MYCOFLORA ASSOCIATED WITH LEAF SURFACE OF CERTAIN VEGETABLES

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Air, phyllosphere and phylloplane fungi of four vegetables, viz., *Luffa cylindrica, Lagenaria siceraria, Cucumis melo* and *Abelmoscus esculentus* have been described which can be conveniently compared with nonrhizosphere, rhizosphere and rhizoplane regions of root. A remarkable correlation was observed between these three regions. *Alternaria tenuis* and *Curvularia pallescens* trapped from air as dominants were also dominantly associated with phyllosphere and phylloplane. Maximum population was recorded from phyllosphere region which may be due to various macro and microenvironment of leaves and their secretion/excretion. It can be concluded from the present work that air spora of a place has considerable effect upon the vegetation concerned.

Leaf, being the principal photosynthetic region of the plant body, plays an important role in the seed output of the plant. Any disturbance in the morphology of leaf, due to external agencies, changes the physiological set up of the organ and consequently the plant as a whole is affected. Leaf surface is coated with a rich microflora of parasitic or saprophytic or both natures. LAST (1955), DICKINSON (1965, 1967), RUINEN (1961, 1966), SINHA (1965), and MISHRI and SRIVASTAVA (1970, 1972b) have paid considerable attention to this sphere. We are well acquainted with the magnitude of the loss caused by parasitic forms to the plants particularly to crop. However, the literature regarding the saprophytes on host leaf is very limited.

MISHRA and SRIVASTAVA (1972 b), keeping the seasonal spread of some plant pathogens in view, suspected that there is a possible correlation in between the air spora and leaf surface microflora of plants growing in adjacent environment. With this view the study of air spora is of considerable importance in order to understand the spread of microbes in the atmosphere and their possible effect (s) on plants particularly to their shoot region. In the present communication this phytopathological topic has been studied by investigating the air spora, phyllosphere and phylloplane fungi of some vegetables growing together. As the work on leaf surface microflora of vegetables and the effect of their close association on phyllosphere is still in infancy so the problem was studied on vegetables.

Materials and Methods

Four plant species, viz., *Luffa cylindrica* (L) Roem. (Syn. *L. aegyptica*), *Lagenaria siceraria* (Mol). Stande., *Cucumis melo* L. Var. *utilissimus,* and *Abelmoscus esculentus* (L) Moen. were selected for the present study. These plants are commonly grown in kitchen gardens as summer vegetables. Vegetable farm selected for the present investigation was about 3 km. south-west of university campus. Leaves from these plants were collected in sterilized containers from top, middle and bottom regions during young, flowering and fruiting stages respectively at the interval of 20 days.

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Two leaves were collected from each plant and 10 plants of each type of plant species were sampled every time. Small leaf pieces each measuring 0.5 cm. (both side area 1.0 cm) were cut with flamed razor blade, one from each leaf and 20 such pieces from each plant species were introduced into 750 ml conical flasks separately under aseptic condition containing 200 ml of sterilized distilled water. Flasks were hand shaken for 20 minutes and the suspensions thus obtained were used for the isolation of phyllosphere fungi using 100 mm Petri-plates containing modified Martin's medium. One ml of suspension was inoculated in each plate and 10 such plates were used for each sample. The washed leaf pieces were removed from the flasks and washed thoroughly with sterilized water giving 15 changes. These pieces were then cut each into 4 small pieces and dried on sterilized filter paper under aseptic conditions and plated on nutrient plates of Dox + yeast extract medium (PH 4.0) for the assessment of phylloplane fungi. Some washed small leaf pieces were also plated directly on sterilized moist filter paper in petri-plates.

Petri-dishes of 100 mm size containing three different nutrient media, viz., Czapek's solution agar, modified Martin's, and Malt-extract agar were used for trapping air fungi over vegetable farm. 10 plates of each medium were exposed at the height of one meter on a exposing bench for 5 minutes over three different places every time in farm area at 10.A.M. Exposings were done just before the phyllosphere sampling. Plates were incubated for 5–6 days at 25±1°c and fungi appeared thereafter were identified and recorded.

Moisture content was determined by drying 20 g. of each type of leaf and PH by electric PH meter.

Results

Present findings are incorporated in tables 1 and 2. The four plant species are dsignated as LC, LS, CM and AE respectively in the tables.

PHYLLOSPHERE:– Fungal species isolated from different phyllospheres were more or less similar but at some places considerable differences were also observed. In general, *Alternaria tenuis* was isolated as a dominant species from most of the samples and occasionally it was found altogether absent. Three different species of *Curvularia* were frequently recorded amongst which *Curvularia pallescens* was the codominant as well as subdominant at some places. Other infrequent dominant was white sterile colony. *Rhizopus nigricans, Aspergillus terreus* and *A. niger* were occasionally isolated as subdominants from most of the phyllosphere samples. Yeast, *Penicillium humicola, Helminthosporium* sp. and Brown sterile colony were restricted to this region. Number of species showed an increasing trend from young to fruiting stage in all the vegetable crops. Quantitative estimation of phyllosphere showed a gradual increase in population from young to fruiting stage in all plant species and maximum flora was obtained from *L. siceraria* and least from *L. cylindrica.*

PHYLLOPLANE:– Three fungal species in this region, viz., *Alternaria tenius, Curvularia pallescens* and White sterile colony were recorded as dominants amongst which the former two were more commonly associated. Mean number of colonies per plate and number of species were least in young stage and maximum during fruiting period of the plants. *Mucor hiemalis* was associated as specific fungus in this region.

AIR SPORA:– Fourteen, 16 and 12 species were trapped from the air over vegetable farm during young, flowering and fruiting stage respectively. Quantitative population i.e. mean number of colonies per plate was the maximum in young stage and least in the last sampling stage. *Aspergillus niger* in young and fruiting stage; *Curvularia pallescens* during young and flowering stage, and *Alternaria tenuis* in all

the three stages were trapped as dominants. Rare subdominants were *Aspergillus niger, Rhizopus, Fusarium* and White sterile colony. *Aspergillus sydowi* was of restricted occurrence in this region.

Physico-chemical analysis of leaf samples (Average of 3 replicates) also exhibited a significant variation. Moisture content was always highest in young stage and least in fruiting stage while PH showed a reverse pattern in all plant species except a little deviation from this trend in *C. melo* (Table 2).

Discussion

It can be concluded from the present findings that there exists a remarkable correlation between the air spora, phyllosphere and phylloplane. These regions can be conveniently compared with non-rhizosphore, rhizosphere and rhizoplane of root. The distribution and succession of fungi on leaf surface can be discussed on the basis of microflora on root regions. SRIVASTAVA and MISHRA (1971) determined a decrease in quality of mycoflora from non-rhizosphere to rhizoplane through rhizosphere while quantitative population was found to be maximum in rhizosphere and least in rhizoplane. Some of the fungal species migrated towards the rhizosphere from nonrhizosphere soil and finally got established in rhizoplane. Likewise rhizosphere, phyllosphere region also harboured the maximum mycopopulation. Air spora contained diverse type of fungi which when fell on leaf surface constitute, the phyllosphere mycoflora and only selective forms capable of infecting leaf tissue penetrate the leaf epidermis and got established in phylloplane region. *Alternaria tenuis* and *Curvularia pallescens* may, be assessed as true leaf infecting fungi where the former is one of the most common leaf parasite and sapro phyte of this area. In earlier study MISHRA and SRIVASTAVA (1970, 1972a,b) also recorded this fungus together with *Cladosporium* as dominant during winter season from leaf surface and air over wheat and barley fields. In present work *Cladosporium* was altogether absent and *Alternaria tenuis* alone accounted approximately half of the total fungal colonies.

A higher colony count in phyllosphere may be due to leaf secretion/excretion and moreover leaves would be getting continuous contact of somewhat changed air spora every hour and microbial layers would be deposited on leaf surface. This ultimately resulted in thick coating of dust containing different types of microbial spores which may eventually be one of the causes contributing for high fungal population in phyllosphere region. Some of the spores which fall upon leaf surface find suitable micro-habitates try to colonize and the excretion of different metabolities like sugar, aminoacids etc. from leaf provide favourable substrate for germination and growth of microbes (BROWN, 1922) resulting in an increased mycoflora. The increase in quality and quantity of fungi in phyllosphere and phyllophane regions at fruiting stage may be ascribed to the moribund nature of leaves which encouraged the growth of certain saprophytes viz., *Fusarium,* Brown sterile colony nd White sterile colony at later stage of plants' growth.

The variation in mycoflora shows that different plant species favour a somewhat changed mycoflora due to the difference in plants' physiological set up and these forms such as *Aspergillus flavus* and White sterile colony in the present study might be selective in nature. Sometimes some dominant air fungi, viz., *Aspergillus niger, paecilomyces fusisporus* and *Fusarium* spp. occurred rarely on leaf surface. This would seem to indicate that the excretion/secretion of metabolites from leaf surface, or some other unknown factor produces and ecological substratum which is unfavourable or less favourable for the growth and multiplication of many air fungi.

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Table 1

Distribution of fungi on leaf surface and in air during different stages of plants' growth

Plants / Fungi	Young (25.4. 1970)					Flowering (15.5. 1970)					Fruiting (5.6. 1970)				
	LC	LS	CM	AE	Air	LC	LS	CM	AE	Air	LC	LS	CM	AE	Air
Rhizopus nigricans	+/—**	—/—	—/—	—/—	+	—/—	—/+	—/—	—/—	+	—/—	—/—	+/—	+/—	—
Mucor hiemalis	—/—	—/—	—/+	—/—	—	—/—	—/—	—/—	—/—	—	—/—	—/—	—/—	—/—	—
M. racemosus	+/—	+/—	—/—	+/—	+	—/—	—/—	—/—	—/—	—	—/—	—/—	—/—	—/—	—
Yeast	—/—	+/—	—/—	—/—	—	+/—	—/—	—/—	—/—	—	—/—	—/—	—/—	—/—	—
Aspergillus nidulans	—/—	—/—	—/—	—/—	+	—/—	+/—	—/—	+/—	—	—/+	—/+	—/—	—/+	+
A. flavus	—/—	+/—	—/—	—/—	+	—/—	—/+	—/—	—/—	+	—/—	+/—	—/—	—/—	+
A. niger	+/—	+/—	—/—	+/—	*	+/—	+/+	—/—	+/—	+	+/—	+/+	+/—	+/+	*
A. sydowi	—/—	—/—	—/—	—/—	—	—/—	—/—	—/—	—/—	+	—/—	—/—	—/—	—/—	+
A. terreus	+/—	—/—	—/—	+/—	+	+/—	—/—	—/—	—/—	+	+/—	—/—	+/—	—/—	+
A. versicolor	—/—	—/—	—/—	—/—	—	—/—	—/—	—/—	—/—	+	+/—	+/—	—/—	—/—	+
Paecilomyces fusisporus	—/—	—/—	+/—	—/—	+	—/—	—/—	+/—	—/—	+	—/—	—/—	—/—	—/—	*
Penicillium humicola	—/—	—/—	—/—	—/—	—	—/—	—/—	—/—	+/—	—	—/—	—/—	+/—	—/—	—
P. frequentans	+/—	—/—	—/—	—/—	+	+/—	+/+	—/—	+/—	+	—/—	—/—	—/—	—/—	—
P. sp.	+/+	+/—	+/—	—/—	—	—/—	—/—	—/—	—/—	—	—/—	—/—	—/—	—/—	—
Alternaria tenuis	* /—	* / *	* /—	* /*	*	* /+	* /—	* /—	* / *	*	* / *	* / *	* / *	* / *	*
Curvularia lunate	—/—	—/—	—/—	—/—	—	—/—	—/—	+/—	—/—	+	—/—	+/—	—/—	—/—	+
C. pallescens	+/ *	—/—	+/—	—/—	*	—/ *	+/—	—/ *	—/ *	*	+/+	+/+	—/+	+/ *	+
C. tetramera	—/—	—/—	—/—	—/—	+	—/+	—/—	—/—	—/—	+	—/—	+/—	+/—	—/+	+
Botrytis cinerea	—/—	—/+	—/—	—/—	—	—/—	—/—	—/—	+/—	+	—/—	—/—	—/—	—/—	—
Helminthosporium sp.	—/—	—/—	—/—	—/—	—	—/—	—/—	—/—	—/—	—	+/—	+/—	—/—	—/—	—
Cephalosporium sp.	—/—	—/—	+/—	—/—	+	—/—	—/—	—/—	—/—	—	—/—	—/—	—/—	—/—	—
Torula sp.	—/—	—/—	—/—	—/—	—	—/—	—/—	+/—	—/—	+	—/—	—/—	—/—	—/—	—
Aure obasidium pullulans	—/—	—/—	+/—	—/—	—	—/—	—/—	+/+	—/—	—	—/—	—/—	—/—	—/—	—
Fusarium spp.	—/—	+/+	—/—	—/—	*	+/—	+/—	+/—	+/—	+	—/—	+/—	+/+	+/+	+
Brown sterile colony	—/—	—/—	—/—	—/—	—	+/—	+/—	—/—	—/—	—	+/—	+/—	—/—	+/—	—
White sterile colony	+/—	—/—	—/ *	—/—	+	* /—	+/—	* /—	—/—	+	+/—	—/—	+/—	+/—	—

Table 2

Myco-population, moisture content and PH of leaf samples

Plants	Stage of growth	No. of spp.	Mn. no. of cols/pl.	Moisture content(%)	PH
	Y	9/2**	5/2	80.45	7.6
LC	F	8/3	5/4	77.80	7.5
	Fr	8/3	10/4	77.00	7.8
	Y	7/3	4/2	83.50	7.8
LS	F	8/4	7/3	82.10	7.9
	Fr	10/4	17/5	73.22	8.2
	Y	6/2	3/2	84.40	8.3
CM	F	7/2	11/2	87.75	8.0
	Fr	8/3	47/5	79.70	7.6
	Y	4/1	4/2	80.64	7.3
AE	F	7/2	6/2	79.25	7.4
	Fr	7/6	29/5	74.60	8.0
	Y	14	20	—	—
AIR	F	16	17	—	—
	Fr	12	13	—	—

Y= Young, F= Flowering, Fr= Fruiting stage

** = Numerators designate the phyllosphere and denomenators the phylloplane.

\+ = Present, - = Absent, *= Dominant.

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