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Phyllosphere Microflora of some Common Plants

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Abstract

The quantitative and qualitative phyllosphere microflora of some crop plants, forest trees, plantation crops and weeds were studied by using Dickinson's leaf surface washing method and leaf maceration method. Both methods showed different trends of poplution in the leaf surface

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of the plants studied. In general, bacterial population was predominant of leaf surfaces of all plants and amongst bacteria gram–ve and coloured species were more in number. Amongst different plant groups maximum population was recorded from leaves of crop plants, followed by weeds, forest trees and plantation crops. By leaf surface washing method no fungal colonies could be detected from the samples of sunflower (*Helianthus annus*), eucalyptus (*Eucalyptus spp.*) and jamboo neralle (*Syzygium cumini*) whereas, by leaf maceration method low fungal population was recorded from the samples of these plants. The bacterial and fungal isolates from phyllosphere were tentatively identified as species of *Alcaligens, Bacillus, Corynebacterium, Klebsiella, Lactobacillus, Pseudomonas, Sarcina, Streptobacillus, Xanthomonas* and *Aspergillus, Beauveria, Candida, Cladosporium, Cunninghamella, Curvularia, Fusarium, Haplosporangium, Isariopsis, Nigrospora, Pestolotia, Pseudoplea, Phoma* and *Sporobolomyces.*

The presence of saprophytic and parastic microorganisms in the surface of leaves has been known since long. The quantitative and qualitative nature of the microorganisms in the leaf surface are influenced to a large extent by the plant species, nature of leaf surface, age of the plant, environmental factors etc., Dickinson, (1971) ; Kulkarni *et al.* (1973) ; Bagyaraj *et al.* (1974) ; Prasad and Edward, (1975). The phyllosphere microflora of bhendi, beet root, cabbage, bean and bengalgram have been studied by Kulkarni *et al.* (1973) and Bagyaraj *et al.* (1974). The present studies were conducted to investigate the phyllosphere microflora of some common plants representing crop plants, forest trees, plantation crops and weeds. The results of quantitative and qualitative estimation of phyllosphere microflora of plants representing different groups are discussed.

Material and Methods

For present investigation 15 plants representing different groups *viz.*, crop plants, forest trees, plantation crops and weeds listed in Table I were selected. The studies were undertaken during the months of November–December 1976 when temperature and relative humidity varied from 15.65°C to 27.4°C and 63.5 to 77.3 per cent respectively. The leaf samples were collected from the plants grown in Gandhi Krishi Vignana Kendra Campus of the University. The samples from plants at different locations and from different plant heights were collected. The quantitative estimation of phyllosphere microorganisms was done by following two methods: (i) leaf surface washing method (Dickinson, 1967) and (ii) leaf maceration method (Menna, 1959). For each plant two samples served as replicates and each sample consisted of bits from randomly selected leaves. The leaf bits of known weight and of known area were transferred to 100 ml sterile water blanks in 250 ml Erlenmeyer flasks and shaken thoroughly for 5 minutes on a mechanical shaker. Serial dilutions were then prepared and plated on nutrient glucose agar and Martin's Rose bengal agar for the enumeration of population of bacteria and fungi respectively. The bacterial and fungal numbers were expressed in terms of number per gram weight or per square centimeter area of leaf tissue.

The representative colonies of various bacteria and fungi were selected from the plates, purified, transferred and subsequently maintained on agar slants of respective media. Various morphological, physiological and biochemical tests were carried out for bacterial isolates by following standard procedures (Anon, 1957). The fungal cultures were identified by studying their morphology and other microscopic observation.

Results and Discussion

The results presented in Table I indicate that the leaf surface of the plants supported growth of various microorganisms. In

TABLE I

Quantitative and qualitative estimates of Phyllosphere microflora of some important plants

Name of Plant		Leaf surface washing method No. of microbes/cm² of leaf × (10^4)		Leaf maceration method No. of microbes/g of leaf × (10^5)		Genera representing bacterial flora	Genera representing fungal flora
Common name	Generic name						
1	2	3		4		5	6
I. Crop plants		Bacteria	Fungi	Bacteria	Fungi		
Redgram	*Cajanus cajan* L.	4.99	0.13	85.00	0.26	*Pseudomonas*	*Cladosporium* and *Aspergillus*
Ragi	*Eleusine coracana* Gaertn	2.79	0.04	51.70	0.37	*Pseudomonas*, *Streptobacillus* and *Corynebacterium*	*Cladosporium*
Sunflower	*Helianthus annus* L.	9.07	—	28.75	0.03	*Pseudomonas* and *Xanthomonas*	—
Beans	*Phaseolus lunatus* L.	14.69	0.56	52.50	0.35	*Pseudomonas* and *Xanthomonas*	*Aspergillus*, *Candida* and *Cladosporium*
Jowar	*Sorghum vulgare* Pers	10.84	0.19	38.05	0.35	*Pseudomouas* and *Lactoblcillus*	*Cladosporium*, *Curvularia* and *Haplosporangium*
II. Forest trees and Plantation Crops							
Cashew	*Anacardium accidantale*	0.07	0.05	0.50	0.28	*Bacillus*	*Aspergillus*, *Cladosporium*, *Isariopsis* and *Pestolotia*
Eucalyptus (Mysore gum)	*Eucalyptus* spp.	0.23	—	0.06	0.007	—	—

1	2	3		4		5	6
Sandal wood	*Santalum album* L.	0.46	0.05	0.36	0.15	*Bacillus*	*Beauveria, Cladosporium* and *Curvularia*
Jampoo Neralle	*Syzygium cumini* L.	0.12	—	0.50	0.05	*Bacillus, Pseudomonas* and *Sarcina*	*Isariopsis Phoma* and *Pseudoplea*
Teak wood	*Tectona grandis*	1.08	0.30	4.27	0.42	*Bacillus, Pseudomonas* and *Lactobacillus*	*Aspergillus, Cladosporium* and *Nigrospora*
III. Weeds							
Starbur	*Acanthospermum*	0.65	0.06	0.73	0.09	*Pseudomonas*	*Aspergillus, Cladosporium* and *Curvularia*
Bill goat weed	*Ageratum conyzoides* L.	4.44	0.07	9.50	0.76	*Bacillus* and *Pseudomonas*	*Aspergillus, Cladosporium* and *Cunnighamella*
Beggar's stick (Spanish needle)	*Biden pilosa* L.	31.82	1.19	10.92	0.15	*Lactobacillus* and *Xanthomonas*	*Cladosporium, Fusarium* and *Spikaria*
Nutgrass	*Cyprus rotendus* L.	2.16	0.07	11.00	0.12	*Alcaligens, Klebsilla, Pseudomonas* and *Xanthomonas*	*Cladosporium*
Lantana	*Lantana camara* L.	2.72	0.08	25.70	0.20	*Bacillus* and *Xanthomas*	*Cladosporium Fusarium* and *Nigrospora*

general, bacterial population was higher than fungal population. The results of quantitative analysis by leaf washing and leaf maceration method showed different poplution trends in case of these plant groups. However, both the methods showed approximately same population of nitrogen fixers except on weeds (Wani *et al.*, 1977).

Amongst crop plants, maximum population of bacteria (14.69×10^4/sq cm) was recorded in the case of bean by leaf surface washing method followed by jowar, sunflower, redgram and ragi. Whereas, by leaf maceration method, a maximum population of bacteria was recorded from redgram and followed by double bean, ragi, jowar and sunflower. The maximum fungal population was recorded from double bean leaves followed by jowar, redgram and ragi by following leaf maceration method. No fungal colony was observed from sunflower leaf surface by following leaf washing method. Whereas, fungal population of 0.03×10^5 was recorded from sunflower leaves wherein leaf maceration method was used.

A maximum bacterial and fungal pupulation was recorded from teak wood leaves amongst the forest trees and plantation crops, wherein the leaf washing method was used. The lowest bacterial population was recorded from cashew nut leaves. *Eucalyptus spp.* and *Syzygium cumini* leaves showed complete absence of fungal flora by leaf washing method. Whereas, leaf maceration method recorded very low fungal population from leaf surfaces of these plants. Even in case of leaf maceration method particularly *Eucalyptus* spp. showed very low fungal population. This might be due to the presence of some toxic substances in the leaves of *Eucalyptus spp.* which may be inhibiting the fungal growth on the leaf surface. However, it needs further confirmation.

Bidens pilosa showed presence of a maximum bacterial and fungal population on its leaves amongst weeds by leaf washing method. Whereas, a maximum bacterial population was noted in case of *Lantana camera* and a maximum fungal population from *Ageratum conyzoides* by following leaf maceration method.

Amongst three groups of plants in general, crop plants showed better bacterial and fungal population followed by weeds and forest trees. These results indicated that population of microorganisms vary from plant species to species and these results are in conformity with the findings Kulkarni *et al.* (1973) and Bagyaraj *et al.* (1974).

Grouping of microorganisms: The bacterial isolates were identified as species of *Alcaligens, Bacillus, Corynebacterium, Klebsiella, Lactobacillus, Pseudomonas, Sarcina, Streptobacillus* and *Xanthomonas*. It was revealed from the identification of bacteria that mostly Gram–ve bacteria were dominating in the phyllosphere of various plants. Ruinen (1976) recorded similar observation. The other observation made during these studies was that mostly pigmented bacteria dominated the bacterial population.

The phyllosphere microflora can also be grouped on their colour charactestics. In the present report, the bacteria can be grouped on the basis of their pigmentation into different groups *viz.*, white, faint yellow, dark yellow, orange colour, light brown, pink colour, blusih tinge etc. The yellow pigmented bacterial population was domi nating in all the plates. Similar observations were made by earlier workers (Voznyakovskaya, 1969 ; Vozynakovskaya and Khudyakov, 1960). The yellow pigmented, Carotene containing forms seem to offer protection against U. V. damage. It is noteworthy that the colour appears on N–containing media in the light, and is often indicates start of nitrogen fixation in cultures on N–free medium (Ruinen, 1974).

The fungal cultures were tentatively identified as species of *Aspergillus*,

Beauveria, *Candida*, *Cladosporium*, *Cunninghamella*, *Curvularia*, *Fusarium*, *Haplosporangium*, *Isariopsis*, *Nigrospora*, *Pestolotia*, *Pseudoplea*, *Phoma* and *Sporobolomyces* etc. The *Cladosporium* was dominating fungus in all the plates. There were different types of *Cladosporium* but could not be identified up to species level. The interesting observation made during these studies was that forest trees inhabited various types of fungi *viz.*, *Isariopsis*, *Pestolotia*, *Nigrospora*, *Pseudoplea*, etc., whereas, the crop plants and weeds leaf surfaces were inhabited by species of *Cladosporium*, *Curvalaria* and *Fusarium* etc.

Further studies relating to interactions of phyllosphere saprophytes with parasitic microorganisms and also with nitrogen fixers are in progress.

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