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LEAF SURFACE MICROFLORA OF HEALTHY AND DISEASED (*PHYTOPHTHORA INFESTANS*) *SOLANUM KHASIANUM*.

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Phyllosphere and phylloplane microflora of healthy and diseased (*Phytophthora infestans*) leaves of *Solanum khasianum* were studied. Maximum species of fungi were observed on diseased leaves, whereas maximum yeasts and bacteria were recorded on healthy leaves. Total micro-organisms were always higher on healthy leaves. In phyllosphere, *Penicillium* sp., *Alternaria alternata*, *Cladosporium cladosporioides*, *Trichoderma viride*, *Aureobasidium pullulans*, *Sporobolomyces roseus* and filamentous yeast were common in both healthy and diseased leaves. In phylloplane, *Cladosporium cladosporioides*, *Sporobolomyces roseus* and *Aureobasidium pullulans* were found on both healthy and diseased leaves.

INTRODUCTION

Solanum khasianum, a solasodine yielding plant, constitutes natural vegetation of north-east India. The plants of *Solanum khasianum* are severely attacked by *Phytophthora infestans* at flowering and fruiting stage which reduce the yield and causes premature death of the plant.

The phyllosphere studies provide a great challenge for plant pathologists for biological control by using antagonistic saprophytic fungi (Mishra & Tewari, 1976; Dickinson, 1967; Fokkema, 1976). The present investigation was undertaken to compare the microflora of *P. infestans*-infected and healthy leaves of *S. khasianum*.

MATERIALS AND METHODS

The present study was carried out in the botanical garden of the North-Eastern Hill University at Shillong. The seeds were sown in the month of April 1977 and severe disease was noticed in the month of December, 1977. The crop was harvested in January, 1978. Healthy and diseased leaves of approximate same age and height were randomly collected in sterilized polythene bags in the month of December 1977 and January 1978. Disks (5 mm diameter) were cut from each of fifteen leaves with a sterile cork borer. One hundred disks were taken in 100 ml of sterilized distilled water and were wrist shaken for 15 minutes. 1 ml of aliquot was plated on Rose Bengal-Streptomycin agar, Nutrient agar and Starch-Casein agar media (Johnson & Curl, 1972) for fungi, bacteria and actinomycetes respectively. Yeasts were counted on Rose Bengal-Streptomycin

agar. Eight disks (half adaxial, half abaxial) were plated out on Malt Extract agar. The plates of fungi and yeast were incubated at 25 ± 1°C for five days, whereas the plates for bacteria and actinomycetes were incubated at 30°C for 24 h and seven days respectively. Triplicate sets were maintained for all treatments. The total microbial population was calculated by following formulae :—

$$\text{Total number of microbes per cm}^2 = \frac{\text{Total number of microbes in 100 ml}}{\text{Total area of leaf disks}}$$

Total area of leaf disks = 100 × area of leaf disk × 2.

RESULTS AND DISCUSSION

The results are presented in Tables 1 and 2. Table 1 shows that maximum filamentous fungi were found on diseased leaves, whereas maximum yeasts and bacteria were observed on healthy leaves. Actinomycetes population was low and the dominant species was *Streptomyces* sp. The total microbial population was found higher on healthy leaves than diseased leaves.

TABLE 1. Microbial population per cm² of leaf area of healthy and diseased leaves.

Micro-organisms	December 24th 1977		January 28th 1978	
	Healthy	Diseased	Healthy	Diseased
Filamentous fungi	34·39	48·41	27·39	52·50
Yeasts	527·39	345·22	708·28	375·16
Bacteria	961·78	629·22	232·48	207·39
Actinomycetes	7·01	9·24	8·60	7·32
Total	1530·57	1032·09	976·75	642·37

In the phyllosphere some as., *Cladosporium cladosporioides*, *Trichoderma viride*, *Penicillium* spp., *Alternaria alternata*, *Sporobolomyces roseus* and *Aureobasidium pullulans* were common on both healthy and diseased leaves (Table 2). *Fusarium* sp., Ascomycete 1 and Ascomycete 2 were found only on healthy leaves in the month of December 1977 and *Mucor hiemalis*, *Papularia* sp., *Helminthosporium* sp., *Pleochaeta* sp., *Cylindrophora* sp. and *Chaetomium* sp., were observed in the month of January 1978. *Acremonium* sp., *Phoma* sp. and *Ascomycete 3* were specific to diseased leaves in the month of December 1977,

TABLE 2. Fungi isolated from the phyllosphere and phylloplane of healthy and diseased leaves of *Solanum khasianum*.

Fungal Species	Phyllosphere				Phylloplane			
	December		January		December		January	
	H	D	H	D	H	D	H	D
Mucor hiemalis	—	—	+	—	—	—	+	—
M. circinelloides	—	—	—	+	—	—	—	—
Sterile phycomycete	—	—	+	+	+	+	—	—
Pythium sp.	—	—	—	—	—	+	—	—
Sterile hyphae (white)	—	—	—	—	—	—	+	+
Trichoderma viride	+	+	+	+	+	—	+	—
Cladosporium cladosporioides	+	+	+	+	+	+	+	+
Penicillium sp.	+	+	+	+	—	—	—	—
Alternaria alternata	+	+	+	+	+	—	—	—
Fusarium sp.	+	—	—	+	—	—	—	—
Papularia sp.	—	—	+	+	+	—	+	—
Cylindrophora sp.	—	—	+	+	—	—	—	—
Helminthosporium sp.	—	—	+	—	—	—	—	—
Pleochaeta sp.	—	—	+	—	—	—	—	—
Monilia sp.	—	—	—	+				
Acremonium sp.	—	+	—	+	—	+	—	+
Phoma sp.	—	+	—	—	—	—	—	—
Verticillium sp.	—	—	—	—	—	+	—	—
Nigrospora sp.	—	—	—	—	+	—	—	—
Fusidium sp.	—	—	—	+	—	—	—	—
Ascomycete 1	+	—	—	+	—	—	—	—
Ascomycete 2	+	+	+	—	+	—	+	—
Ascomycete 3	—	+	—	—	—	—	—	—
Chaetomium sp.	—	—	+	+	—	—	—	—
Sporobolomyces roseus	+	+	+	+	+	+	+	+
Aureobasidium pullulans	+	+	+	+	+	+	+	+
Filamentous yeast	+	+	+	+	—	—	—	+
Total species	19	11	15	17	9	7	8	6

H=Healthy leaves; D=Diseased leaves.

whereas *M. circineloides*, sterile phycomycetes, *Fusarium* sp., *Papularia* sp., *Monilia* sp., *Fusidium* sp., Ascomycete 1 and *Chaetomium* sp., were found in the month of January, 1978. *Acremonium* sp. was observed only in case of diseased leaves. Total fungi species was found higher in diseased leaves.

The phylloplane fungi *Cladosporium cladosporioides*, *Sporobolomyces roseus* and *Aureobasidium pullulans* were found common in both healthy and diseased leaves (Table 2). Sterile Phycomycetes, *Acremonium* sp., *Alternaria alternata* and *Nigrospora* sp., were observed only in healthy leaves in the month of December 1977 whereas *M. hiemalis*, white sterile septate mycelium, were found in the month of January 1978. *T. viride*, *Papularia* sp. and Ascomycete 2 were found in both the months. *Pythium* sp., sterile phycomycetes, *Acremonium* sp. were found only in diseased leaves in the month of December 1977 and sterile septate, *Verticillium* sp. and filamentous yeast were observed in the month of January 1978. *T. viride* and Ascomycete 2 were observed in healthy leaves in both months.

The present study revealed the quantitative differences of microbial population of *Phytophthora infestans*-infected and non-infected leaves although the study was not conducted for long duration to encounter the other parameters due to the harvesting of the plants. There may be many explanation for the differences of fungal population, qualitatively as well as quantitatively, in infected and uninfected leaves of *Solanum khasianum*. One of them may be that *P. infestans* may provide nutrient in the form of decay of nyphae. Probably it also helped in liberation of more substances through the breakage of the leaf tissue. Smith *et al.* (1969) observed that the accumulation of carbohydrate in fungal mycelium and tissue may also affect the growth of certain microorganisms. Gianinazi *et al.* (1977) demonstrated that four new leaf proteins synthesized surrounding the viral infected lesions, are resistant to secondary infection but they favoured the saprophytic yeast population. Last (1970) found increased number of *Sporobolomyces roseus* on diseased leaves than healthy leaves. Derx (1948) and Brady (1960) observed the differences in surface microflora of rust infected and non-infected leaf. Our results reveal that total yeast and bacteria population was always higher in healthy leaves than infected ones. Another possible reason for the difference in fungal population may be early senescence of leaves infected with *P. infestans* and as such the leaves which were collected for sampling were though of the same age but in different physiological conditions.

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