

MICROBIAL DECOMPOSITION OF LEAF LITTER OF *TERMINALIA* IN A TROPICAL FOREST BIOME : BIOCHEMICAL CHANGES DURING DECOMPOSITION

V. P. SINGH* and R. S. DWIVEDI

Botany Department, Banaras Hindu University, Varanasi 221 005

(Received 16 June 1978)

Microbial decomposition of leaf litter of four species of *Terminalia*, viz. *T. tomentosa* Bedd., *T. arjuna* Bedd., *T. chebula* Retz. and *T. belerica*, Roxb. was studied with reference to fluctuation in microbial populations, biochemical changes and energy content. The highest number of fungi belonged to Deuteromycetes and the least to Ascomycetes. Biochemical nature and energy contents of the leaf litter varied at different stages of decomposition.

INTRODUCTION

Leaf litter contains considerable amount of nutrients and bound energy which are released during decomposition. The significance of ecology of litter-decomposing fungi has been emphasized by several workers (Garrett, 1962, 1966, 1975; Webster, 1956; Webster & Dix, 1960; Hudson, 1968; Sharma, 1973; Sharma & Dwivedi, 1975). Litter decomposition in the forest ecosystem was studied by Witkamp (1966a, b, 1969), Witkamp and Van der Drift (1961) and Dwivedi and Singh (1974a, b). The biochemical changes during decomposition of wheat straw were studied by Chang & Hudson (1967). There is little information available regarding changes in biochemical nature and energy contents of litter during microbial decomposition in the tropical forest biome and this forms theme of the present paper.

EXPERIMENTAL WORK AND RESULTS

Four species of *Terminalia*, viz. *T. tomentosa* Bedd., *T. arjuna* Bedd., *T. chebula* Retz. and *T. belerica* Roxb. were selected for the present study. Aluminium pots (30 cm diam.) were filled with 2 kg forest soil collected from beneath the respective plant cover. 50 g air dry litter was spread over the soil surface in five pots. Control was maintained with soil without litter. Pots were watered regularly to

*Present address : Department of Botany, Digvijai Nath Degree College, Gorakhpur, 273 001

TABLE I
Distribution of fungi colonizing the leaf litter of four Terminalia spp. during ten months of decomposition (1971) in laboratory condition

| Classes of fungi | <i>T. tomentosa</i> | | <i>T. arjuna</i> | | <i>T. chebula</i> | | <i>T. belerica</i> | | <i>In all the litter</i> | |
|----------------------------------|---------------------|------------------------|---------------------|------------------------|---------------------|------------------------|---------------------|------------------------|--------------------------|------------------------|
| | % distri- bution | No. of sp. isolated | % distri- bution | No. of sp. isolated | % distri- bution | No. of sp. isolated | % distri- bution | No. of sp. isolated | % distri- bution | No. of sp. isolated |
| PHYCOMYCETES | 6.1 | 4 | 5.8 | 4 | 5.2 | 3 | 7.1 | 4 | 10.5 | 10 |
| ASCOMYCETES | 4.6 | 3 | 7.3 | 5 | 5.2 | 3 | 3.5 | 2 | 6.3 | 6 |
| DEUTEROMYCETES | 87.6 | 57 | 85.2 | 58 | 87.7 | 50 | 87.5 | 49 | 82.1 | 78 |
| Sphaeropsidales | 1.5 | 1 | 4.4 | 3 | 3.5 | 2 | 3.5 | 2 | 3.1 | 3 |
| Melanconiales | 1.5 | 1 | 2.9 | 2 | — | — | 3.5 | 2 | 2.1 | 2 |
| Moniliales | 70.7 | 46 | 64.7 | 44 | 75.4 | 43 | 71.5 | 40 | 63.1 | 60 |
| Moniliaceae | 44.6 | 29 | 35.2 | 24 | 45.6 | 26 | 46.4 | 26 | 34.7 | 33 |
| Dematiaceae | 18.4 | 12 | 19.1 | 13 | 17.5 | 10 | 16.07 | 9 | 18.9 | 18 |
| Tuberculariaceae | 7.6 | 5 | 10.2 | 7 | 12.2 | 7 | 8.9 | 5 | 9.1 | 9 |
| Mycelia sterilia | 13.8 | 9 | 13.2 | 9 | 8.7 | 5 | 8.9 | 5 | 13.6 | 13 |
| Unidentified species | 1.5 | 1 | 1.4 | 1 | 1.7 | 1 | 1.7 | 1 | 1.05 | 1 |
| Total number of species isolated | — | 65 | — | 68 | — | 57 | — | 56 | — | 95 |

maintain the moisture level. pH of the decomposing litter was determined at the time of sampling for analysis of microflora.

Isolation of fungi from litter

Fungi were isolated by dilution plate technique. 10 g litter was suspended in sterile water and 1 ml suspension was inoculated in Petri dishes containing Czapek-Dox-Yeast extract nutrient medium (sucrose, 30 g; NaNO₃, 2 g; K₂HPO₄, 1g; KCl, 1g; MgSO₄.7H₂O, 0.5 g; Yeast-extract, 0.5 g; FeSO₄.7H₂O, 0.01 g; agar, 18 g; distilled water, 1l; pH adjusted to 5.5). Streptomycin (0.07 g/litre) was added to check the bacterial growth. Plates were incubated at 25 ± 1°C and examined regularly after 6 days of incubation to record fungal population.

Isolation of cellulose-decomposing bacteria from litter

Cellulose-decomposing bacteria were isolated and recorded from the decomposing litter employing Fuller and Norman's (1942) water-insoluble cellulose dextrin agar.

TABLE II

Average number of cellulose-decomposing bacteria (in ten thousands)/g oven dry litter isolated from four Terminalia spp. at different stages of decomposition during 1971

| Species | Oct. | Nov. | Dec. |
|---------------------|------|------|------|
| <i>T. tomentosa</i> | 15.8 | 8.4 | 3.5 |
| <i>T. arjuna</i> | 18.9 | 13.8 | 3.5 |
| <i>T. chebula</i> | 15.9 | 7.0 | 5.2 |
| <i>T. belerica</i> | 24.6 | 6.8 | 5.1 |

Moisture content and pH of decomposing litter

Moisture content of decomposing litter was determined by drying known amount at 105°C for 24 hr in an oven and calculating per cent loss in dry weight.

pH was determined by preparing suspension of litter powder in distilled water and using BDH indicator.

Chemical analysis of decomposing litter

Litter was analysed for chemical constituents. Ash was determined by igniting the litter sample in muffle furnace at 60°C for 4 to 6 hr and expressed in per cent dry weight. Total nitrogen was estimated by Micro-Kjeldahl distillation apparatus (Peach & Tracey, 1955) and protein was calculated by multiplying the values of total nitrogen with a constant factor 6.25. Sugar was determined by hydrolysing the material with dilute HCl (2%) and estimated titrating against Fehling solution and amount calculated in per cent. Cellulose was determined by the method of

TABLE III
 pH and % moisture content (on oven dry wt. basis) of litter of four Terminalia spp. at different stages of decomposition during 1971

| Species | March | April | May | June | July | Aug. | Sept. | Oct. | Nov. | Dec. |
|---------------------|-------|-------|------|-------|------|-------|-------|------|------|------|
| <i>T. tomentosa</i> | | | | | | | | | | |
| pH | 5.5 | 6.0 | 7.0 | 6.7 | 8.5 | 7.2 | 8.2 | 8.2 | 8.2 | 8.2 |
| Moisture | 4.9 | 49.1 | 53.0 | 134.9 | 25.1 | 42.7 | 105.9 | 36.4 | 27.6 | 26.6 |
| <i>T. arjuna</i> | | | | | | | | | | |
| pH | 4.9 | 4.3 | 7.2 | 8.2 | 8.5 | 8.0 | 8.1 | 8.2 | 8.2 | 8.1 |
| Moisture | 5.2 | 116.4 | 60.0 | 79.1 | 42.8 | 22.9 | 58.6 | 21.4 | 17.1 | 26.5 |
| <i>T. chebula</i> | | | | | | | | | | |
| pH | 4.5 | 4.0 | 6.7 | 8.2 | 8.2 | 7.1 | 8.2 | 7.9 | 10.0 | 8.2 |
| Moisture | 4.3 | 142.6 | 42.0 | 162.1 | 60.6 | 53.0 | 99.9 | 29.6 | 75.9 | 60.0 |
| <i>T. belerica</i> | | | | | | | | | | |
| pH | 4.9 | 6.8 | 6.7 | 7.2 | 7.0 | 8.1 | 8.2 | 8.5 | 8.5 | 8.5 |
| Moisture | 7.2 | 176.3 | 75.4 | 117.8 | 99.2 | 109.9 | 104.6 | 26.5 | 66.1 | 66.9 |

Waksman & Tenny (1927). Hemicellulose was determined by treating the residue from the alkali extract (Waksman & Tenny, 1927) with 100 ml of 2% H₂SO₄ at boiling temperature for 30 min and finally determining sugar content in the extract by Somogyi's method (1945). Lignin was estimated by treating the material with 80% HCl and residue obtained after hydrolysis was considered as lignin. Total organic carbon was estimated by Walkley & Black (1935) rapid titration method.

The data on biochemical analysis of litter are given in Tables IV and V.

ENERGY CONTENT OF DECOMPOSING LITTER

The calorific values were determined in an Oxygen Bomb Calorimeter (Golley, 1961). A known amount of powdered and dried litter material, not exceeding 1 g, was made into pellets with the help of a mechanical compressor. The pellets were then burnt in Bomb Calorimeter under the current of oxygen (15 lb/inch²) with the help of an ignition wire. The rise in temperature of surrounding distilled water was noted. A blank estimation using Benzoic acid was also done to determine the water equivalent of Bomb Calorimeter. Powdered litter samples were ignited separately in weighed crucibles in muffle furnace at 550–600°C to determine the ash content.

Rise in temperature of distilled water and ash-free dry weight of pellets was known, and the energy content calculated by the following formula :

$$E = \frac{570 \times t + 1350 \times t}{W}$$

where E = Energy content in Cal./g; 570 = Water equivalent of Bomb Calorimeter; 1350 = Volume of distilled water in Bomb; W = Weight of pellets; t = Rise in temperature of distilled water;

The data on energy contents of decomposing litter are given in Table VI.

DISCUSSION

During colonization of decomposing litter, percentage distribution of different classes of fungi varied with different species of *Terminalia* (Table I). The highest number of fungi belonged to Deuteromycetes and the lowest number to Ascomycetes. There was a rise in number of fungi g⁻¹ dry litter followed by a fall till August and again a slow rise in September. In case of *T. arjuna* a continuous decrease in number of mycoflora from September onward was noted. An irregular trend of slow rise and fall up to December was observed in case of rest of the *Terminalia* spp. Generally number of fungi g⁻¹ dry litter decreased during July to December. However, as long as the chemical composition of litter was favourable, mycopopulations increased. During late June to December the maximum number of fungi colonized the litter leading ultimately to disappearance of some species which might have been unable to withstand the competition in relation to other fungi (Webster, 1956; Hudson & Webster, 1958; Sharma & Dwivedi, 1975).

TABLE IV
Ash, organic carbon (C), and total nitrogen (N) (on % oven dry wt. basis) of the leaf litter of four Terminalia spp. at different stages of decomposition during 1971

| Species | March | | | June | | | September | | | December | | |
|---------------------|-------|-------|------|------|------|------|-----------|------|------|----------|------|------|
| | Ash | C | N | Ash | C | N | Ash | C | N | Ash | C | N |
| <i>T. tomentosa</i> | 11.6 | 40.2 | 1.5 | 24.4 | 37.0 | 1.5 | 26.4 | 33.6 | 1.4 | 40.9 | 31.6 | 1.35 |
| <i>T. arjuna</i> | 15.6 | 40.5 | 1.3 | 32.9 | 32.9 | 1.15 | 35.2 | 29.5 | 1.15 | 37.0 | 25.5 | 1.0 |
| <i>T. chebula</i> | 10.7 | 44.1 | 1.3 | 21.1 | 33.7 | 1.0 | 29.4 | 33.2 | 1.0 | 33.7 | 29.5 | 1.0 |
| <i>T. belerica</i> | 10. | 438.1 | 1.15 | 26.6 | 35.8 | 1.15 | 27.8 | 34.2 | 1.1 | 28.9 | 30.9 | 1.0 |

TABLE V
Amount of various organic constituents (on % oven dry wt. basis) in litter of four Terminalia spp. at different stages of decomposition during 1971

| Name of species | March | | | June | | | September | | | December | | | | | | | | | | |
|---------------------|-------|-----|------|------|------|-----|-----------|------|-----|----------|-----|-----|------|-----|------|-----|-----|------|-----|------|
| | S | P | C | S | P | C | S | P | C | S | P | C | S | P | C | HC | L | | | |
| <i>T. tomentosa</i> | 4.8 | 9.9 | 26.6 | 10.5 | 22.2 | 4.6 | 9.9 | 22.3 | 8.4 | 22.2 | 4.0 | 9.2 | 20.0 | 6.5 | 22.0 | 4.0 | 8.9 | 18.1 | 5.8 | 24.0 |
| <i>T. arjuna</i> | 5.6 | 8.5 | 24.8 | 8.7 | 20.0 | 4.2 | 7.5 | 19.2 | 6.2 | 21.0 | 4.1 | 7.5 | 15.1 | 5.1 | 21.1 | 4.1 | 6.6 | 17.1 | 4.3 | 24.9 |
| <i>T. chebula</i> | 5.6 | 8.5 | 23.6 | 8.4 | 23.4 | 5.5 | 6.6 | 22.0 | 7.8 | 23.5 | 4.5 | 6.6 | 20.1 | 6.2 | 24.3 | 4.1 | 6.6 | 18.9 | 5.8 | 25.1 |
| <i>T. belerica</i> | 4.9 | 7.5 | 21.7 | 7.6 | 25.6 | 4.5 | 7.5 | 20.0 | 6.8 | 25.7 | 4.5 | 7.2 | 18.7 | 5.9 | 26.0 | 4.5 | 6.6 | 17.3 | 5.0 | 28.0 |

S= Sugar; P=Protein; C=Cellulose; HC=Hemicellulose; L=Lignin

TABLE VI
*Energy content (Ca²g ash free dry wt.) of leaf litter of four Terminalia spp.
 at different stages of decomposition during 1971*

| Name of species | March | June | September | December |
|---------------------|--------|--------|-----------|----------|
| <i>T. tomentosa</i> | 4551.8 | 4023.3 | 3974.7 | 4023.3 |
| <i>T. arjuna</i> | 4506.2 | 3347.4 | 3208.0 | 3887.1 |
| <i>T. chebula</i> | 4304.8 | 3782.5 | 3797.3 | 4254.0 |
| <i>T. belerica</i> | 3898.1 | 3347.4 | 3380.7 | 3449.6 |

Phycomycetes were represented by only 10.5% of the total fungal population (Table I). *Cunninghamella bertholletiae* and *Rhizopus* sp. were frequently isolated as the primary colonizers. Ascomycetes were also poorly represented (6.3%). *Saccharomyces* sp. appeared at the initial stage, *Chaetomium indicum* at the middle, *Thielavia terricola* at the middle and also at later stage, *Penicillium roseum* at the end, and *Eurotium chevalieri* and *Emericella nidulans* throughout the decomposition. Deuteromycetes were represented by 82% of the total fungal population; *Phoma humicola*, *P. hibernica*, *Pestalotia* sp., *P. macrotricha*, aspergilli, penicillia, *Alternaria humicola*, *Cladosporium herbarum* and *Scolecobasidium constrictum* were the dominant colonizers.

Population of cellulose-decomposing bacteria g⁻¹ dry litter decreased from October onward to its minimum in December (Table II), this corresponded with the decrease in the cellulose content of litter.

pH of the extract of litter of all the four species of *Terminalia* increased with advancement of decomposition, and moisture content varied almost in the similar pattern at all the stages of decay (Table III).

T. chebula and *T. belerica* had high quantity of lignin in the litter (Table V). Protein content was almost moderate but in case of *T. tomentosa* it was comparatively higher. Sugar content was maximum in *T. arjuna* and *T. chebula* and minimum in *T. tomentosa*. A gradual decrease in organic contents of decomposing litter, except lignin, was recorded. Lignin content was higher at the later stage of decomposition in December.

Energy content of the fresh and decomposing litter falls under two categories (Table VI). In the first category energy decreased till September (*T. tomentosa* and *T. arjuna*) and increased slightly in December; while in the second category (*T. chebula* and *T. belerica*) it decreased till June and later showed a gradual increase till December. This increase in energy level of decomposing litter was possibly due to an increase in lignin content which may be explained by the fact that during early stages of decomposition simpler compounds, viz. sugar, cellulose, protein, etc., were broken down into still simpler substances and energy content decreased; but at the final stages of decomposition when simpler compounds were sufficiently broken down, the percentage content of lignin increased which might have given higher

value of energy content. It may also be explained owing to carbon bonds in lignin being more complex than those in other compounds.

Four species of *Terminalia* had different levels of organic constituents. Maximum ash and good amount of nitrogen with least lignin contents were present in *T. arjuna* and *T. tomentosa*. *T. chebula* had medium ash, nitrogen and lignin content; while *T. belerica* had the lowest ash and nitrogen but the highest lignin content (Tables IV and V).

ACKNOWLEDGEMENTS

We are thankful to the Head of Botany Department, B.H.U. for facilities. One of us (V.P.S.) acknowledges the financial help by the Director, P. G. I. I. M. of this University during the study.

REFERENCES

- Chang, Y. & Hudson, H. J. (1967). The fungi of wheat straw compost. II. *Biochem. Physiol. studies, Trans. Br. mycol. Soc.* **50**: 667-677.
- Dwivedi, R. S. & Singh, V. P. (1974a). Competitive cellulolytic ability of some litter inhabiting fungi. *Proc. Indian natn. Sci. Acad.* **B 40**: 420-423.
- (1974b). Effect of different nutritional factors on the rate of leaf litter decomposition of four species of *Terminalia*. *Trop. Ecol.*, **15**: 90-94.
- Garrett, S. D. (1962). Decomposition of cellulose in soil by *Rhizoctonia solani* Kuhn. *Trans. Br. mycol. Soc.*, **45**: 115-120.
- (1966). Cellulose decomposing ability of some cereal foot rot fungi in relation to their saprophytic survival. *Trans. Br. mycol. Soc.*, **49**: 57-68.
- (1975). Cellulolysis rate and competitive saprophytic colonization of wheat straw by foot rot fungi. *Soil Biol. Biochem.*, **7**: 323-327.
- Golley, F. B. (1961). Energy Values of Ecological Materials. *Ecology*, **42**: 581-584.
- Hudson, H. J. (1968). The ecology of fungi on plant remains above the soil. *New Phytol.*, **67**: 837-874.
- Hudson, H. J. & Webster, J. (1958). Succession of fungi on decaying stems of *Agropyron repens*. *Trans. Br. mycol. Soc.*, **41**: 165-177.
- Peach, K. & Tracey, M. V. (1956). *Modern Methods of Plant Analysis* Vol. I. Springer-Verlag, Berlin (G.D.R.).
- Sharma, P. D. (1973). Succession of fungi on decaying *Setaria glauca* Beauv. : A qualitative analysis of the mycoflora. *Ann. Bot. (Lond.)* **37**: 203-208.
- Sharma, P. D. & Dwivedi, R. S. (1975). Mycoflora colonizing the cut-down shoots of *Setaria glauca* Beauv. *Trop. Ecol.*, **16**: 96-103.
- Somogyi, M. (1945). *J. Biol. Chem.* **160**: 61.
- Waksman, S. A. & Tenny, F. G. (1927). Composition of natural organic materials and their decomposition in soil. I. Methods of quantitative analysis of plant materials. *Soil. Sci.*, **24**: 275-283.
- Webster, J. (1956). Succession of fungi on decaying cocks foot culms. I. *J. Ecol.*, **44**: 517-544.
- Webster, J. & Dix, N. J. (1960). Succession of fungi on decaying cocks foot culms. III. A comparison of the sporulation and growth of some Primary Saprophytes on stem, leaf blade and leaf sheath. *Trans. Br. mycol. Soc.*, **43**: 55-99.
- Witkamp, M. (1966a). Decomposition of leaf litter in relation to environment, microflora and microbial respiration. *Ecology*, **47**: 194-201.
- (1966b). Rate of CO₂ evolution from the forest floor. *Ecology*, **47**: 492-494.
- (1969). Environmental effects on microbial turnover of some mineral elements. Part II. Biotic factors. *Soil Biol. Biochem.*, **1**: 177-184.
- Witkamp, M. & Van der Drift (1961). Breakdown of the forest litter in relation to environmental factors. *Pl. Soil.*, **15**: 295-311.