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Amounts of Fungal Mycelium in the Soil by Soil Sectioning Technique in West Bengal, India

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ABSTRACT: The microscopic analysis of fungal populations in comparatively undisturbed soil samples from the layers of iron-humus soil in West Bengal was made possible by the application of the soil-sectioning approach. Additionally, a seasonal analysis of the quantitative variations in fungal populations in the soil's five mineral horizons and the A_0h layer of the organic horizon was conducted using this technique. The most sensitive method for these quantitative evaluations was measuring the mycelium length per unit amount of soil; this method outperformed measuring the density of mycelium or the percentage frequency of mycelium occurrence. The outcomes of this research demonstrate the significance of microscopic analysis of soil in offering crucial additional information to that which can be gained from cultural studies.

Keywords: Fungal mycelium, soil, soil sectioning technique, West Bengal.

INTRODUCTION

For many years, one of the biggest challenges facing soil microbiologists has been the quantitative evaluation of fungal populations in soil. Warcup (1955) clearly showed that the use of dilution plate counts for the soil fungus only provided information on the soil spore content and did not allow for the measurement of the mycelium amount that was present. A number of visual techniques have been proposed to enable such assessments, but these have not been used to in-depth studies of the soil mycoflora, with the work exception of Brown (1958). The number of fungal hyphae in soil can be measured using a soil-sectioning approach, as explained by Burges (1956). Extensive research on the fungal population in West Bengal's iron-humus soil has been conducted using this methodology van Aarle & Olsson (2008). This study is comparable to that of the nature and fluctuations of the fungus populations in the same soil since it considers both the vertical distribution of the fungus population and its seasonal variations in the soil of West Bengal (Williams and Parkinson 1964).

MATERIALS AND METHOD

Soil samples have been taken from various horizons in July 2020 and July 2021, with follow-up sampling in October 2020. A pit measuring about one square meter in size and eighty centimeters in depth was excavated in an unaltered section of the sampling site for every sample. Three faces of the pit were sampled for soil from the A_0h, A_1, A_2, B_1, B_2, and C layers. A single, sizable chunk of dirt, measuring roughly 20 by 20 by 15 centimeters and covering the entirety of the A, A_1, and part of the A_2 layers, was removed in the case of the A_0h and A_1 horizons. Smaller slabs of soil, measuring roughly 6 by 6 by 2cm, were taken from the center of each of the A_2, B_1, and B_2 horizons. Comparable little soil blocks were discovered 60 cm below the surface of the C horizon. Fresh soil samples were gathered in the field, quickly frozen in liquid nitrogen, and kept in a deep freeze until needed. Large field samples were sliced in half using a hacksaw to remove smaller samples. The A_0h-A_1 field samples were divided into single blocks (2.5×1.0×1.0cm, with the long axis vertical), with the A0h horizon located in the upper half and the A1 horizon in the lower half. From the field samples of the other horizons, blocks measuring 1.5×1.0×0.5cm have been cut.

Soil sectioning: The tiny samples of soil have been completely freeze-dried before being submerged in a mixture of micro resin. Marco resin S.B. 28/c, along with the corresponding monomer C, accelerator E, and catalyst paste H, made up this composition. By adjusting the ratios of the different ingredients in the combination, the resin's pace of setting may be adjusted Borisov *et al*. (2006). The freeze-dried soil samples were carefully put into the compartments of a polythene ice cube tray, which had been filled with freshly produced resin. After that, the ice cube tray was put in a vacuum desiccator, and resin was injected into the soil at a lower pressure. With every soil tested, this method produced results that were completely satisfactory. Adding the mixture of resin to soil already under decreased pressure could increase the impregnation in much finer-textured soils or in larger samples of soil where greater penetration is required. The soil samples that had been impregnated with resin were taken out of the desiccator and allowed to solidify at room

temperature in the ice cube tray. After the rock-hard resin-impregnated samples of soil were cut, ground, and polished according to standard geological procedures, sections 5½ thick were obtained. Lakeside 700 was then used to bond the soil blocks to the slides of glass, and Canada balsam was used for mounting. It was decided not to attempt staining the fungus that was present in the soil before sectioning after tests showed that the staining along with the washing process significantly disturbed the soil. Hyaline mycelium was made more detectable by staining; however, only a small portion of this mycelium absorbed the pigment. The frequency values in these tiny measurements would only be significant with respect to the dimensions and layout of the sampling region. Following a number of early observations, it was determined that the sampling area's size needed to be relatively considerable given the quantity of mycelium present Maillard *et al.* (2019). Due to the lesser edge effect for any given size, circular sampling areas were thought to be better than square ones. Selecting a standard field size that would allow for the observation of mycelium in all soil strata proved to be challenging. A differential count based on six morphological mycelium groups—groups divided into easily distinguishable characters—was applied in an effort to track qualitative differences in the fungal populations of the various soil horizons sampled, in addition to monitoring the field's overall mycelial content. These 6 groups were: *thin hyaline, dematiaceous hyphaeshort fragments, broad, aseptate, "Dematiaceous hyphaein situ of growth, Broad, septate hyphae, brown-stained hyphae, hyaline hyphae, septate, sparsely-septate fragments of purple-black hyphae* Brabcova *et al.* (2016). The relative quantities of the various morphological groups of mycelium were estimated in the differential count, a quantitative assessment method (length of the hyphae per cc of" soil).

Table 1: Mean Percentage Frequency of Occurrence of Overall Mycelium in the Different Soil Horizons over 1 Yr. Data obtained from observations of the soil section (average values obtained from 200 fields on six slides, each having a diameter of 460µ).

Horizon	Sampling time							
	July	Sept.	Oct.	Nov.	Jan.	Mar.	May	July
A_0h	56.1	49.3	63.3	70.2	69.8	56.6	71.5	65.4
A_1	44.7	29.9	61.5	75.3	78.8	38.0	79.8	48.7
A_2	33.9	21.4	45.2	50.8	68.4	47.0	56.9	38.0
B_1	21.1	19.0	48.1	40.3	37.8	49.9	39.3	36.3
B_2	8.2	6.6	27.0	5.2	9.7	14.5	18.0	16.1
C	1.3	2.7	5.3	1.5	4.4	2.4	2.5	2.4

Table 2: Based on the soil sections observation, the mycelium mean estimated length (measured in meters/cc soil) in the various horizons at the period of sampling.

Horizon	Sampling time							
	July	Sept.	Oct.	Nov.	Jan.	Mar.	May	July
A_0h	5.42	2.44	8.18	9.94	7.50	4.10	8.31	4.97
A_1	3.87	1.35	7.03	15.00	12.87	1.97	10.69	2.95
A_2	3.79	1.34	5.40	5.87	9.80	3.95	5.98	2.94
B_1	1.10	0.79	6.65	2.96	2.19	3.68	2.19	2.02
B_2	0.37	0.26	1.84	0.21	0.52	0.62	0.92	0.74
C	0.04	0.08	0.25	0.06	0.23	0.07	0.09	0.08

RESULTS

Total amount of fungi on the basis of 6 slides per horizon (200 fields per slide), the mean % frequencies of mycelium occurrence for each of the 8 periodic samples in Table 1 are given. The average person believed that measuring length was the more sensitive and accurate way to evaluate fungal mycelium in soil quantitatively. These results are summarized for each soil horizon in Table 3, along with the lowest and maximum values recorded for the eight periodic samples. Additionally, Table 3 provides a straightforward indication of the degree of variability in these data by listing the max:min ratios.

Table 3: Condensed Data for Percentage Frequency of Occurrence and Length of Mycelium (m/cc), containing Max and Min Values obtained during 1Yr of Sampling.

Horizon	% frequency of occurrence			Mycelial length (m/cc soil)		
	Minimum value	Maximum value	Maximum Minimum	Minimum value	Maximum value	Maximum Minimum
A_0h	49.3	71.5	1.5	2.44	9.94	4.08"
A_1	29.9	79.8	2.7	1.35	15.00	11.1
A_2	21.4	68.4	3.2	1.3	9.80	7.3
B_1	19.0	48.1	2.5	0.79	6.65	8.5
B_2	5.2	27.0	5.3	0.21	1.84	8.7
C	1.3	5.3	4.0	0.04	0.25	6.5

The data suggests that there is a clear and consistent increase in fungal mycelium production within the top three horizons studied (A_0h, A_1, and A_2) at the time of the autumn-winter period (October-January). However, there is less strong evidence supporting this enhanced mycelium formation in the B_1 horizon, and there is no sign of a clear and consistent rise in mycelium in the B_2 and C horizons. Additionally, there may be a possibility of a rise in mycelium production at the time of the early summer within the A_0h, A_1, as well as A_2 horizons.

SD findings in the spatial test have been much lower as compared to those in the periodic test, indicating that the degree of variation observed for the A_0h and A_1 horizons in the samples of periodic could not be explained exclusively in terms of spatial variation (even though the spatial test's overall means in several cases were significantly greater as compared to those of the periodic samples) (Oliach *et al*., 2020). This provided proof that the concentration of mycelium varied over time to a significant extent.

Table 4: Comparison of Data obtained from Soil Samples from Profiles (numbered 1, 2, and 3) exposed in November 2021.

Profile no.	% frequency of occurrence				Mycelial length (m/cc soil)			
	1	2	3	Maximum / Minimum	1	2	3	Maximum / Minimum
A_0h	78.8	81.3	79.8	1.04	12.52	11.43	11.81	1.09
A_1	81.8	83.0	87.0	1.07	16.14	13.13	20.73	1.58
A_2	60.5	69.0	79.3	1.31	5.89	8.05	16.28	2.76
B_1	51.8	60.5	72.8	1.40	3.66	4.11	7.72	2.11

Table 5: Comparison of the Degree of Variation in the Measurements of Length of Mycelium obtained from Eight Periodic and Three Spatial Profiles (length of mycelium expressed in meters per cc of soil).

Horizon	Periodic test		Spatial test	
	Overall mean	SD	Overall mean	SD
A_0h	6.36	2.36	11.92	0.45
A_1	6.97	4.94	16.67	3.12
A_2	4.88	2.37	10.07	4.48
B_1	2.70	1.73	5.17	1.82

The same data for the A_2 and B_1 horizons were compared; however, the results did not support the idea that mycelium lengths vary with time. The SD in the spatial test has been higher than that in the periodic test on both of these horizons. Although the data may be partially explained by the higher total means attained for mycelial lengths at the A_2 and B_1 horizons, it seems inexplicable to conclude otherwise as compared to the fact that the degree of variation observed in these horizons in periodic sampling may very well be explained in terms of spatial variation. Therefore, it was incorrect to propose earlier—based on information from Tables 1 and 2—that there was a significant temporal fluctuation in mycelial length A_2 and B_1 horizons (Polyanskaya *et al.*, 1998).

The following summary of the data collected can be used to determine whether a seasonal pattern of higher mycelium production occurs:

— Strong evidence has been found within the A_0h and A_1 horizons pointing to a notable, seasonal rise in mycelium creation that occurs repeatedly at the time of the autumn-winter season Rudsari *et al.* (2015).

Table 6: Differential Measurement of the 6 Morphological Groups of Mycelium on Soil Sections of the A_0h, A_1, and A_2 Horizons Sampled Eight Times During 2020 and 2021 (estimated lengths in meters/cc of soil).

Horizon	Mycelial Group	Sampling time							
		July 2020	Sept. 2020	Oct. 2020	Nov. 2020	Jan. 2021	Mar. 2021	May 2021	July 2021
A_0h	1	0.82	0.71	1.10	1.62	1.24	0.85	1.66	1.14
	2	0.25	0.24	1.04	2.05	1.12	1.96	1.07	0.99
	3	0.65	0.10	0.41	0.51	0.39	0.01	0.19	0.15
	4	3.00	0.39	4.43	4.00	3.26	0.42	4.15	1.99
	5	0.32	0.71	0.58	1.16	1.19	0.52	0.83	0.43
	6	0.40	0.27	0.63	0.59	0.30	0.33	0.44	0.27
A_1	1	0.10	0.09	0.42	0.48	0.44	0.34	0.50	0.34
	2	0.35	0.16	2.80	2.45	2.10	0.89	5.30	0.49
	3	0.32	0.01	0.32	0.44	0.77	0.02	0.25	0.21
	4	2.75	0.35	2.17	9.40	8.60	0.37	4.25	1.73
	5	0.22	0.67	0.99	2.07	0.77	0.25	0.28	0.12
	6	0.14	0.07	0.34	0.23	0.20	0.10	0.11	0.05
A_2	1	0.03	0.01	0.05	0.04	0.11	0.08	0.13	0.07
	2	0.52	0.42	2.28	1.68	3.05	0.20	2.16	0.89
	3	0.18	0.07	0.13	0.89	0.98	0.25	0.60	0.43
	4	2.28	0.44	0.87	3.03	5.40	3.13	2.69	1.08
	5	0.76	0.38	2.06	0.18	0.27	0.25	0.32	0.45
	6	0.06	0.02	0.02	0.01	0.03	0.40	0.06	0.02

— Within the A_2 and B_1 horizons, indication of the same pattern of mycelial expansion attained from periodic samples is refuted by the spatial variation research in mycelial expansion Sharma and Mishra (2020).
— All available data indicates that there is no seasonal variation in the amount of mycelium within the B_2 and C horizons.

DISCUSSION

Strong evidence suggests that during the fall and winter months of the year, there is a noticeable rise in fungal mycelium, particularly in the upper soil strata. It doesn't appear likely that the climatic conditions in West Bengal are responsible for this periodic condition of mycelial growth Shumilov *et al.* (2017). A broad tolerance to the temperature range observed at the site of sampling is demonstrated by fungi. Furthermore, despite the fact that soil moisture content has a significant impact on fungal activity, there was no discernible pattern of variation in the rainfall during the sampling period, which would have suggested that it was crucial in identifying the pattern of mycelium creation.

CONCLUSIONS

Numerous researchers have used culture techniques to study seasonal variations in the makeup and activity of fungal communities. However, their findings have frequently been inconclusive. When combined with the data from this investigation, the data from West Bengal have been previously discussed (Williams and Parkinson 1964), and they significantly support Warcup's (1955) claim that "In studies on activity of fungi microscopic and isolation techniques". A range of techniques combined with as much physically observation as feasible will likely produce the most information, as no single approach seems to be fully suitable at this time Vinichuk *et al.* (2013).

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Conflict of Interest. None.

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