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Phylloplane microbial populations on *Amaranthus tricolor L.* and *Achyranthes aspera L.* from Sakoli Taluka of Bhandara District (M.S.)

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Abstract

The present study is an approach to investigate the microbial population in leafy vegetable crop-Amaranthus tricolor L. and medicinal herb- Achyranthes aspera L. Studies on the phylloplane microflora of both plants revealed that the bacterial population was found to be more than the fungal population in various growth stages of selected plants. The mean fungal population was highest at stage of flowering (5.58 x 10^2/cm^2 leaf area), whereas the mean bacterial population was more at the senescence stage (28.94 x 10^2/cm^2 leaf area). Predominant micro-flora observed as *Aspergillus, Penicillium, Curvularia, Trichoderma, Saccharomyces,* (from Fungi) and *Bacillus, Micrococcus, Pseudomonas, Streptomyces* (from Bacteria).

Keywords: Phylloplane, Microbial population, Growth stages, Amaranthus tricolor, *Achyranthes aspera*

Introduction

Aerial leaf surfaces, like the other plant parts, are colonized by a number of microorganisms like fungi, virus, bacteria and actinomycetes [1-3]. Some surface inhabitants show pathogenic behavior towards the host and causes leaf infection whereas some of them may promote or inhibit the growth and development of the pathogens on plant surface [4]. Successful landing of aero-microflora on leaf surfaces results in their colonization and engage to build a significant relationship between these microbes and symptom development [5,6]. yeasts and filamentous fungi [7-9].

Phylloplane microdiversity intimately depends on several host attributes such as, nature, age and polarity of leaves, and epidermal architects and appendages as well. Phylloplane, landing tribunes for micrcobiota, is the surface of plant leaves represents complex terrestrial habitat, characterized by a variety of microorganisms. Majority of the phylloplane microbiota constitutes Bacteria, Yeasts, and filamentous fungi represents lower densities in juvenile and fresh, tender leaves and higher in mature leaf stage [10,11]. The impact of various environmental factors (such as light, temperature, humidity, rainfall), and physiological changes in the plants (like vegetative phase to flowering phase and later in senescence), witness the diversities in microbial population on phylloplane. Some of the phylloplane microorganisms play a significant role in management of foliar diseases. Aboriginal fungal as well as bacterial populations tend to suppress the diseases by competing with pathogen to grow and thereby reducing the disease severity [12-15].

Amaranthus tricolor L. is an annual, erect, branched herb, attaining 1-1.5 m high, stem stout, usually much-branched, leaves rhomboid to deltoid, ovate, flowers in rounded dense axillary clusters forming long, distantly interrupted spikes, flowers and fruiting in winter, specially cultivated for leafy vegetables, popularly known as "Laal Bhaaji".

Achyranthes aspera L. is a perennial herb, 30-90 cm high. Leaves few, generally thick, softly pubescent, ovate, flowers small, greenish-white, inflorescence elongate spikes. Fruit oblong or ovoid, pointed, utricles enclosed by hard perianth, Flowering and fruiting in August – December, popularly known as "Kutri.

In the present investigation, phylloplane microorganisms on *Amaranthus tricolor L.* and *Achyranthes aspera L.* were quantified.

Material and Methods

For Phylloplane microflora investigations, healthy leaf samples were collected from several different locations of Sakoli taluka, in different stages of growth (Vegetative, Flowering, senescence). Locations for sampling were selected arbitrarily, like cultivated fields, highway roadside, and forest areas, of nearby villages (Jambhali, Lawari, Kumbhali, Bodra, Virsi) including sakoli town.

Healthy, asymptomatic leaf samples were collected and immediately put into sterile ziplock bags, and brought to the laboratory for further processing. The isolation of leaf microflora was based on the plating of leaf washings. A preliminary washing step was carried out in order to avoid plating of phyllosphere propagules deposited on the leaf surfaces. Leaf discs of 5 mm diameter were punched with the help of sharp sterilized cork borer. 100 discs were placed in 250 ml conical flask containing 100 ml sterile distilled water, followed by shaking on rotary shaker for 20 minutes to get a homogenous suspension of the microbial propagules. From this, one ml of suspension was pipetted out into sterilized petri plates. The plates were poured with potato dextrose agar medium (for fungi) and nutrient agar medium (for bacteria) and mixed thoroughly. The plates were incubated at 27±1°C temperature. 5-7 days after incubation, the fungal colonies were counted, subcultured and purified by single hyphal tip method. The plates for bacterial colonies were observed in 1-2 days after incubation. The bacterial population was counted, subcultured and purified by streak plate method [15-17]. Total microbial population per square cm of leaf surface was calculated by using the formula given below

Results and Discussion

Phylloplane Fungal Population:

The mean phylloplane fungal population on selected plants at various crop stages ranged from 2.4 to 5.58 x $10^2/cm^2$ leaf area (*Amaranthus tricolor*), and 1.92 to 3.66 x $10^2/cm^2$ leaf area (*Achyranthes aspera*). Highest fungal population recorded as 5.58 x $10^2/cm^2$ leaf area in *Amaranthus tricolor during* flowering stage, which interprets as the presence of higher nutrients and pollen mass on their surface [8,9,15]. And least population

recorded in senescent stages might be partially due to their low content of soluble sugars, which is primarily related to the loss of chlorophyll [2,5,9]. Fungal species frequently encountered during the investigations of selected plants in different phases are *Aspergillus, Penicillium*, *Curvularia, Trichoderma, Saccharomyces, etc.*

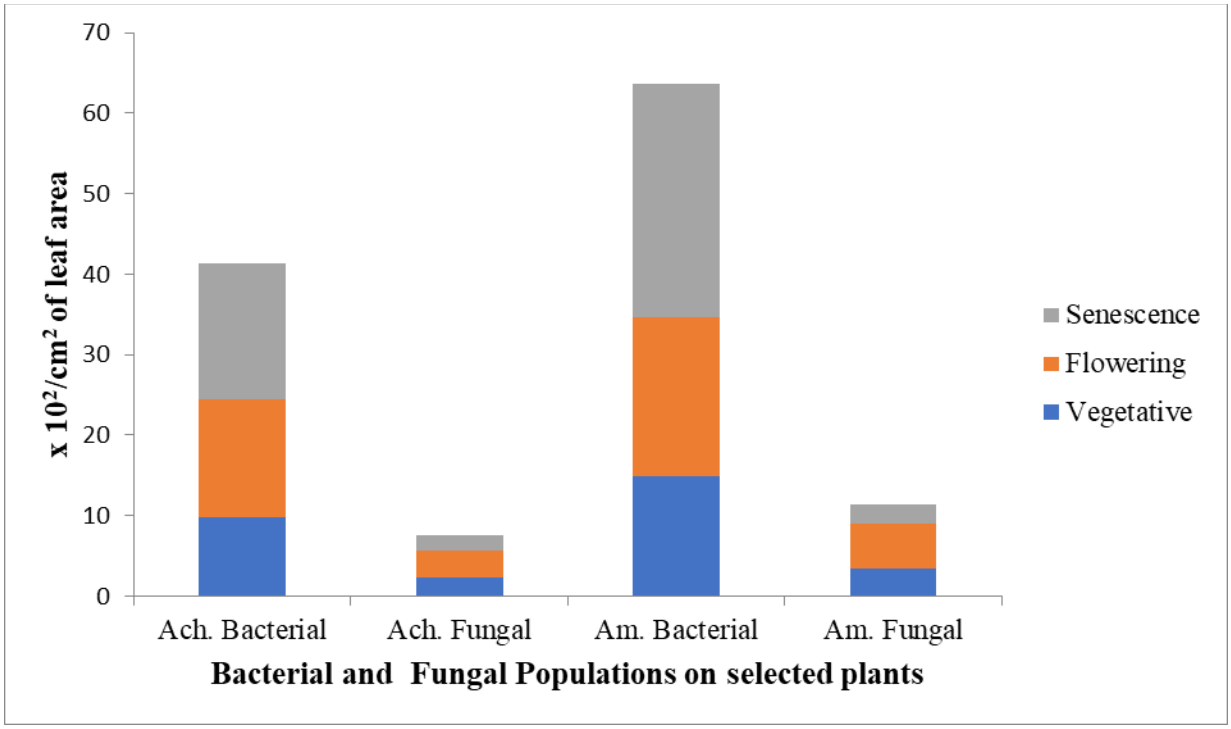
Phylloplane Bacterial Population:

The mean phylloplane bacterial population on selected plants at various crop stages ranged from 14.88 to 28.94 x 10^2/cm^2 leaf area (*Amaranthus tricolor*), and 9.8 to 16.42 x 10^2/cm^2 leaf area (*Achyranthes aspera*). Highest bacterial population recorded as 28.94 x 10^2/cm^2 leaf area in *Amaranthus tricolor during* senescent stage, might be due to the change in micro-climate of the leaves and also due to the fact that older leaves are rich in more leachates released on their surface [10,11,14,18]. Bacterial species frequently encountered during the investigations of selected plants in different phases are *Bacillus, Micrococcus, Pseudomonas, Streptomyces, etc.*

The variation in phylloplane microflora of two plants belongs to same family Amaranthaceae might be due to the environmental factors, genetic makeup and other structural leaf surface attributes [7,8,15].

Table 1:

S. N.	Growth Stage	Fungal Population (x 10^2/cm^2 of leaf area)		Bacterial Population (x 10^2/cm^2 of leaf area)	
		Achyranthes	Amaranthus	Achyranthes	Amaranthus
1.	Vegetative	2.25	3.44	9.8	14.88
2.	Flowering	3.66	5.58	14.6	19.77
3.	Senescence	1.92	2.4	16.42	28.94



Conclusion

From the present investigation, it can be concluded that phylloplane microbial diversity is multifactorial and interdisciplinary in nature. It needs to reveal the hidden and complex interkingdom pathways affecting the phylloplane microflora in terms of ecological and environmental variables, seasonal changes, microclimate of leaves, growth stages, leaf epidermal appendages, leachates or leaf exudates, genetic makeup of plants, the study of disease transmission, and plant pathology, at molecular level. [18,19]

Conflicts of interest: The author stated that no conflicts of interest.

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