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Succession of Microfungi on Attached Leaves of Sunflower (*Helianthus annuus* L.) at Different Heights

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Succession of fungi on lower leaves preceded that on the upper leaves. Fungal population and number of taxa were higher on lower leaves at all stages of samplings. Quantitatively distribution of fungi was maximum at the pre-flowering stage followed by a decline in both samples. A dramatic change occurred in the species composition of fungi at the onset of senescence. Forty species of fungi comprising 2 zygomycetes, 3 Ascomycetes and 35 Deuteromycetes were isolated from the phylloplane at different heights. Difference in progress of leaf senescence at varying heights in sunflower (*Helianthus annuus* L.) is implicated for the variation in the fungal succession.

Key Words: Microfungi, Succession, Sunflower, Phylloplane

Introduction

The existence of an active fungal population and a consequential succession of mycoflora have been reported by many workers (Sharma & Mukerji 1976, Wildman & Parkinson 1979). The contamination of leaf by microorganisms is dependent upon morphology, age and exposure period of its surface, composition of air spora, climatic conditions and the arrangement and location of leaves in the canopy of the plant (Dickinson 1976).

Microbial population and succession in leaves of different heights in the canopy of trees have been studied by Lamb and Brown (1970) and Wildman and Parkinson (1979). Such a study in relation to ephimeral and seasonal crops may be of special interest. The microbial population and the infection level on leaves of different heights play a significant role in determining the yield of the crop. Mishra and Tewari (1969, 1976) have reported the influence of heights on microbial population in some annual plants. The present work was taken up to study fungal succession on leaves borne at different heights on the sunflower plants.

Materials and Methods

Sunflower seeds (Cultivar EC 68414) were procured from Allahabad Agriculture Insti-

tute and were grown in 2 m^2 plots in the Botanical Garden of the Banaras Hindu University in the monsoon season (June-Oct). Plants were spaced 20 cm apart in rows and 45 cm between the rows.

Two leaves, the second and the third from the top (upper sample), and the basal leaves other than those moribund and senesced (lower sample), were detached from 50 plants with the help of a flamed forceps. The leaves from both the positions were pooled separately in new polyethylene bags and were processed on the same day for the isolation of mycoflora. The collection of samples was carried out at the following five stages of development:

1. *Seedling stage* (S_1): When shoot was 30 cm tall and had on an average six leaves. One leaf from each sample was excised at this stage and one hundred plants were sampled.

2. *Pre-flowering stage* (S_2): When plants were nearly 68 cm in height and possessed 10–12 leaves. Defoliation of the basal leaves had started.

3. *Flowering stage* (S_3): One week after the flowering had commenced. Plants were nearly 90 cm tall and had 15-18 leaves.

4. *Post-flowering stage* (S_4): When the achenes had started setting and pappus had begun to fall. The peripheral achenes were at the milk stage. The plants were nearly 108 cm high.

5. *Senescent stage* (S_5): When achenes had started becoming full and hard. Plants were nearly 125 cm tall and upper leaves started turning yellow.

Details in respect of heights (from the ground level) at which the leaves were sampled at each of the above stages have been provided in the table.

Isolation Procedures

Preliminary studies of isolation employing differential methods showed that the maximum number of species of filamentous fungi were obtained by the dilution plate method: Czapek Dox + yeast extract + streptomycin (100 mg/l) was most suitable for the recovery of a maximum number of fungi.

Two discs of 5 mm diam, each from fifty leaves (sampled) were punched with the help of a sterilized cork borer, avoiding the mid rib region. The leaf discs were shaken in a 250 ml Erlenmeyer flask containing 100 ml sterile distilled water on a horizontal electric shaker (120 throws min^{-1}, displacement 1.4 cm) for 20 min. The washing was diluted further with sterile distilled water in different series. One ml aliquots were pipetted out in ten 9 cm Petri dishes at each dilution and then overpoured with 15 ml of molten cooled down (40°C) aforesaid medium. Incubation was done at 24 ± 2°C under a bank of 12 h day^{-1} fluorescent light (NUV with continuous spectrum from 320 to 420 nm). Determinations of fungal colonies were made 5 days after incubation until no further development of colonies was observed. Results of the fungal colony counts have been expressed in cm^{-2} of the leaf area using the following formula:

$$Fungi\ cm^{-2} = \frac{Total\ number\ of\ fungi\ in\ 100\ ml}{Total\ area\ of\ discs}$$

$$Total\ area\ of\ discs = Number\ of\ discs \times area\ of\ one\ surface\ of\ the\ disc \times 2$$

Results

Numerical distribution ($cm^{-2} \times 10$) of individual phylloplane fungi and a comparative account of total phylloplane population (cm^{-2}) at different stages on leaves of both heights is presented in table 1 and figure 1 respectively.

It is evident from figure that the distribution of fungi located at lower heights on the plant was higher than that on leaves borne higher up at all stages of samplings. The trend

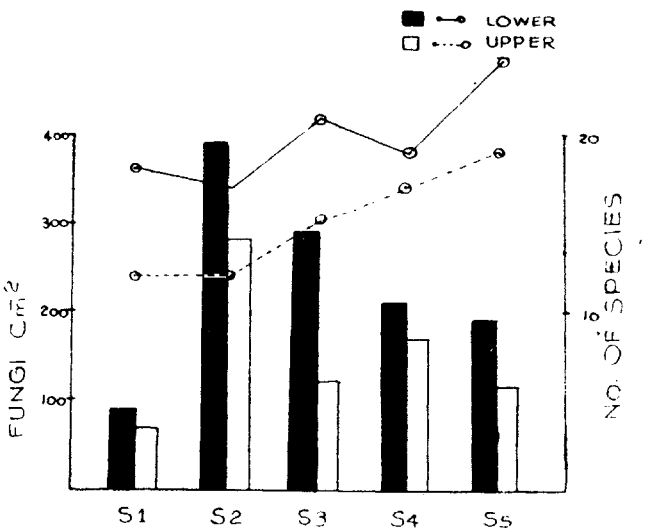

Figure 1 Numerical distribution of fungi (per unit area and total number of taxa) on leaves of *Helianthus annuus* L. at different heights and at five stages (S_1–S_5) of sampling

of fungal distribution revealed maximum population at pre-flowering stage, followed by a decrease in the subsequent stages on leaves of both heights.

A comparison of the species spectrum (table 1) at both heights showed that leaves at lower heights harboured higher number of fungi in all the samplings. In the early stages of isolation, a greater number of uncommon fungi were observed in the two samples. This difference, however, decreased and in the isolations made at the senescent stage there was similarity in the species composition of both the samples. A majority of species detected on the leaves at lower height at any one sampling were observed on the leaves of upper height in the subsequent sampling.

In all 40 species of fungi were isolated in both samples. Aspergillii, Fusaria, *Mortierella subtilissima*, *Myrothecium roridum*, *Nigrospora sphaerica*, *Phoma glomerata* and *Rhizopus nigricans* were isolated from the seedling, pre-flowering and flowering stages; *Alternaria alternata*, *Aspergillus luchuensis*, *Botryodiplodia theobromae* and *Curvularia lunata* were recovered from all stages of plant growth. There was a dramatic change in the composition of phylloplane with the onset of post-flowering and senescent stages. The species spectrum at these stages was marked by the presence of *Acremonium parsicinum*, *Arthrinium phaeospermum*, *Cephalosporium acremonium*, *Gliomastix murorum*, *Leptosphaerulina trifolii*. *Penicillium citrinum*, *Pyrenochaeta* sp., *Robillarda sessilis*, *Sordaria fimicola* and *Stemphylium* sp.

Table 1 *Distribution of phylloplane fungi ($cm^{-2} \times 10$) on leaves of sunflower at different heights*

Species of fungi	Stages									
	Seedling		Pre-flowering		Flowering		Post-flowering		Senescent	
Height in cm	11	24	34	64	46	84	58	104	60	120
Acremonium parsicinum	—	—	—	—	3.53	—	—	—	3.53	3.53
Alternaria alternata	—	3.53	5.89	3.53	5.89	3.53	13.27	5.30	8.84	5.89
Arthrinium phaeospermum	—	—	—	—	1.76	—	8.84	2.35	3.53	—
Aspergillus candidus	—	—	11.77	23.59	—	2.35	—	2.35	—	—
A. flavus	8.84	5.30	—	—	—	—	8.84	4.42	8.84	5.89
A. fumigatus	1.76	—	—	3.53	—	—	—	—	3.53	5.30
A. lunchuensis	12.38	5.30	—	—	2.35	3.53	8.84	8.84	3.53	5.30
A. niger	—	—	11.77	11.77	5.89	11.79	—	—	—	—
A. nidulans	—	—	2.35	5.89	—	—	5.89	—	—	—
A. sydowi	—	—	171.09	123.89	—	—	—	—	—	—
A. terreus	1.76	3.53	11.77	—	—	—	—	—	3.53	—
Botryodiplodia theobromae	3.53	4.42	23.59	11.77	70.77	17.69	44.24	70.77	52.85	28.85
Curvularia lunata	12.38	19.46	17.69	11.77	41.30	23.59	23.59	18.84	14.15	9.61
C. pallescens	1.76	—	—	—	23.59	5.89	8.84	—	—	—
Cephalosporium acremonium	—	—	1.76	—	17.69	17.69	8.84	4.42	7.07	4.42
Chaetomium globosum	1.76	—	—	—	2.35	—	—	—	—	—
Drechslera australiansis	5.30	—	—	—	—	—	—	—	—	—
Fusarium semitectum	3.53	10.61	5.89	—	—	—	13.27	8.84	—	—
F. solani	1.76	3.53	—	—	35.39	5.89	—	—	3.53	5.30
F. equiseti	1.76	3.53	5.89	5.89	—	—	—	—	3.53	3.53

Gliomastix murorum	—	—	—	—	—	—	4.32	—	—	—
Leptosphaerulina trifolii	—	—	—	—	3.53	—	8.84	2.35	3.53	2.35
Mortierella subtilissima	7.07	1.76	—	—	23.59	5.89	—	—	—	—
Myrothecium roridum	—	5.30	—	—	17.69	—	—	4.42	10.61	10.61
Nigrosporo sphaerica	3.53	—	17.69	5.89	11.79	5.89	—	—	—	—
Oospora sulphurea	—	—	11.77	3.53	—	5.89	—	—	—	—
Pestalotiopsis disseminata	—	—	—	—	2.35	—	4.42	2.35	—	—
Papulaspora sp.	—	—	—	—	—	2.35	—	—	—	—
Penicillium citrinum	1.76	—	—	—	—	—	8.84	7.07	4.42	3.53
P. decumbens	—	—	76.69	70.79	2.35	—	—	—	8.84	—
P. implicatum	1.76	—	—	—	—	—	—	—	3 53	—
Phoma glomerata	—	1.76	2.35	—	11.79	11.79	—	—	7.07	5.30
Pink sterile mycelia	14.15	—	5.89	—	3.53	—	—	—	8.84	7.07
Pyrenochaeta sp.	—	—	—	—	—	—	4.42	4.42	3.53	—
Rhizopus nigricans	5.30	—	5.89	—	—	2.35	—	—	—	—
Robillarda sessilis	—	—	—	—	—	—	8.84	7.07	3.53	2.35
Sordaria fimicola	—	—	—	—	3.53	—	4.42	—	8.84	7.07
Stemphylium sp.	—	—	—	—	—	—	13.27	8.84	3.53	5.30
Trichophyton sp.	—	—	—	—	—	—	8.84	8.84	—	—
Yellow sterile mycelia	—	—	—	—	—	—	—	—	7.07	5.30

— = Absent

Discussion

The higher count of fungal colonies on leaves borne at lower height at early stages of plant development seems to be connected with the nearness of basal leaves to the soil surface. This is explainable because of the isolation of a large number of soil forms from basal leaves in early samplings. But at later stages of samplings such a difference appears to be due to variation in age and exposure period to the atmosphere of the two leaves. Mishra and Tewari (1976) suggested that the higher population of microflora on the leaves of lower height was because of favourable conditions of humidity and temperature close to the soil surface. Water splash is also resposible for contamination of leaf surface (Gregory 1971). Bernstein and Carroll (1977) are of the opinion that the leaf surface community is influenced by variations in temperature, relative availability of dissolved substances in the canopy-wash and to differing degree of grazing pressures. The lower leaves receive a large amount of substances leached out from the canopy which may be responsible for increasing the microbial population. The influence of height of leaves in the canopy was shown to influence the growth of fungi on leaf surface in *Cassia tora* (Mishra & Tewari 1969). *Paspalum dilatatum*, *Salix babylonica* and *Eucalyptus stellulata* (Lamb & Brown 1970) and *Populus tremuloides* (Wildman & Parkinson 1979).

The difference in the succession of fungi on leaves of upper and lower height is explained on the basis of their age. The basal leaves in the sunflower stem remain at lower strata of the canopy where they age and senesce earlier to the upper leaves. Difference in the age of leaves in this study was clearly manifested in the successional pattern in fungi also which revealed an early progression in basal leaves. Variations in the physiology, exposure period, resistance and microclimate of leaves are apparent factors which influence colonization of leaf surface microflora.

The species spectrum of fungi in both samples revealed the isolation of a number of common phylloplane inhabitants. *A. alternata* and *B. theobromae* recovered from all the sampling are pathogens of sunflower. The composition of the mycoflora changes with the onset of senescence (Sharma & Mukerji, 1976 and Lindsey & Pugh 1976a,b). These changes are associated with the capacity of individual species to utilize the decomposed substances, to tolerate prevalent microbial antagonism and environmental factors. A number of species detected in the last stages of sampling have been recognised by Hayes (1979) as the organisms taking part in decay process.

The objection that reliance on the isolation technique employed in the present study might have favoured some forms selectively is countered by the fact that the forms isolated in any one sampling were recovered in different proportion in the subsequent samplings besides many species being dropped out altogether till the last sampling. In addition, when a comparison with other methods was made, dilution plate method was found suitable for recovering the maximum amount of viable propagules. Although the possibility of a few undetected species occurring on leaf surface cannot be ruled out, the method used in this study proved suitable for gross quantitative and qualitative comparisons.

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