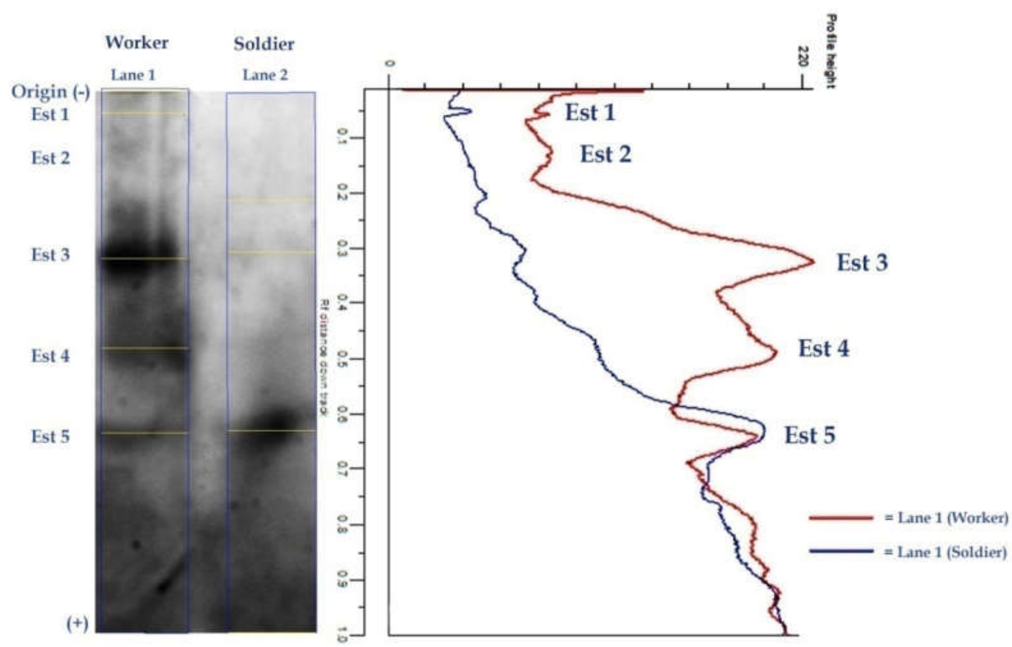


Figure 5.18: Comparative densitometric electrophoregram of general esterases between worker and soldier castes of *M. obesi*

CYP450, respectively were observed in workers as compared to the soldiers (Table 5.16). The esterase bands observed in the worker castes of *M. obesi* were more intensely stained in comparison to soldiers signifying higher activities of GE in workers (Figure 5.18).

5.4.5. Discussion:

A phytophagous insect often has to encounter either plant allelochemicals (which is a form of direct plant defense chemical) or man-made synthetic pesticides (indirect defense). With time, insects have acquired mechanisms to deal with these chemicals coming from plants and human beings. An insect has defensive mechanisms that involve physiological machinery for insecticides detoxification. These machinery can either metabolically neutralize the toxicity of an insecticide before it reaches its target or the target site itself gets altered to become insensitive to these insecticides. In an organism the metabolic detoxification is achieved with the help of enzyme system such as, general esterases (GE), glutathione *S*-transferases (GSTs) or cytochrome P450 monoxygenases which are expressed excessively in tolerant group of insects in comparison to susceptible ones (Brown and Brogdon, 1987; Hemingway, 1989; Hemingway et al., 1995).

As evident from the above results management practices for controlling pest has direct effect on the physiology of termite pests. *O. obesus* and *M. obesi* both seem to have adapted to changing environment of conventionally managed tea plantations as both have been observed to have higher activities of detoxifying enzymes (Table 5.13-5.14; Figure 5.15 and 5.16). It is well explained by different workers how a continuous selection can occur and more tolerant variety of insects emerges under insecticidal pressure (Brogdon and McAllister, 1998; Karaağaç, 2012; Kunz and Kemp, 1994). Among all the physiological tolerance system duplication or hyperactivation of detoxifying enzyme genes is very common. Many insects like house fly, bed bug, tea pests like Tea Mosquito Bug, Geometrid Loopers, Red Spider Mites have been reported to exhibit higher activity of detoxifying enzymes under insecticide stress. The comparative accounts of many of these insects from less xenobiotic and highly stressed environment have shown a distinct difference in the expression of the detoxifying enzymes (Nardini et al., 2012; Ishaaya, 1993).

Among termites, a true worker caste is the one responsible for maintaining the entire colony, foraging, harvesting, feeding, cleaning and any other odd job that colony demands. Eusociality allows only few specialized one to reproduce and rest of the members of colony generally follows altruistic behavior helping colony to maintain. It is hypothesized that workers while foraging come across many allelochemicals and xenobiotics which puts a constant stress on the physiology of these insects (Soleymaninejadian et al., 2014). A comparison between the expression of different detoxifying enzymes of worker and soldier castes does seem to support that. More the exposure to insecticides, more expression in detoxifying enzymes does seem applicable to different environment, species and individual forms (Ishaaya, 1993).