Termite sampling methods: A comparative study in four habitats of north Kerala

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ABSTRACT: Initially, three termite sampling methods were compared in a coastal area of North Kerala to check the efficiency of the widely followed standardized belt transect protocol (Jones and Eggleton, 2000) with a simplified belt transect protocol and random search method. Of the total 11 species that belong to 4 genera collected during the study, the standardized belt transect protocol recorded only two genera and 5 species in 20 hours of sampling effort, while the simplified protocol with half the effort (10 hours) recorded two genera and 4 species. Random search method with least effort (6h) recorded 4 genera and 11 species that included all the species collected in the earlier methods. There was a marked difference in sampling efficiency; the random sampling method yielded 1.87 species per hour while standardized belt transect protocol and simplified belt transect protocol yielded only 0.25 and 0.4 species per hour respectively. The result of the study was further verified in three more habitats viz. natural forest, coffee plantation and tea plantation which gave similar results. The study indicates that the random search method which covers more area in less time yield more representative termite fauna in all the four habitats tested, than the standardized belt transect protocol which spends more time covering less area.
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Appropriate sampling methods are important for studying the diversity of any organism. There are different sampling protocols for different organisms and different habitats. Absence of adequate sampling strategies is an impediment to our understanding of many groups of organisms. Soil organisms in general and termites in particular are among such organisms. In spite of their high diversity and importance, an efficient and foolproof sampling strategy is still lacking.

Davies *et al*. (2012) reported that a particular sampling method may not be the most appropriate or effective for all habitats and active searching (modified version of standardized belt transect of Jones and Eggletton, 2000) was most effective method of termite sampling in mesic savannas and baiting in arid savannas. Zeidler *et al.* (2004) also suggests baiting experiments to arid environments. The major limitation with bait experiment is that it only document cellulose feeding termite species, thereby excluding the soil feeding termites and it is less effective in wet season (Davies *et al*., 2021).

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The standardized belt transect (Jones and Eggleton, 2000) protocol is considered as the most appropriate method for sampling of soil termites in tropical areas (Eggleton *et al.,* 2002; Donovan *et al.*, 2002; Varma and Swaran, 2007; Shanbhag and Sundararaj, 2013). Other suggested methods include different forms of baiting (Abensperg-Traun, 1993; Taylor *et al.,* 1998; Gromadzki, 2003), mound density counts (Traore and Lepage, 2008) and litter bags (Yamashita and Hiroshi, 1998). The standardized belt transect protocol is designed to cover only a small area (100m x 4m transect), within which, much time (20 h) is spent for thorough searching. There is a possibility that if more area is sampled, more species could be obtained. The present study was designed to check this hypothesis by comparing the efficiency of the standardized belt transect protocol with simplified methods of termite sampling in different habitats with lesser effort covering more area.

The study was completed in two steps - Initial comparison of three methods in the coastal area and then verification of the results in three other habitats in Wayanad district.

Study area: The initial field trials were conducted in the coastal area at Mavilakadappuram, a small village near Cheruvathur in Kasaragod district, (12.191733 N; 75.124300 E). The area was selected after a pilot survey that indicated high termite diversity. The plot selected for study had dimensions of 150m x 30m and located about 60m away from the coastline. Further verification of the results was done in three habitats in Wayanad district viz., a forest ecosystem at Thrissileri (11.8325 N; 76.0392 E), a coffee plantation at Thonichal (11.4550 N; 75.5910 E) and a tea plantation at Thalappuzha (11.8332 N; 75.9677 E).

Sampling Methods:

A. Testing three sampling methods in coastal area: Three sampling methods compared in the study were: standardized belt transect protocol (Jones and Eggleton, 2000), a simplified belt transect protocol and random search method.

1) Standardized belt transect protocol (Jones and Eggleton, 2000) – 20h. sampling: Standardized sampling protocol is based on a belt transect of 100 m length and 4 m width. The transect was divided into 20 contiguous sections of 5m x 2m and numbered sequentially (Figure 1). A total of one hour (20 minutes each by 3 investigators) time spent searching for termites in every alternate section. So a total of 20 man hours spent for sampling. Microhabitat like surface soil, accumulations of litter and humus at the base of trees; all subterranean nests, inside of dead logs, mounds and runways on vegetation were observed in each section up to a height of 2m above the ground level. Belowground searches were also made in 12 small plots per section each about 12×12cm, to 10cm depth.

2) Simplified belt transect protocol – 10h. sampling: This is a modified form of the standardized belt-transect protocol by reducing the sampling time from one hour to half an hour (10 minutes by 3 investigators) per each section. Only 6 plots of 12×12cm, to 10cm depth were sampled per each section, instead of 12. All other things remain the same.

3) Random search method – 6h sampling: The whole area of the plot (150m x 30m) was divided into six parts and randomly searched for termites. One hour was spent searching for termites in each part. Searching was done over the soil surface, dead
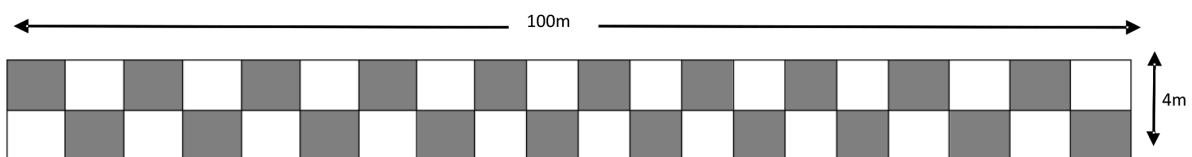

Fig. 1 Lay out of the sampling methods (Shaded area-Standardized belt transect protocol, unshaded areas - Simplified belt transect protocol)

wood, subterranean nests and other microhabitats up to a height of 2m above the ground. Search was not done in soil below ground level.

B. Testing sampling methods in three other habitats: The result of the study in coastal area was further verified in three ecologically different areas: a natural forest (unaltered natural ecosystem), a coffee plantation (least managed monoculture land), and a tea plantation (intensively managed monoculture land). Plots selected with 150m x 30m area in each habitat. Only the standardized belt-transect protocol (with reduced time of 6hrs.; 18 minutes per section and 3 plots of 12×12cm, to 10cm depth per each section) and random search method (6h) were compared. The collection methods followed were the same as mentioned before.

Collection and preservation of samples: Specimens were sampled from every encountered termite population. Preferably workers and soldiers were collected by using wet brush or forceps; placed in a vial containing (70%) ethanol and labeled with specimen number, habitat, collection locality, date and time. The preserved specimens were examined under a stereo zoom microscope at 45x magnification and identified up to species level using key published by Roonwal and Chhotani (1989) and Chhotani (1997). All collected specimens were deposited in the Zoology museum, Payyanur College, Edat, Kerala, India.

Statistical analysis: The efficiency of sampling methods is calculated as the number of samples and species obtained for unit effort as follows.

Sampling efficiency = total number of samples and species / total hours spent.

Efficiency of different methods were compared through analysis of variance (ANOVA) using SPSS and Shannon index of biodiversity (H) using the PAST software (Hammer *et al.,* 2001).

Shannon index $\qquad H = -\sum_{i=1}^{s} (p_i * \ln p_i)$

where:

H = the Shannon diversity index

Pi = proportion of the population made up of species i

s= numbers of species in sample

A. Sampling in coastal area

The number of termite samples and species recorded from the three termite sampling methods in coastal area are presented.

1) Standardized belt transect protocol: Twenty two samples were collected during the 20 hours of observation, 18 samples from surface soil and 4 samples from belowground soil (upto 10 cm depth). All the samples collected from deeper soil were devoid of soldiers, which could be identified only upto genus level. They belonged to two genera which were already recorded - *Odontotermes* and *Microcerotermes*. Thus a total of 5 species under 2 genera were documented (Table 1).

2) Simplified belt transect protocol: A total of 7 samples were collected during the 10 hours of observation that belonged to 4 species under 2 genera (Table 1). Two samples could be identified only up to generic level and they both belonged to *Microcerotermes*.

3) Random search method: A total of 19 samples were collected during the 6 hours of observation and they belonged to 11 species under 4 genera (Table 1). Three samples were identified only up to generic level and they belonged to the three genera - *Odontotermes, Heterotermes* and *Microcerotermes*.

The random search method is distinctly efficient when compared to the other two methods. The highest number of termite species per hour (1.83) and samples per hour (3.17) were recorded by the random search method. Standardized belt transect protocol recorded only 0.25 species and 1.1 samples per hour and simplified belt transect protocol recorded 0.4 species and 0.7 samples per hour sampling effort. The Shannon index of diversity was also found much higher in the random search method (2.253) compared to standardized belt transect protocol (1.413) and simplified belt transect protocol (1.332).

B. Sampling in three other ecosystems

Results of the two termite sampling methods in three

Table 1. Species and number of samples collected in three sampling methods in the coastal area

Species	No. of samples per protocol/ method			Total
	Standardized belt transect (20h)	Simplified belt transect (10h)	Random search (6h)	
Heterotermes balwanti	-	-	1	1
Heterotermes indicola	-	-	1	1
Odontotermes feae	-	-	1	1
Odontotermes giriensis	-	-	2	2
Odontotermes obesus	4	-	4	8
Odontotermes redemanni	5	1	2	8
Odontotermes vaishno	1	1	1	3
Odontotermes yadevi	-	-	1	1
Microcerotermes fletcheri	2	2	1	5
Microcerotermes pakistanicus	1	1	1	3
Synhamitermes quadriceps	-	-	1	1
Unidentified samples	9	2	3	14
Total samples	22	7	19	48
Total species	5	4	11	11
Samples per hour	1.1	0.7	3.17	
Species per hour	0.25	0.4	1.83	

different ecosystems are given in Table 2. A total of 14 species from 96 samples were recorded in this study. Thirteen species were collected in random search method and standardized belt transect protocol could collect only eight species. The highest number of termite species per hour was recorded in the forest ecosystem (1.33) by random search method. In the standardized belt transect protocol it was only 0.67. The lowest number of termite species per hour was recorded in coffee plantation and tea plantation by standardized belt transect protocol (0.5 each). In the random search method, it was 1.17 and 0.67 respectively.

Number of samples per hour recorded by random search method in coffee plantation was 3.5 and in forest ecosystem and tea plantation it was 3.33. In standardized belt transect protocol it was 1.83 for both forest ecosystem and coffee plantation and 2.17 for tea plantation).

The highest number of termite samples was found in random search method. Out of 96 samples, 61

samples were collected in random search method. Standardized belt transect protocol recorded 35 samples only. During the study, the maximum efficiency was obtained in the random search method.

Comparing the effectiveness of the two sampling methods, there was a significant difference (ANOVA, $P<0.05$). The diversity indices calculated, also depicts the efficiency of random search over standardized belt transect protocol. By random search method, Shannon diversity index value for forest ecosystem was 2.056, coffee plantation was 1.493 and tea plantation was 1.277 and it was 1.121, 0.759 and 0.687 by standardized belt transects protocol. The values show more termite diversity obtained via random search method in all the three different ecosystems when compared with standardized belt transect protocol. The result of the current study reveals that the random search method with least effort yield the maximum number of samples and species.

Table 2. List of species recorded from three ecosystems by different sampling protocol/method

Species	Forest ecosystem		Coffee plantation		Tea plantation		Total
	Standardized belt transect	Random search	Standardized belt transect	Random search	Standardized belt transect	Random search	
Odontotermes anamallensis	-	-	1	1	-	-	2
O. assmuthi	-	-	-	-	1	5	6
O. boveni	-	-	-	-	2	-	2
O. ceylonicus	-	-	2	1	-	-	3
O. feae	1	3	-	1	-	1	6
O. obesus	-	-	-	-	-	3	3
O. redemanni	6	1	8	12	10	10	47
O. vaishno	1	1	-	-	-	-	2
O. sps	3	3	-	-	-	-	6
O. yadevi	-	2	-	2	-	-	4
Narulitermrs indicola	-	5	-	-	-	-	5
N. sp.	-	1	-	2	-	-	3
Ampoulitermes wynaadensis	-	2	-	-	-	-	2
Ceylonitermellus peryarensis	-	-	-	1	-	-	1
Unidentified sp.	-	2	-	1	-	1	4
Total samples	11	20	11	21	13	20	96
Total species	4	8	3	7	3	4	14
Samples per hour	1.83	3.33	1.83	3.5	2.17	3.33	
Species per hour	0.67	1.33	0.5	1.17	0.5	0.67	

There were some attempts to modify the standardized belt transect protocol by reducing sampling size and/or sampling time (Davies *et al*., 2013; Schyra and Korb, 2019; Effowe *et al*., 2021). In the present study, the modified version with reduced time did not yield promising results. The random search method with least effort yielded the maximum number of samples and species for all the four habitats tested. It is also important to note that except for a single species (*O. boveni;* in the tea plantation), all the species recorded from the standardized belt transect protocol were recorded from the random search method at all the four sampling sites. On the other hand, the random sampling recorded 6, 4, 4 and 2 species each, in addition to that recorded in standardized protocol, from the four sites.

The random search method, covering more surface area, yielded more samples and species and the time and effort spent for standardized belt transect protocol did not yield comparable results. The probable reason for low efficiency being the high effort for sampling below ground (12 sections of 10 cm depth as mentioned before) which yielded only few samples; that too mostly devoid of the soldier caste, that made identification difficult. Majority of the termite samples and species were collected from the soil surface within mudplasters. There is every chance to get majority of the termites from the surface soil as the deeper termites too come up to the soil surface for foraging. Thus covering maximum surface area and microhabitats seems more important, at least for preliminary termite sampling of a tropical habitat. Searching

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belowground can be still relevant for extensive sampling as it may help to collect any missed out species.

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