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Environmental influence on physico-chemical and biological activities of fungus growing termite (Isoptera: Macrotermitinae)

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Fungus cultivating termites are important, highly specific insect microbe symbionts in Asia and Africa: The aim of this study was to determine physical, chemical and biological parameters of the fungus comb of *Macrotermitinae* collected from the different locations of Western Ghats of Tamil Nadu. The pH of the fungus comb was 4.4 to 4.8 and moisture content about 46.92 to 52.80%. The Carbohydrate: Lignin ratio of old combs varied between 1.55 to 2.08 (w/w) and C: L ratio of New comb varied between 0.6 to 0.86 (w/w). The nitrogen, phosphorus and potassium content of fungus comb varied from 0.75 to 1.8%, 0.175 to 0.193% and 0.075 to 0.08% on dry weight of fungus comb, respectively. The distribution of other fungi and also the symbiotic fungi *Termitomyces* in the nest of *Macrotermitinae* was also investigated in this study.

Key words : *Macrotermitinae*, Fungus comb, Microbial population, Nutrient content, Lignin content.

INTRODUCTION

In tropical ecosystems, the main influence of macro invertebrate such as earthworms and termites on soil characteristics is by the creation of biogenic structure *viz.*, gallery, voids and tunnels (Lavelle, 1997). The symbiotic relationship with fungi were observed in various insects (Kendrick, 1991), including scale insects (Hemiptera), gall midges (Diptera), wood- wasps (Hymenoptera), beetles (Coleoptera), aphids (Homoptera), ants (Hymenoptera) and termites (Isoptera). Among these insects, ants (fungus-growing ants, sub family Myrmicinae) and termites, (fungus-growing termites, subfamily *Macrotermitinae*) are well known to have a major impact on tropical ecosystems (Cherrett *et al.*, 1989; Wood and Thomas, 1989). The group of fungus growing termite is interesting, have symbiotic relationship with basidiomycetes fungi of the genus *Termitomyces*, in this relationship temite cultivate the symbiotic fungi with in their nest. The insect cultivates its symbionts *Termitomyces* on a special medium, the fungus comb, which is maintained within the nest. The symbiotic fungi grow on a sponge like structures (called a fungus comb) constructed by the temites from a litter. They are found as mycelia and white round structures (called fungus nodules) on the fungus comb surface. The temites water their fungal gardens with temite excretions and thus maintain the necessary humidity for fungal growth. The temites building ventilation ducts into their mound so that the fungi are properly aerated.

The fungus comb are constructed from temite faecal pellets with partially digested plant debris; old combs are consumed by the temites (Darlington, 1994). To construct fungus comb, different Macrotemitinae species feed on different plant materials; usually one of wood, leaf litter or grass. For example, *Macrotermes carbonarius* feeds on leaf litter (Abe and Matsumoto, 1979; Jones and Brendell, 1998; Sands, 1998) are likely to be predominantly wood feeders. The chemically analyzed fungus comb of different ages to assess lignin degradation with in them.

MATERIALS AND METHODS

Samples of fungus comb of *Macrotermitinae* collected from the temite mound at five different locations of Western Ghats of Tamil Nadu *viz.*, Location, Mettupalayam; Location, Ooty; Location, Kodaikanal; Location, Anaikatti; Location, Thadiyankudisai and Location, Sotthuparai. The samples were taken to the laboratory for experiment under controlled condition. The collected samples were analyzed for their physico, chemical and biological properties.

Isolation of microorganism from fungus comb:

The bacteria, fungi and actinomycetes were isolated from the fungus comb by dilution plates method in respective media. All the plates were incubated at 28^0 - 30^0C for 3-5 days and counts were made. The results were expressed as number of organisms per g of sample on dry weight basis.

Chemical composition of the fungus comb

The chemical composition like moister content, pH, carbohydrate and lignin ratio and major nutrients like nitrogen, phosphorus and potassium were estimated in collected fungus comb as per standard procedure.

Determination of carbohydrate content in fungus comb:

The total carbohydrate content of the fungus combs were estimated by Anthrone method. The samples were hydrolyzed with HCl and neutralized with sodium carbonate. The volume was made upto 100 ml and centrifuged, 0.5 and 1.0 ml of aliquots were taken from supernatant for analysis. The amount of carbohydrate present in the sample was calculated using glucose as standards.

Determination of lignin content in fungus combs:

The lignin content in the fungus combs were determined by gravimetric method (Zadrazil and Brunert, 1960). One g of fungus comb, 5 ml of conc. H_2SO_4 and 50 ml of 37% HCl was taken and kept for 16, h at 25°C. The mixture was transferred into 1 liter flask with 450 ml distilled water and boiled for 10 min and filtered through geena G3 glass filter. The acidic residues were washed to neutrality with distilled water, then dried at 105°C and weighed. The results were expressed as Carbohydrate / Lignin ratio.

Estimation of major nutrients in fungus combs:

The fungus combs were dried, powdered and 0.5g of representative sample was taken for the estimation of major nutrients. The samples were digested with diacid for the estimation of total nitrogen and with triple acid for the estimation of phosphorus and potassium. The total nitrogen content (Humphries, 1956), Phosphorus content (Jackson, 1973) and Potassium content (Stanford and English, 1949) were estimated as per the standard procedure.

RESULTS AND DISCUSSION

Microbial population in fungus combs:

Fungus combs of different location were collected and analyzed for its microbial population. The result showed that bacterial population of the fungus combs ranged from 25.6-36.2 x 106 cfu / g sample dry weight. The fungi and actinomycetes populations ranged from 92.8-112.8 x10^4 and 8.4 -13.1 x 10^3 cfu / g sample dry weight, respectively. The results indicated that the fungal population was higher when compared to bacteria and actinomycetes (Table 1). This showed that the acidic environment in the fungus comb reduce the population of bacteria. The isolated bacterial population was identified as *Bacillus*, *Azotobacter*, *Beijerinckia* and *Pseudomonas*. Fungi were identified based on morphological characteristics as *Termitomyces*, *Aspergillus*, *Penicillium*, *Trichoderma*, *Fusarium*, *Xylaria* and the actinomycetes as *Streptomyces*.

Table 1 : Analysis of microbial population in fungus combs

Locations	cfu /g sample on dry weight basis		
	Bacteria x 10^6	Fungi x 10^3	Actinomycetes x 10^3
1.	36.2	102.6	8.4
2.	32.4	92.8	13.1
3.	25.6	112.8	11.5
4.	42.5	96.5	10.2
5.	82.8	108.7	14.4
6.	79.4	84.6	11.5

Chemical composition of the fungus combs:

Moisture percentage and pH in the collected samples were estimated as per the standard procedure. The results revealed that the moisture content of the fungus combs ranged from 46.92- 52.80% and the pH varied from 4.4 - 4.8. Though there was not much difference between samples, the highest moisture content and pH were recorded in the sample from location 5 (Table 2). The low pH in the fungus combs would tend to prevent the development of bacteria, which favours fungal population.

Table 2 : Estimation of moisture content and pH of the fungus comb

Locations	Fresh comb	
	Moisture content	pH
1.	51.23	4.6
2.	48.50	4.4
3.	49.85	4.5
4.	46.92	4.4
5.	52.80	4.8
6.	50.64	4.6

Carbohydrate and lignin content of fungus comb:

The collected fresh comb and old comb were assayed for carbohydrate(C) and lignin(L) ratio. The result indicated that C:L ratio was higher in the old comb when compared to new comb. The C:L ratio of old comb varied between 1.55 - 2.08 (w/w) and C:L ratio of New comb varied between 0.6 - 0.86 (w/w) (Table 3).

Table 3 : Analysis of carbohydrate and lignin content of fungus comb

Locations	C/L ratio (w/w)	
	New Comb	Old comb
1.	0.78	1.98
2.	0.64	1.67
3.	0.60	1.55
4.	0.86	2.08
5.	0.84	2.02
6.	0.76	1.90

Nitrogen, phosphorus and potassium content in fungus comb:

The major nutrients like Nitrogen, Phosphorous and Potassium content of fungus comb were estimated. The results of the present study indicated that the nitrogen content of fungus combs varied from 0.75 - 1.8 % on dry weight basis. The phosphorus and potassium contents of fungus combs varied from 0.175 - 0.193% and 0.075 to 0.08% on dry weight basis, respectively (Table 4).

Table 4 : Analysis of nitrogen, phosphorus and potassium content in fungus comb

Locations	Nutrient (%) on dry weight basis		
	N	P	K
1.	1.76	0.172	0.076
2.	1.68	0.175	0.075
3.	0.75	0.168	0.077
4.	1.78	0.193	0.069
5.	1.06	0.186	0.078
6.	1.80	0.177	0.080

Many different species of fungi also isolated from the fungus comb reported by Batra and Batra, 1966. In the active nests of mycelium, mycotetes of *Termitomyces* are the only visible fungal growth, whereas when the comb is removed from the nest, it is soon enveloped by the growth of other fungi reported by Petch (1906). Neither the food store, nor the nest structure, consisting of soil moistened with saliva, support more fungal growth. It has been suggested that the development of other fungi on the comb is prevented by the mechanical activity of the termites, inhibition by the termite secretion (Batra and Batra, 1966), nest microclimate (Sands, 1969), antibiotic production and the chemical composition of the comb (Grasse, 1944). Thomas (1987) reported that the pH of the fungus comb (4.1-4.6) and food store (5.3-5.8) were suitable for the growth of fungi, with the lower pH of the fungus comb also being suitable for the activity of *Termitomyces* cellulases. The moisture content of the fungus comb (36-58%) was suitable for wood rotting fungi. This was also suggested by Grasse (1944). The pH ranged from 3.9 to 4.35 suitable for *Termitomyces* cellulases for its activity (Martin and Martin, 1978). The results of the study on carbohydrate and lignin content estimation was agreed with studies conducted by Hyodo *et al.* (2003) that in *Macrotermes* spp., the carbohydrate lignin ratio of the combs increased with increased comb age, but it decreased or remained the same in *Odontotermes* spp., *H. makhamensis*, *A. pakistanicus* and *P. militaris*. This efficient degradation of lignin in fungus combs has also been reported for *M. gilvus* and *M. carbonarius* (Hyodo *et al.*, 2000). In terms of delignification, the high carbohydrate content in the primary faeces may be very important, since white- rot fungi which include *Termitomyces* spp are not able to use lignin alone as growth substrate, so that other carbon and energy sources, such as glucose and cellulose are required (Kirk *et al.*, 1976). From the ecosystem point of view, we can note that by associating with the lignin decomposer, the fungus growing termites make it possible to utilize lignocellulose nearly completely, reflected in the small volume of their final faces (Darlington, 1994) and therefore, play dominant role in decomposition processes in many areas of the tropics (Abe, 1980; Buxton, 1981). Fungi serve as nitrogen rich food, which is advantageous because the dead plant consumed by termites contains very little nitrogen (Matsumoto, 1976; Collins, 1983). The results revealed that the nitrogen content was high when compared to phosphorus and potassium in the fungus combs. The occurrence of lignin and polysaccharide degradation in the fungus comb during aging is predicted to be accompanied by nitrogen enrichment by eliminating carbon from the substrate. However, there is no evidence to support this prediction. In fact, the published results from *M. subhyalinus* (Abo-khatwa, 1977), *M. natalensis* and *M. ukuzii* (Rohrmann, 1978) revealed that the nitrogen concentration in the old comb is lower than that in the fresh comb or unchanged. Nitrogen depletion during the aging might be caused by the uptake of nitrogen by conidia in the mature comb (Rohrmann, 1978). Thus, it is unlikely that the fungus growing termites selectively consume the old comb on the basis of their nitrogen requirement alone, although the nitrogen concentration of the whole fungus comb is high, relatively to the plant litter collected by the termites and fungus comb produces conidia rich in nitrogen which is consumed to support larvae and nymphs (Collins, 1983). Therefore, the fungus comb is suggested to serve as the most suitable food, because it combines general nitrogen enrichment with specific accessibility of cellulose for digestion.

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