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A PRELIMINARY INVENTORY OF SUBTERRANEAN TERMITES IN THE PREMISES OF FACULTY OF SCIENCE, UNIVERSITY OF KELANIYA AND THE POTENTIAL OF A PONERINE ANT SPECIES, NEEMAZAL-F AND CITRONELLA OIL IN THE CONTROL OF TWO TERMITE SPECIES

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ABSTRACT

Termites are an important biotic component in terrestrial ecosystems and the termites in seven localities of the Faculty of Science of University of Kelaniya were investigated by employing soil sifting, litter sifting, hand collection, baited pitfall trapping and long term baits from September, 2008 to April, 2009. The collected termites were preserved in 80% ethanol. Soil temperature and pH, and percentage soil moisture at each location were also recorded. Members of Termitidae and Rhinotermitidae, *Dicuspeditermes incola* Wasmann, *Heterotermes ceylonicus* Holmgren, *Nasutitermes ceylonicus* Holmgren, *Nasutitermes horni* Wasmann, *Odontotermes ceylonicus* Wasmann, *Odontotermes horni* Wasmann and *Trinervitermes rubidus* Hagen, were recorded. *Odontotermes horni* had a wide distribution in the seven localities. The interactions between a common ponerine ant, *Odontomachus simillimus* F. Smith and *O. ceylonicus* Wasmann revealed that *O. simillimus* acts as a natural enemy of this termite species. Termiticidal potential of Neemazal-F and citronella oil on the soldiers and workers of *O. horni* was evident while LD_{50} values were 11.15 mg and 8.21 mg per body weight g^{-1} of each product, respectively.

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Keywords

Termites of University premises, citronella oil, Neemazal-F, *Odontotermes horni, Odontotermes ceylonicus, Trinervitermes rubidus, Odontomachus simillimus*

INTRODUCTION

Termites (Order: Isoptera) are a very important biotic component on earth as they play a significant role in recycling materials and, act as serious pests of agricultural crops and many other materials (Harris1961; Roonwal & Chhotani 1989; Pearce 1999; Thakur 2000). Subterranean termites that live in soil or diffused chambers are very destructive pests among them (Harris 1961). The diversity of the world termite fauna is estimated to be of seven families, 282 genera and 2,761 species (Rathore & Bhattacharyya 2004). Although termites belonging to three families, Kalotermitidae, Rhinotermitidae and Termitidae, twenty seven genera and fifty four species have been recorded from Sri Lanka (Roonwal & Chhotani 1989; Chhotani 1997) very little is published recently (Hemachandra *et al.* 2010) on the termites that inhabit different regions of the country.

Termites have a wide range of natural enemies such as insects, birds, reptiles and mammals. Ants (Order: Hymenoptera, Family - Formicidae), specially, the dorylines and ponerines, can cause high mortality in termites (Harris 1961; Pearce 1999). Commercially available insecticides, e.g. Chlorpyrifos, endosulfan, carbofuran and permethrin, are usually applied to control termites that act as pests (Thakur 2000; Pearce 1999) irrespective of their adverse effects on the environment. Natural plant extracts such as neemazal-F and citronella oil also show insecticidal properties and are less harmful to the environment (Pearce 1999; Wijesekara 1973).

The aim of this investigation was to prepare a preliminary inventory of subterranean termites while studying their distribution in the Science Faculty premises of University of Kelaniya and provide information on the effects of neemazal-F and citronella oil on the subterranean termite, *Odontotermes horni* Wasmann, a known pest of coconut (Harris 1961). *Odontomachus simillimus* F. Smith is a common ponerine ant species recorded from the Science Faculty premises of University of Kelaniya (Dias 2006) and this paper also presents the

interactions between this ant species and the subterranean termite, *Odontotermes ceylonicus* Wasmann under the laboratory conditions.

MATERIALS AND METHODS

Study sites

Termites were surveyed in seven regions (A-G) of the Faculty of Science (Figure 1). Site A (06^0 $58^/$ N 79^0 $54^/$ E) is about 500 m^2 and situated around building A5. Site B (06^0 $58^/$ N 79^0 $54^/$ E) is about 500 m^2 and situated behind the Department of Statistics and Computer Science. Site C (06^0 $58^/$ N 79^0 $54^/$ E) is about 700 m^2 and is the botanical garden of Science Faculty. Site D (06^0 $58^/$ N 79^0 $54^/$ E) is about 150 m^2 and situated behind Sri Dharmaloka Convocation Hall (building A1). Site E (06^0 $58^/$ N 79^0 $54^/$ E) is about 50 m^2 and situated behind the Department of Zoology. Site F (06^0 $58^/$ N 79^0 $54^/$ E) is about 150 m^2 and a grassland situated behind the Department of Chemistry. Site G (06^0 $58^/$ N 79^0 $54^/$ E) is about 100 m^2 and an open grassland situated behind the building A8.

Field sampling methods

Table 1 shows the total number of samples collected by each method (Taylor *et al*. 1998; Pearce 1999) from each site. Termite soldiers, workers and reproductives in each soil sample (20 x 20x 5 cm^3) were collected after sifting soil with a sieve and a tray. All individuals were preserved in 80% ethanol. Plastic cups (5cm x 5 cm x 5cm) were filled with 20 ml of 30% ethanol and set randomly at a 5 cm depth with the mouth of the cups flush with the surface soil level. Five traps were set throughout each site and collected after 24 hours. Pieces of decaying wood and unscented toilet paper tissues were placed as baits at each pitfall trap (Taylor *et al*. 1998).

Litter and decaying pieces of wood carrying mud galleries were collected randomly from each site. Termites were sorted by a pair of forceps and preserved in 80% ethanol. Sawdust, cattle dung or toilet paper wetted with water was wrapped in an aluminium foil (each of 100g) to make the baits. Holes were made in the foil to facilitate the entry of termites. The bait packs (n = 5) were set randomly at a 5 cm depth on the 26^{th} of September, 2008 and observed after 3 months.

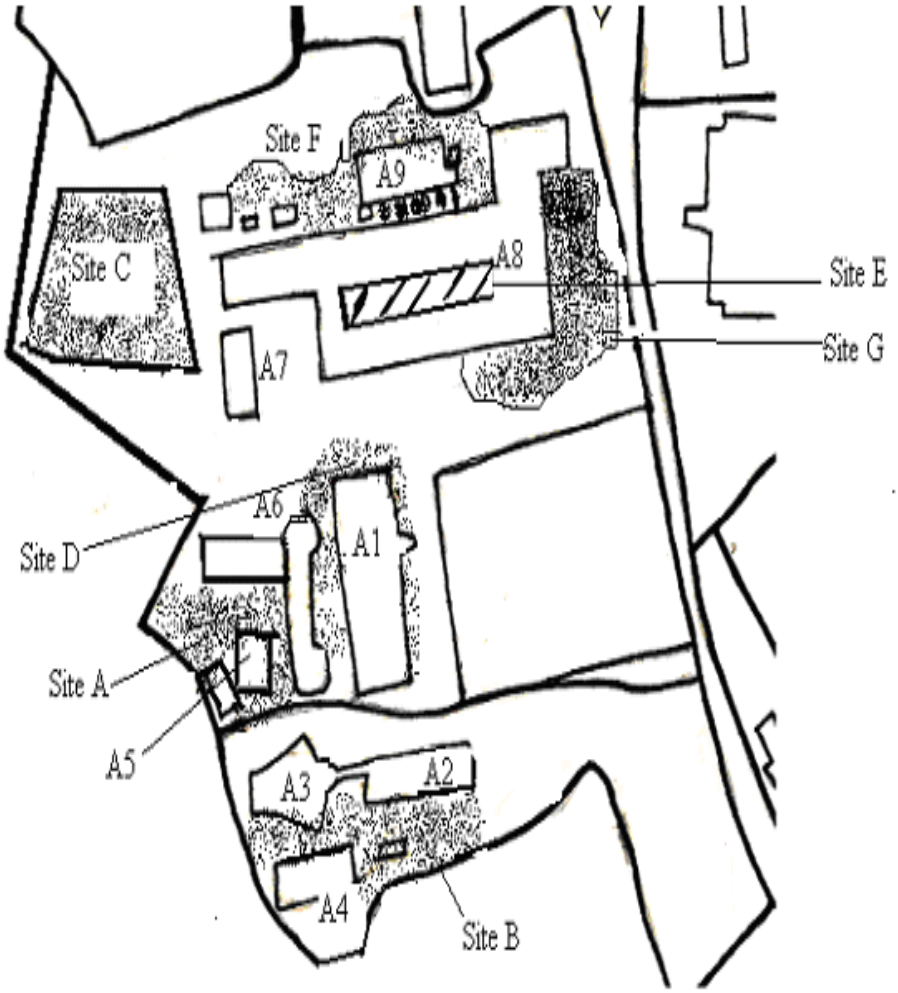

Figure 1: A schematic diagram showing the sites in the premises of Faculty of Science, University of Kelaniya

Table 1: Total number of samples collected by each method from each site

Region	Sampling method	Number of samples taken by each method
A	Soil sifting	25
	Litter sifting	6
	Hand collection	5
B	Soil sifting	15
	Litter sifting	5
	Hand collection	4
C	Soil sifting	25
	Hand collection	6
	Baited pitfall traps	5
D	Soil sifting	10
	Litter sifting	2
	Baited pitfall traps	5
E	Soil sifting	10
	Litter sifting	2
	Hand collection	1
F	Soil sifting	20
	Litter sifting	4
	Hand collection	2
G	Soil sifting	10
	Hand collection	3
	Baited pitfall traps	5
Building A5	Long term baits	4
Building A6	Long term baits	4
Building A8	Long term baits	4

Collection of termites from the buildings

The bait packs (n = 12) described previously were set randomly at selected places in the building A5, A6 and A8.

Measurement of environmental parameters

Soil temperature (n = 3) and pH (n = 4) at each site were measured using a thermometer and a pH meter. Percentage soil humidity was estimated by collecting four soil samples, each of 100 g, from each site and keeping them in an oven at 105 ^{0}C for twenty four hours.

$$\text{Soil moisture\%} = \frac{(A - B)}{B} \times 100$$

A = Initial weight of the soil sample

B = Weight of the soil sample after drying at 105 ^{0} C for 24 hours.

Laboratory analysis of termites

Termites in each sample were identified to the furthest possible taxon under a low power stereo-microscope at suitable magnifications with reference to Roonwal & Chhotani (1989) and Chhotani (1997). A calibrated micrometer eye piece fixed to a high power microscope at x10 magnification was used to record each measurement. In mandibulate soldiers, total length, head length and width, mandible length, pronotum length and width, length and width of post-mentum of individuals were measured. Head index (maximum width of head/ length of head to lateral base of mandibles), mandible-head index (maximum length of mandibles/length of head to lateral base of mandibles), pronotum-head index (maximum width of pronotum/ maximum width of head) were estimated according to Roonwal & Chhotani (1989). Length of head, length of rostrum and posterior bulge of head of five individuals were measured in non-mandibulate soldiers. Rostrum-head index (length of head rostrum/ length of head without rostrum) was calculated for each soldier termite according to Roonwal & Chhotani (1989).

Procedure for studying the interactions between *Odontomachus simillimus* and *Odontotermes ceylonicus*

The trials were performed in a regiform structure (Figure 2) that consisted of a larger cavity (diameter: 10 cm) and two small cavities (diameter: 5 cm). A 10 cm long channel connected the three cavities with each other. This structure was dorsally covered with a glass. Soldiers and workers of *O. simillinus* were collected from the Science Faculty premises of University of Kelaniya and kept in a covered plastic basket in the laboratory at a temperature range of 28 - 29 $^{\circ}$C. Soldiers and workers of *O. ceylonicus* were collected from the Science Faculty premises on the date of experiment. First, major workers of ants (n = 20) were introduced to the larger cavity with colony materials. Soldiers (n = 2) and workers (n = 7) of termites were introduced to a small cavity with 1g of moist soil and a piece of gauze cloth wetted with distilled water. Small cavity at the middle was kept blocked initially with a piece of regiform. The insects were allowed to acclimate for half an hour. The small cavity was unblocked after half an hour and the activities of test insects were observed for eight hours. Two other trials were also conducted and the mortality of termites was recorded after eight hours. Termites which were kept in an ant-free environment served as the control for each trial.

Figure 2: The structure used to study the interaction between *O. simillimus* and *O. ceylonicus*

Procedure for testing the effects of citronella oil and Neemazal-F on *O. horni*

A bottle of citronella oil (100%) was purchased from Beem Hela Osu Lanka & Neemazal-F was provided by Islandwide Marketing Service (Pvt) Ltd. Both soldiers and workers of *O. horni* were collected from the Science Faculty premises of University of Kelaniya on the date of experiment. Topical application of each product to ten individuals was carried out by using a micro-applicator. A series of volumes of citronella oil (0.5µl, 1.0µl, 2.0µl, 3.0µl, 4.0µl, 5.0µl and 6.0 µl) and a series of volumes of Neemazal-F (0.5µl, 0.8 µl, 1.0 µl, 2.0µl, 3.0 µl and 4.0 µl) were applied to the prothorax of each termite separately. This experiment was repeated twice with two other sets of termites. Control termites were kept without applying botanical products simultaneously. Time (s) taken by each individual to die after the application of each product was recorded. Numbers of dead termites in each trial were analyzed using Chi-square test with Yates' correction to investigate whether *O. simillimus* acts as a natural enemy of *O. ceylonicus.* Lethal dosage of each product (LD_{50}) was estimated by the probit analysis.

RESULTS

Although several methods were employed soil sifting, direct hand collection and litter sifting were only positive for termites. Soil sifting was successful in collecting termites more often than other methods carried out in each region (Table 2). Termites were not attracted to the baits kept at indoor sites.

Based on the major morphological features and morphometric measurements of soldiers (Table 3), termites belonging to two families, Termitidae and Rhinotermitidae, five genera and seven species were identified (Table 4).

Distribution of termite species in the premises of Science Faculty and the environmental parameters of each locality are shown in Table 5. The most common *O. horni* showed a wide distribution whereas the least common species, *T. rubidus* was observed at the site G only.

Interactions between *O. simillimus* and *O. ceylonicus*

Number of dead termites observed in each trial (Figure 3) in the presence of *O. simillimus* was significantly higher than that observed in each control (Chi-square test, $p < 0.05$).

Effects of Citronella oil and the Neemazal-F on *O. horni*

Mean survival time of *O. horni* gradually decreased with the increase of citronella oil volume from 0.5 µl to 6 µl (Figure 4) and increase of neemazal- F volume from 0.5 µl to 4 µl (Figure 5). The lethal dosages (LD_{50}) of citronella oil and Neemazal-F were 11.15 mg and 8.21 mg per body weight g^{-1}, respectively.

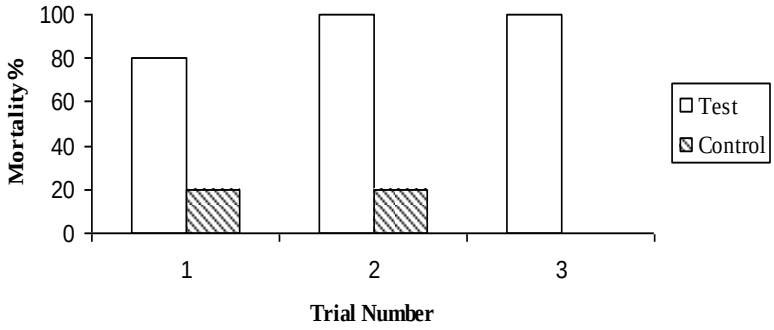

Figure 3: Percentage mortality of termites observed in each trial

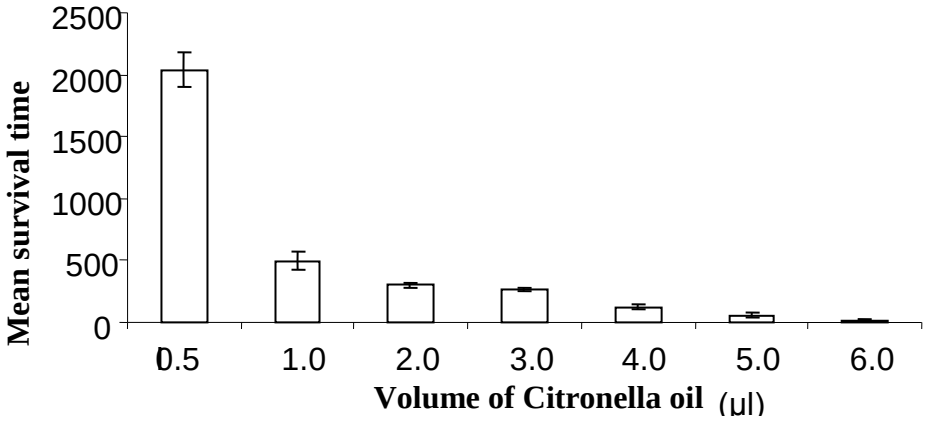

Figure 4: Mean survival time (s) ± SE of *O. horni* for each volume of citronella oil

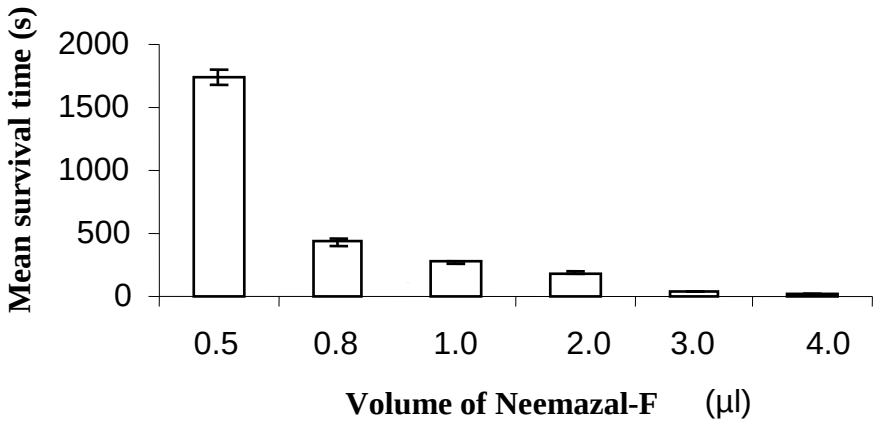

Figure 5: Mean survival time ± SE of *O. horni* for each volume of Neemazal-F

Table 2: Methods that resulted termites and the frequency of occurrence of termites by each method in each region.

Study region	Sampling method	Frequency of occurrence of termites (sample wise)
A	Soil sifting Litter sifting Hand collection	10/17 3/17 4/17
B	Soil sifting Litter sifting Hand collection	7/10 1/10 2/10
C	Soil sifting Hand collection	14/16 2/16
D	Soil sifting Litter sifting	5/6 1/6
E	Soil sifting Hand collection	4/5 1/5
F	Soil sifting Litter sifting Hand collection	5/7 1/7 1/7
G	Soil sifting Hand collection	3/4 1/4

Table 3: Morphometrics (mm) of the seven termite species identified during this survey

Total length	-	3.2-3.5	2.9-4.0	3.0-3.8	5.7-5.9	7.0-8.5	4.2-4.3	3.25-3.4
Head length	1.75-2.0	1.2-1.25	1.35-1.45	0.8-0.9	1.8-1.9	2.0-2.5	2.25-2.32	1.5-1.6
Head width	1.2-1.25	0.75-0.8	0.8-1.8	0.7-1.0	1.38-1.4	2.0-2.15	-	1.4-1.5
Mandible length	L-1.3-1.45 R-0.97-1.1	0.5-0.53	-	-	1.1-1.2	1.4-1.6	-	-
Posterior bulge of head	-	-	0.28-0.30	0.23-0.38	-	-	0.4-0.6	1.75-2.0
Pronotum width	0.65-0.75	0.56-0.58	0.40-0.45	0.38-0.45	1.0-1.5	1.6-1.7	0.4-0.55	0.38-0.4
Pronotum length	-	0.3-0.32	0.18-0.20	0.18-0.22	0.56-0.58	0.75-0.95	3.6-3.75	0.18-0.23
Post-mentum length	-	0.75-0.78	-	-	0.8-0.95	1.5-1.75	-	-
Post-mentum width	-	0.25-0.3	-	-	0.5-0.6	0.75-0.95	-	-
Rostrum length	-	-	0.45-0.5	0.45-0.5	-	-	0.75-0.8	0.6-0.63
Rostrum head length	-	-	0.9-1.2	1.25-1.35	-	-	1.45-1.53	1.42-1.5
Head index-A	0.6-0.7	0.63-0.64	-	-	0.75-0.8	0.86-1.0	-	-
Mandible-Head index	-	0.42-0.43	-	-	0.6-0.64	0.64-0.7	-	-
Pronotum-head index	0.37-0.38	0.46-0.50	-	-	0.55-0.6	0.68-0.80	-	-
Rostrum head index	-	-	0.42-0.5	0.36-0.37	-	-	0.5-0.52	0.4-0.42
Species name	*Dicuspeditermes incola*	*Heterotermes ceylonicus*	*Nasutitermes ceylonicus*	*Nasutitermes horni*	*Odontotermes ceylonicus*	*Odontotermes horni*	*Trinervitermes .rubidus* Soldier major	*Trinervitermes rubidus* Soldier minor

Table 4: Preliminary inventory of termites (Order: Isoptera) of the Science Faculty premises of University of Kelaniya

Family	Genus	Species	Plate No.
Rhinotermitidae	*Heterotermes*	*H. ceylonicus* Holmgren	Plate 2 - soldier
Termitidae	*Dicuspeditermes*	*D. incola* Wasmann	Plate 1- soldier
Termitidae	*Nasutitermes*	*N. ceylonicus* Holmgren	Plate 3 - soldier form 1 Plate 4 – soldier form 2
Termitidae		*N. horni* Wasmann	Plate 5-soldier form 1 Plate 6 - Soldier form 2
Termitidae	*Odontotermes*	*O. ceylonicus* Wasmann	Plate 7 - Soldier
Termitidae	*Odontotermes*	*O. horni* Wasmann	Plate 8 - soldier
Termitidae	*Trinervitermes*	*T. rubidus* Hagen	Plate 9 - Soldier major Plate 10 - Soldier minor

Table 5. Distribution of termite species in the premises of Science Faculty and environmental parameters

Site	Soil T^0 C Mean ± SD	% Soil moisture	Soil pH Mean ±	*D. incola*	*H. ceylonicus* Holmgren	*N. ceylonic*	*N. horni* Wasmann	*O.ceylonic us*	*O. horni* Wasman	*T. rubidus* Hagen
A	30.3 ± 0.6	14.9 ± 1.6	6.7 ± 0.4	-	1	4	-	-	13	-
B	28.0 ± 1.0	15.3± 4.7	6.4± 0.2	1	1	2	1	-	5	-
C	28.0 ± 1.0	15.0 ± 1.8	7.0 ± 0.2	1	2	3	3	2	5	-
D	30.3 ± 0.6	7.0 ± 3.6	7.5 ± 0. 5	-	-	-	-	-	6	-
E	29.0 ± 1.0	8.4 ± 1.1	6.8 ± 0.4	-	-	-	-	-	5	-
F	29.0 ± 1.0	9.0 ± 2.4	6.4 ± 0.6	1	-	1	-	1	4	-
G	29.3 ± 1.5	8.20 ± 2.4	5.5 ± 0.7	-	-	-	-	1	2	1
Frequency of occurrence (region wise)				3/7	3/7	4/7	2/7	3/7	7/7	1/7

Plate 1: *Dicuspeditermes incola*: soldier

Plate 2: *Heterotermes ceylonicus*: soldier

Plate 3: *Nasutitermes ceylonicus* soldier I

Plate 4: ***Nasutitermes ceylonicus*** **soldier II**

Plate 5: ***Nasutitermes horni*** **soldier I**

Plate 6: ***Nasutitermes horni*** **soldier II**

Plate 7: *Odontotermes ceylonicus* soldier

Plate 8: *Odontotermes horni* soldier

Plate 9: *Trinervitermes rubidus*: soldier I

Plate 10: *Trinervitermes rubidus*: *soldier II*

DISCUSSION

Termite fauna at two different elevations of Hantane hills, Kandy consisted of the members of Termitidae only and the genera *Nasutitermes*, *Dicuspiditermes* and *Odontotermes* recorded there (Hemachandra *et al.* 2010) were also observed during the current survey. The two species, *Odontotermes ceylonicus* and *O. horni* were also present at Hantane hills showing their wide distribution in the country. The genus *Ceylonitermellus* recorded from Hantane hills was not found during this survey. The systematics of termites that inhabit other regions of Sri Lanka has not been well-documented and therefore these findings cannot be discussed with regard to any other similar survey conducted in Sri Lanka. Among the sampling methods used in this survey, soil sifting can be highly recommended for similar surveys to be conducted in the future and litter sifting method would also be useful when employed together with the sifting of soil (Table 2). Although termites could not be collected by keeping bait packs at indoor and outdoor locations, mud galleries observed in the bait packs indicated that termites had invaded them for a short period of time.

The preliminary inventory of termites (Table 4) has been prepared by sampling them for a relatively short period, from September, 2008 to April, 2009, due to the limited time period available, and this survey should be

conducted at least throughout an year including both dry and rainy months in the future. Also, the survey of termites should be extended to other areas in the premises of University of Kelaniya and Sri Lanka (Kumarasinghe 2008). The reasons for the absence of the members of family Kalotermitidae during this survey are unknown.

Seven termite species recorded during the survey were not similarly distributed in the seven regions (Table 5) and *O. horni* showed a wide distribution because its larger size and the higher degree of sclerotization of the cuticle allow it to survive under wide ranges (Table 5) of environmental conditions (Pearce, 1999). Presence of *Trinervitermes rubidus* at a single location only should be due to its preference for lower soil moisture and lower pH existed at that region (Table 5).

Odontomachus simillimus is a very common ant species in the Science Faculty premises of University of Kelaniya (Dias 2006). Current results showed that this species may play a significant role in the natural control of *O. ceylonicus* in the area. Also, there is a possibility to use *O. simillimus* as a biological control agent of *O. ceylonicus*. Not only *O. ceylonicus* but also most of the subterranean termite species recorded from this survey act as pests of crops and human belongings.

Citronella oil and Neemazal-F can be used to control *O. horni* effectively and it will have an insignificant hazard to the environment. In addition, it will be less expensive when compared with the use of hazardous synthetic chemicals for control of this termite species (Kumarasinghe 2008). Field studies for the control of other termite species that are pests and encountered during current survey, should be conducted using citronella oil and Neemazal-F in the future for the confirmation of the findings of the laboratory investigation.

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