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DECOMPOSITION OF ^{14}C-LABELLED MELANOID FUNGAL RESIDUES IN A MARGINALLY SODIC SOIL

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Summary—Three melanoid fungi, namely *Alternaria alternata*, *Curvularia lunata* and *Drechslera australiensis* were grown on ^{14}C-glucose and then fractionated into cell wall, cytoplasm and melanin. The decomposition of these fractions and their contribution to the stable organic matter fraction was studied in a marginally sodic soil. The rate of decomposition of fungal melanins was less than that of the cell wall or cytoplasm which had the highest rate of decomposition. However, the contribution of these fractions to the humic acid fraction was very low. Most of the ^{14}C-activity was recovered in the humin fraction.

INTRODUCTION

A great number of soil organisms, including fungi, actinomycetes and bacteria, are known to contribute towards the formation of humic compounds by decomposing and transforming organic residues reaching the soil (Kononova, 1966; Martin and Haider, 1971). Some of these microorganisms are known to synthesize phenolic compounds which are considered to be essential constituents of humic acid polymers (Visser, 1968; Martin and Haider, 1969; Haider and Martin, 1979). In this connection, dematiaceous fungi which accumulate melanin in their cell walls are of great interest as it has been reported that melanin polymers have certain similarities to soil humic acids with respect to elemental chemical composition, constituent units released upon Na-amalgam degradation and by oxidative procedures, exchange acidity and infrared and pyrolysis mass spectra (Felbeck, 1965; Kumada and Hurst, 1967; Filip *et al.*, 1974; Martin *et al.*, 1974; Schnitzer and Neyroud, 1975; Meuzelaar *et al.*, 1977). In addition to these properties, melanin resistance to biodegradation is also similar to the soil humic acid fraction (Linhares and Martin, 1978).

Malik and Sandhu (1973), Malik and Azam (1978) and Malik *et al.* (1979) found that a number of dematiaceous fungi were associated with the decomposition of organic residues added to the soil. Some of these fungi when cultured on a synthetic medium incorporated the melanins within their mycelia. The melanins were not extractable with alkali, and the fungi do not secrete any pigment into the medium. They differed in this respect from the behaviour of *Stachybotrys atra* and *Epicoccum nigrum* (Martin and Haider, 1969). We present a study of the degradability of various ^{14}C-labelled components of three dematiaceous fungi, namely *Alternaria alternata*, *Curvularia lunata* and *Drechslera australiensis*.

MATERIALS AND METHODS

The three dematiaceous fungi selected for this investigation were previously isolated at the Nuclear Institute of Agriculture and Biology, Faisalabad, from salt-affected soil amended with fresh plant residues (Malik and Azam, 1978; Malik *et al.*, 1979) and were deposited at the American Type Culture Collection under the accession numbers *Alternaria alternata* ATCC 42012, *Curvularia lunata* ATCC 42011, *Drechslera australiensis* ATCC 42022.

The fungi were grown in a glucose–asparagine medium (Martin and Haider, 1969); 300 ml of the medium was contained in a 1 l conical flask. Glucose was sterilized separately and added to the sterile medium just before inoculation. Each flask was given uniformly ^{14}C-labelled glucose having a specific activity of 50 μCi g^{-1} C. Fungal inoculum was given in the form of a spore suspension. All the flasks were incubated at 30°C for 8 weeks, by which time the entire surface of the medium in each flask was covered with a thick dark-brown mycelial mat. Since no dark-brown colouration was observed in the culture medium, it was discarded and the mycelium was separated and washed with distilled water. A part of the mycelial mat was fractionated into cell wall and cytoplasm (Hurst and Wagner, 1969). Another portion of the mycelium was hydrolysed with 2 N HCl for 12 h to give the melanin fraction. Hydrolysis with 6 N HCl as described by Nicolaus *et al.* (1964) delivered the same amount of melanin but was more likely to cause alterations. All these fractions were freeze dried.

A partially reclaimed marginally-sodic soil was obtained from a field at NIAB where *Sesbania aculeata* was growing (Malik and Haider, 1976; Sandhu and Malik, 1975). The pH of the soil was 8.3; the electrical conductivity was 0.27 S^{-1} and the exchangeable sodium percentage 22.6. The soil was air dried, sieved (<2 mm) and 50 g portions were placed in

200 ml Erlenmeyer flasks. Three replicate portions were thoroughly mixed with 100 mg fungal mycelium, cell wall, cytoplasm or melanin fractions, respectively. The amended soil was brought to 60% water-holding capacity and the flasks were aerated with a constant stream of CO_2-free humidified air. The $^{14}CO_2$ evolved from the soil was absorbed in gas-washing flasks containing 50 ml 10% NaOH which was changed periodically during incubation. At each sampling 1 ml of the alkali was placed in a scintillation vial containing 2 ml water; 7 ml Dimilune scintillator (Packard, Frankfurt) was added and the radioactivity measured in a liquid scintillation counter (Nuclear Chicago, Model Mark II).

The soil amended with fungal components was incubated at 30°C for 7 weeks. After incubation the soil was freeze dried and a portion of it extracted with a mixture of 0.1 N NaOH and 0.2 N $Na_4P_2O_7$ (Kononova, 1966) and fractionated into humic acid, fulvic acid and humins. The radioactivity of various freeze-dried fractions and of the extracted soil was determined from aliquots using a combustion apparatus (Packard Tricarb Sample Oxidizer).

RESULTS

The mineralization rates of different fractions of the three dematiaceous fungi are presented in Figs 1–3. The rate of evolution of $^{14}CO_2$ from the melanins of the three fungi was similar from the very beginning: 32–33% of the ^{14}C was lost as $^{14}CO_2$ after 7 weeks. This fraction was the most resistant of the fungal components tested. Melanins also showed a relative lag in $^{14}CO_2$ evolution during the first 2 days.

The decomposition rates of the cell wall, cytoplasm and whole mycelium of the three fungi were similar. $^{14}CO_2$ evolution was greatest with the cytoplasm fraction; 58–62% of ^{14}C being lost as $^{14}CO_2$ in 7 weeks. The cell-wall fractions of *A. alternata* and *C. lunata* evolved $^{14}CO_2$ at similar rates; about half of the ^{14}C was mineralized after 7 weeks. The cell-wall fraction of *D. australiensis* was relatively resistant as only 38% of the ^{14}C was respired as $^{14}CO_2$ after 7 weeks. The decomposition of the whole mycelium of the three fungi ranged from 48 to 52%. After incubation, the soil was fractionated into humic acid, fulvic acid and humin. The percentage distribution of ^{14}C activity in these fractions is summarized in Table 1. Maximum ^{14}C activity was recovered in the humin fraction in nearly all the treatments. In the melanin

Table 1. Percentage distribution of ^{14}C-activity in various organic-matter fractions of the soil amended with different fungal residues after incubation for 7 weeks at 30°C

Treatments	%of applied activity in			
	Soil after incub.	Fulvic acid	Humic acid	Humins (extr. soil)
Melanins				
Alternaria alternata	67.2	12.0	2.3	57.8
Curvularia lunata	68.0	12.3	2.6	47.5
Drechslera australiensis	67.0	8.3	3.8	46.0
Mycelium				
A. alternata	50.3	7.7	3.6	30.7
C. lunata	47.7	9.1	2.6	35.1
D. australiensis	51.3	9.3	2.4	34.5
Cytoplasm				
A. alternata	39.6	10.5	2.8	26.4
C. lunata	38.0	11.6	1.8	24.6
D. australiensis	41.5	10.5	2.6	27.1
Cell wall				
A. alternata	49.3	8.5	3.5	36.4
C. lunata	51.9	11.5	2.6	32.9
D. australiensis	62.0	6.5	2.0	51.1

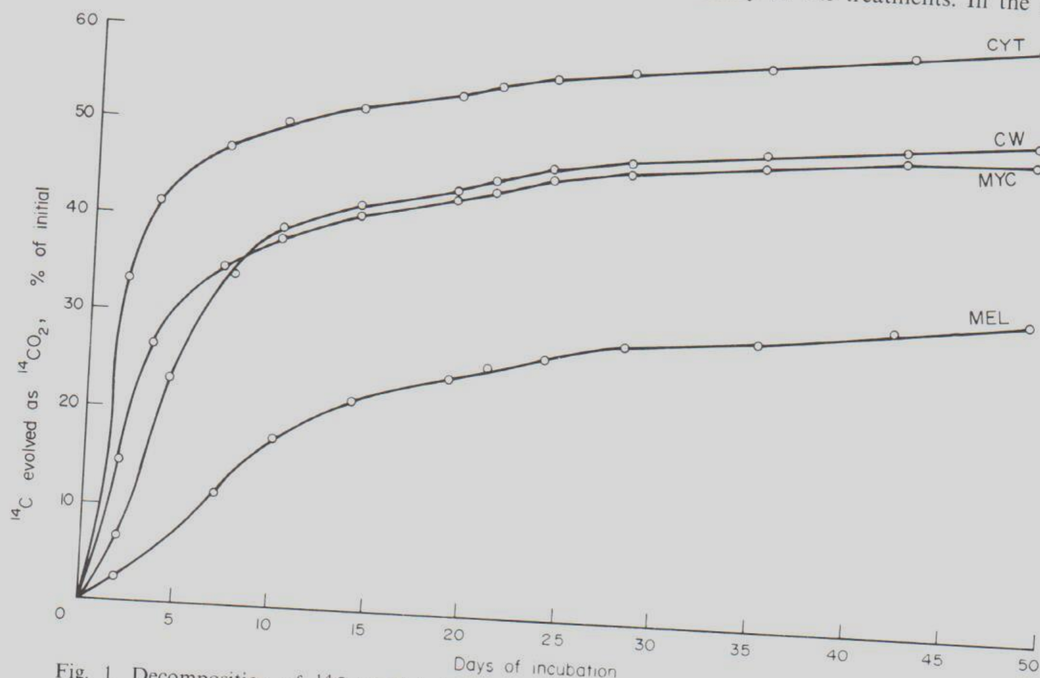

Fig. 1. Decomposition of ^{14}C-labelled fungal fractions of *A. alternata* during incubation in soil. MEL = melanin; CW = cell wall; MYC = mycelium; CYT = cytoplasm.

incubations, the ^{14}C activity in the humin fraction ranged from 70 to 85% when it was based on activity remaining in soil. In the fulvic- and humic-acid fraction it ranged from 12 to 18 and 3 to 6%, respectively. Mycelium, cell wall and cytoplasm gave similar pattern of distribution of ^{14}C in the various organic-matter fractions. Activity in the humins was somewhat lower than in the melanin incubations while that of the fulvic-acid fraction was somewhat higher.

DISCUSSION

The fungal melanins derived from mycelia when added to the soil had the lowest rate of decomposition, compared to other fractions. Hurst and Wagner (1969) reported about 35% $^{14}CO_2$ evolution from the fungal cell-wall fraction of a melanoid fungus after 160 days of incubation. As nearly all the melanins are deposited on the cell wall, its stability can be compared to that of melanins. In our results,

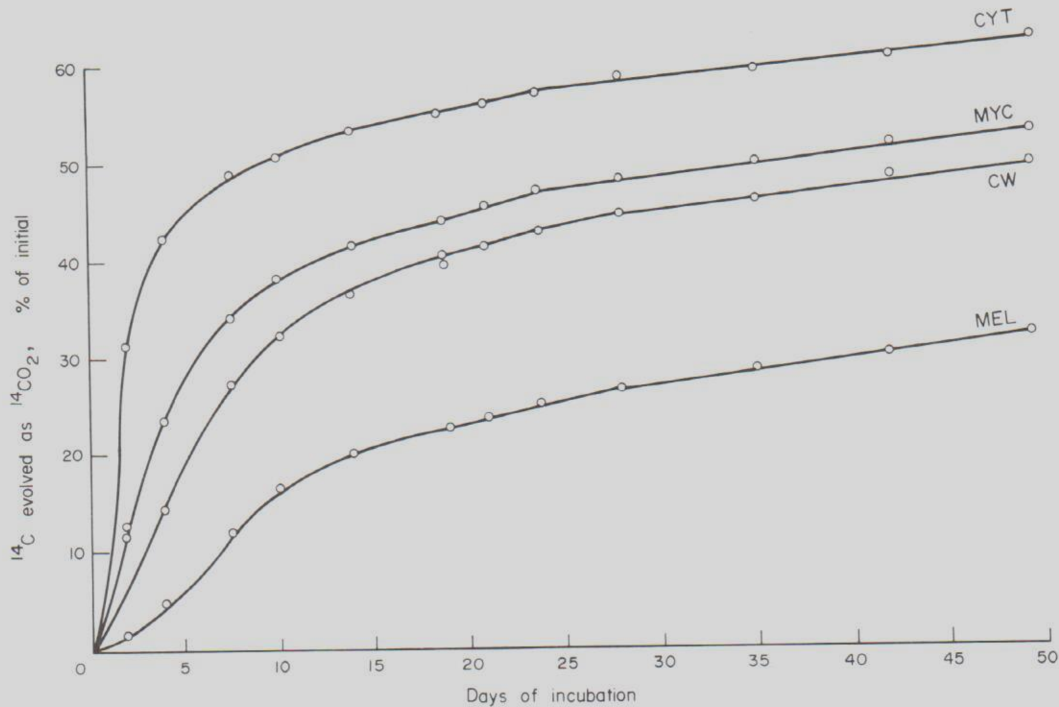

Fig. 2. Decomposition of ^{14}C-labelled fungal fractions of *C. lunata* during incubation in soil. Abbreviations see Fig. 1.

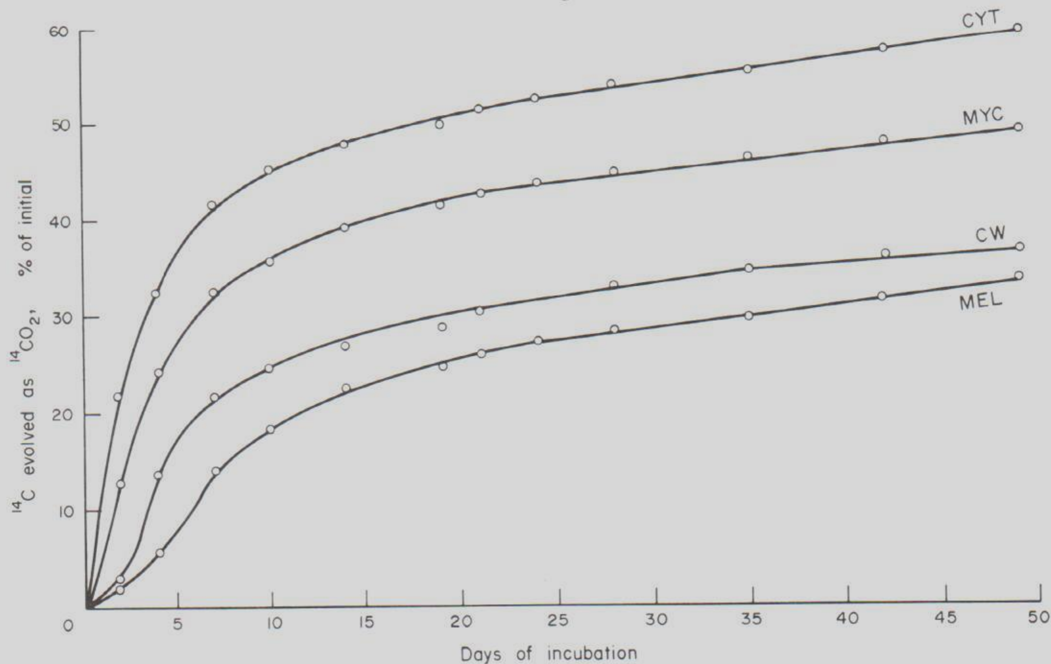

Fig. 3. Decomposition of ^{14}C-labelled fungal fractions of *D. australiensis* during incubation in soil. Abbreviations see Fig. 1.

the rate of decomposition of melanins was similar to that reported by Hurst and Wagner (1969). Wolf and Martin (1976) reported decomposition of 14 and 24% respectively for *Hendersonula toruloidea* and *Stachybotrys atra* melanins incubated in soil for 3 months. Martin and Haider (1969) reported even less decomposition (2–10%) for *S. atra*, *S. charatrum* and *E. nigrum* melanins.

The decomposition percentage of cytoplasmic fractions was quite high. However, the rates of decomposition of the cell-wall fraction were also relatively high. It may be that some cytoplasm had contaminated the cell-wall fraction. If so, the rate of decomposition of the cell wall would be slower than reported here. Similar observations were also made by Hurst and Wagner (1969).

The stability of melanoid fungal residues in soil has been shown to be related to the presence of melanins in the cell wall (Martin *et al.*, 1959; Kuo and Alexander, 1967; Jones and Webley, 1968). Such stable fungal residues ought to make a large contribution to the soil-humic substances. Our results regarding the stability of melanins and cell walls of dark-coloured fungi are in general agreement with the results reported by other workers (Haider and Martin, 1979). This evidence indicates that such fungal residues constitute an important fraction of the soil humus. But there is no direct evidence in the literature as to the actual contribution of these fungal residues to the different humus fraction as separated by conventional soil extraction procedures (Kononova, 1966).

Our investigation indicates that little of the various fungal residues had entered the humic-acid fraction. The greatest ^{14}C activity was observed in the humin fraction. The low contribution of these residues to the humic-acid fraction could be due to the short period of incubation (7 weeks) or to inadequate extraction procedures.

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