MICROBIAL DEGRADATION OF GREEN LEAVES OF *HIBISCUS ROSA-SINENSIS* L.*

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Abstract

The colonisation of green leaf surfaces of China rose (*Hibiscus rosa-sinensis* L.) were traced from the time of full maturity of leaves till its complete degradation. *Aurevbasidium pullulans* and few bacterial species have been isolated as active and dominant colonisers of green leaves which colonised till mid-degradation. *Colletotrichum malvarum* has been isolated as a dominant and active decomposer of leaf surfaces during the period of investigation.

Introduction

Litter is essentially produced by the deposition and decomposition of dead plant parts on the soil surface. This process involves interaction of micro-organism with dead plant. The microbial colonisation of green leafy tissue assumes special importance because of their high pigment and starch content and the microbial flora differ in such leafy substrates from dead leafy plant material. The studies have clearly demonstrated that yeast like organism generally predominate the filamentous fungi and bacteria on the green leaf surfaces (Ruinen, 1961), the frequency of which gradually decrease (Pugh & Mulder, 1971). The abundance and colonisation of leaves also changes with the change in season (Preece & Dickinson, 1971; Last & Deighton, 1965; Pugh, 1958). Microbial succession again changes when the leaves reach the soil surface (Pugh, 1958). Therefore, the microbial colonisation is a complex process which has not been studied in many of the plant species.

The present investigation aims at the isolation of microorganisms which colonise the green leaves before and after they reach the soil surface. Comparative studies were also undertaken in rainy and non-rainy season so as to find the effect of seasonal changes on mocrobial succession.

Material and methods

Fully matured green leaves of china rose (*Hibiscus rosasinensis* L.) were placed on the garden soil surface and flora identified before placement. Phylloplane flora of green leaves was isolated by leaf washing technique prior to studies for degradation. Regular isolation of organisms was conducted every alternate day in both rainy (April to late September) and non-rainy season (October to February). The washing were transferred to the Czapeck dox and Sabouraud agar medium in triplicate. The plates were incubated at room temperature, fungi isolated and identified. The percentage abundance of the micro-organisms was calculated (Agarwal & Prasad, 1972).

Results and discussion

Phylloplane flora of the green leaves at the time of placement on soil surface remained the same in both rainy and non-rainy season except for one more bacterial isolate which was observed in rainy season. However, the differences in their abundance and time period of landing of micro-orga-

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Table 1—Percentage abundance of fungi colonising green leaves during rainy season

Organism	Day of Isolation												
	1	3	5	7	9	11	13	15	17	19	21	23	25
Fungi													
Aspergillus candidus Link.	8.74	2.0	0.83										
A. fumigatus Fresenium	6.99	5.0	3.33	2.0									
A. niger Van-Teighem.	—	1.0	—	—									
Aspergillus sp. I	3.49	2.0	3.33	2.0	1.01								
Aureobasidium pullulans (de-Bary) Arnaud	20.03	35.00	30.83	38.0	30.30	26.67	23.36	18.18	13.52				
Colletotrichum malvarum South-worth	2.01	7.0	7.52	12.0	15.15	16.19	16.82	20.20	33.78	36.99	34.78	71.32	45.45
Cylindrocarpon sp.	—	1.0	3.33	1.0	1.01	—	—	—	—	—	—	—	—
Mucor variance Povah	—	—	5.83	8.0	10.10	10.48	13.08	7.05	—	—	—	—	—
Penicillium species	—	—	2.50	4.0	2.02	0.95	—	—	—	—	—	—	—
Unidentified fungal cultures	—	2.0	0.83	.0	2.02	1.90	1.87	2.02	2.70	2 2.74	1.45	—	33.77
Bacteria													
Mycobacterium species	24.4	20.00	18.33	12.0	11.11	16.19	16.82	20.20	18.92	19.18	24.64	24.64	19.48
Bacteria species I	13.98	—	—	—	—	—	—	—	—	—	—	—	—
Bacteria species II	20.62	25.00	25.33	20.00	27.27	27.62	28.04	32.32	31.68	41.09	39.13	4.03	1.29

Table 2—Percentage abundance of fungi colonising green leaves during non-rainy season

Organism	Day of Isolation														
	1	3	5	7	9	11	13	15	17	19	21	23	25	27	29
Fungi															
Aspergillus candidus Link.	7-51	4.90	2.43												
Aspergillus fumigatus Fresenium.	6.66	6.86	4.87												
Aspergillus niger Van-Tighem.	0.75	0.98	3.65	1.33											
Aspergillus sp. I	0.37	—	—	1.33											
Aureobasidium pullulans (de Bary) Arnaud	15.08	29.41	12.19	22.67	15.62	10.71	4.25	—							
Colletotrichum malvarum Southworth	0.97	2.94	18.29	26.67	46.87	57.14	74.47	75.00	75.47	75.86	75.00	71.18	66.67	74.38	72.73
Cylindrocarpon sp.	—	2.94	1.22	2.67	—	—	4.25	—	—	—	5.00	3.39	1.59	—	—
Microvariance Povah	—	4.90	8.54	10.33	7.81	5.36	2.13	—	—	—	—	—	1.59	—	—
Penicillium sp. Unidentified	—	1.96	3.65	2.67	—										
Fungal cultures	4.39	1.96	3.65	3.00	3.12	1.78	2.13	3.85	3.77	1.72	—	—	—	—	—
Bacteria															
Mycobacterium sp.	33.45	24.51	24.39	13.33	10.94	12.5	10.64	13.46	15.09	15.52	14.33	11.86	14.28	11.86	10.00
Bacteria species I															
Bacteria species II	30.82	18.60	17.01	16.0	15.62	12.5	2.13	7.69	5.66	6.89	6.67	13.56	15.87	13.68	16.36

nisms changed with the season. Bacterial colonies were isolated throughout the period of investigation but were more dominant during rainy season. It appears from the study that rainy season facilitated the fungal and bacterial growth on the leaf surfaces and degradation was faster. It took about 25 days during the rainy season and 29 days during non-rainy season for complete degradation of the substrate.

Altogether, 9 species were recorded on the green leaf surface. These comprised five species of fungi, one species each of yeast, actinomycetes, and two strains of bacteria, constituting nearly 62.5, 12.5, 12.5 and 12.5% respectively of total population. These organisms include—*Aspergillus candidus*, *A. fumigatus*, *A. niger*, *Aspergillus* spp. *Colletotrichum malvarum*, *Aureobasidium pullulans*, a member of Mycobacterium and two bacterial isolates. There was an inerease in total number of organisms colonising the leaf surface to 12 by 3rd day during non-rainy season and by 5th day during rainy season (Table 1, 2). *Mucor variance*, *Cylindrocarpon* species, *Penicillium* species, and one unidentified fungus colonised the leaf surface. Most of these organisms colonised the surface for the shorter period of time and disappeared by the end of 15/17 day of leaf placement.

Aureobasidium pullulans and other bacterial isolates outnumbered in their abundance over fungal types during the early phase of fungal attack. The former yeast species gradually disappeared with degradation age of leaves and by 15/17 days of placement on the soil surface.

Colletotrichum malvarum was primarily present as a part of the microbial flora before placement of leaves to the soil, increased in abundance with the onset of degradation and was isolated until the process of degradation was completed. Since *C. malvarum* was isolated during course of degradation as dominant coloniser, this strain could be regarded as final and the sole fungal decomposer of green leaves of *Hibiscus rosasinensis*.

In the present investigation, the pattern of phylloplane flora of green leaf surface represented by the members of bacteria, yeasts and fungi were almost similar as isolated on leaf surfaces of most of the species (Dickinsons, 1965). Bacteria and yeasts were found to be the common residents of green leaves phylloplane flora with fewer fungi (Last & Warren, 1972: Ruinen, 1961). Isolation of *Aureobasidium pullulans* on green leaves surfaces has been regarded as regular and dominant coloniser (Preece & Dickinson, 1971; Friends, 1965; Last & Warren, 1972). In the present study, *Aureobasidium* and *Mucor* spp. colonised early and lasted considerably for longer periods. This suggests that might play a role in the degradation proeess. *Aureobasidium pullulans* is known to utilize sugars on leaf surfaces (primary sugar fungi), which might help in degradation of substrate by waste product utilization.

The finding of occurrence of *Colletotrichum malvarum* on the leaves of china-rose cofirms those of James and Plakidas (1952), Rama Krishnan and Sethalakmi (1956) as resident coloniser of plants. In the present investigation, *C. malvarum* has been isolated as the dominant coloniser of leaf surface as well as during the decomposition. This species was most abundant and represented major flora of decomposing leaves besides bacterial flora. *Colletotrichum* has been reported to utilize hemicellulose and cellulose *in vitro* and *in vivo* (Burges, 1939; Garrett, 1960) and therefore can be considered as active decomposer. The present study also suggests that actual decomposers colonise the leaf surface at the time of maturity and activity of organism is initiated as soon as the leaf is detached and falls on the ground for degradation. They remain colonised till the complete degradation has finished the substrate.

The colonisation of organism suggests that organisms belong to two major categories, one which are the initial colonisers and remain dominant for a shorter period on leaf surface and the second group of fungi belong to organisms which were present on the leaf surface before they fall to the ground and colonise the surface till the complete degradation. The active decomposing organisms belong to this group.

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