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STUDIES ON THE NON-PARASITIC MYCOFLORA OF LEAVES OF FOUR ECONOMIC ROSACEAN PLANTS

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ABSTRACT

The non-parasitic mycoflora on the surfaces of the living leaves of four economic plants viz: Pyrus malus; Prunus domestica; Prunus armeniaca and Prunus persica was studied using the washing and impression techniques. The washing dilution plate culture technique showed higher fungal counts, however, the impression technique revealed the topology of fungal isolates on various sites of the leaf.

Four filamentous fungal genera viz: Alternaria, Curvularia, Helmenthosporium and Spondylocladium; a chromogenic and non-chromogenic yeasts were detected on both surfaces of leaves of the investigated plants. Yeasts constitute an appreciable - percentage of the fungal counts on lower surfaces of investigated leaves, Distinctive fungal genera characteristic for the leaf surface and plant type were recorded. In this investigation 18 fungal - genera were identified.

INTRODUCTION

Study of the incidence of fungi on the leaf surface of cereals by Last, (1955) introduced the term phyllosphere in analogy with the term rhizosphere given by Hiltner, (1904) However , the significance of phylloplane as an ecologically neglected mileu has not been sufficiently

assessed as compared to the rhizoplane, mainly due to the dearth of comprehensive studies of the involved - microorganisms.

Surfaces of healthy leaves are colonized by a variety of microorganisms as recorded by some authors e. g. Di Minna, (1955); Leben, (1961); Ruinen, (1961) ; Last and Deighton, (1965) ; Dickinson, (1967) & (1971) Zaki, (1975) ; Lindsey & Paugh , (1976) ; Eicker(1976) Ali, (1978) and El Naggar & Emara, (1981).

The plants under investigation are fruit crops - economically important, especially in this region, and investigation on their phylloplane non-parasitic mycoflora might help revealing fundamentals related to their nutrition.

MATERIALS AND METHODS

Plants:

The plants *Pyrus malus; Prunus domestica: Prunus armeniaca* and *Prunus persica* are growing in a limited area (The Botanic garden, College of Education at Abha) so as to ensure uniform conditions regarding the climate and airspora.

Sampling:

Samples of foliage leaves were collected aseptically to be investigated for their surface fungi.

Medium:

Dox medium was used for counting the developing -

fungal colonies. It contains sucrose, 15 g. ; Na NO_3 , 2g; KH_2 PO_4 , 1g.; Mg $SO_4 . 7H_2O$, 0.5g.; Fe $SO_4 . 7H_2O$ traces , rose bengal , 0.04g; agar 20g ; distilled water 1 litre . PH was adjusted at 6.4 after sterlization.

Phylloplane fungal counts:

Dilution technique: The wshing dilution technique of Dickinson, (1967) was used. In this technique - discs (diameter, 0.75 cm) were punched from leaves using sterlized cork porer. Ten discs were shaken vigorously by hand for 10 minutes in a known volume of sterile distilled water, decimal dilutions were made as required. One ml of each dilution served as an inoculum for a sterile plate. Molten sterile Dox medium was then Poured for each treatment and triplicates of each dilution were made to get mean values.

Impression technique:

A detached leaf was gently impressed on surface of Dox agar medium using a sterile glass rod. The same - leaf surface was impressed again on a series of plates (usually 2) containing the same medium in an attempt to pick up cells not released in the first print; the same was followed for the other leaf surface. The area of the impressed leaf was measured. At least triplicates were made throughout.

Isolation, Purification and Identification of the fungal isolates:

Yeast colonies were picked up and streaked on Dox medium . The isolates were purified by further streaking for several consecutive times, on the same medium and checking by microscopic examination of stained films till getting pure isolates.

Hyphal tips of developing filamentous fungal colonies were detached and allowed to develop on Dox's - agar medium. No difficulty was encountered in getting pure cultures of the filamentous fungi. Filamentous - fungal isolates were identified using Gilman, 1966 and Barnett & Hunter 1972.

R E S U L T S

The total fungal counts using both the dilution plate and the impression techniques were given in Tables 1 , 2 , 3 . Data given in these tables show that counts were higher in case of the dilution than the - impression technique throughout. Results of the impression technique indicates that the lower surface of *Pr. domestica; Pr. armenica* and *pr. persica* leaves harboured higher counts than the upper surface. In case of

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TABLE 1 - counts of fungi in the phylloplane (Upper "U" & lower "L" surfaces) of five full grown foliage leaves down upwards of *P. malus*, *Pr domestica*; *Pr. armeniaca* and *Pr. persica* after flowering, appearance and growth of foliage leaves, as assessed by the impression* (Imp) & the dilution** (Dil) techniques.

Leaves	Surface	Method	Total fungal counts/100Cm^2 leaf surface of			
			P. malus	*Pr. domestica*	*Pr. armeniaca*	*Pr. Persica*
	U	Imp	112	86	72	64
First	L	"	74	94	88	82
	U+L	DIl	256	248	226	214
	U	Imp	146	108	94	80
Second	L	"	118	128	106	94
	U+L	Dil	362	294	276	284
	U	Imp	162	122	112	96
Third	L	"	134	138	132	118
	U+L	Dil	412	336	312	322
	U	Imp	142	116	108	102
Fourth	L	"	126	132	124	126
	U+L	Dil	344	286	266	292
	U	Imp	94	88	92	84
Fifth	L	"	68	102	116	96
	U+L					

* Yeasts constitute 18 - 38% and 26 - 54% of the counts on upper and lower leaf surfaces respectively.

** Yeasts constitute 14 - 43% of the Counts.

TABLE 2 - Counts of fungi in the phylloplane upper "U" & lower "L" surfaces) of five full grown foliage leaves down upwards of *P. malus; Pr. domestica; Pr. armeniaca; Pr. persica,* 6 weeks after flowering as assessed by the impression*(Imp) and the dilution ** (Dil) techniques.

Leaves	Surface	Method	Total fungal counts/100 cm^{-2} leaf surface of			
			P. malus	*pr. domestica*	*pr. armeniaca*	*Pr. persica.*
First	U	Imp	144	106	94	102
	L	"	92	128	116	122
	U+L	Dil	312	286	294	282
Second	U	Imp	170	116	124	136
	L	"	134	134	154	148
	U+L	Dil	396	322	392	368
Third	U	Imp	188	134	156	176
	L	"	164	176	172	212
	U+L	Dil	466	412	426	446
Fourth	U	Imp	152	122	126	128
	L	"	114	145	142	172
	U+L	Dil	322	318	342	388
Fifth	U	Imp	104	86	84	98
	L	"	76	108	102	139
	U+L	Dil	266	216	284	312

* Yeasts constitute 12 - 32% and 17 - 46% of the counts on upper and lower leaf surfaces respectively.

** Yeasts constitute 15 - 41% of the counts.

TABLE (3) - Counts of fungi in the phylloplane (Upper "U" and lower "L") of five full grown foliage leaves down upwards of *P. malus; Pr. domestica; Pr. armeniaca* and *Pr. presica* at twelve weeks after flowering as assessed by the Impression *(Imp) and the dilution** (Dil) techniques.

Leaves	Surface	Method	Total Fungal Counts/100 cm^2 leaf surface of			
			P. malus	*Pr. domestica*	*Pr. armeniaca*	*Pr. persica*
First	U	Imp	184	152	132	144
	L	"	142	166	148	153
	U+L	Dil	468	424	366	386
Second	U	Imp	206	184	172	192
	L	"	168	226	208	226
	U+L	Dil	496	482	436	492
Third	U	Imp	238	222	212	240
	L	"	212	286	246	256
	U+L	Dil	534	568	522	538
Fourth	U	ImP	196	176	148	154
	L	"	152	206	174	166
	U+L	Dil	386	442	410	428
Fifth	U	Imp	158	126	112	126
	L	"	136	144	134	152
	U+L	Dil	332	332	312	326

* Yeasts constitute 19 - 37% and 22 - 49% of the counts on upper and lower leaf surfaces respectively.

** Yeasts constitute 16 - 45% of the counts.

Pr. malus , lower surface of the leaves harboured lower counts than the upper surface. The top leaves showed lower fungal counts than the older lower leaves. Results also show an increase in fungal counts with increase in age of the leaves. Table 4 shows the occurrence of fungal isolates on both surfaces of the investigated leaves. *Alternaria ; Curvularia ; Helminthosporium; Spondylocladium ;* chromogenic yeasts and non-chromogenic yeasts, colonized both surfaces of all the investigated leaves. *Fusarium* was recorded on both surfaces of *P. malus ; Pr. domestica* and *Pr. armeniaca* leaves. *Geotrichum* and *Tricoderma* were detected on impressing both surfaces of *P. malus* and *Pr. armeniaca* leaves . *Penicillium* was recorded on both surfaces of *Pr. armeniaca* leaf and the lower surface only of *Pr. domestica* leaf *Paecilomyces* was recorded on the lower surface of both *Pr. domestica* and *Pr. persica* leaves. *Hermodendrum* was recorded on the lower surface of both *Pr. domestica* and *Pr. armeniaca* leavs. *Chaetomium* and *Cephalosporium* colonized the upper surface only of *P. malus*. *Stemphylium* and *Cladosporium* were recorded only on the upper surface of *Pr. persica* leav *Scopulariopsis* was recorded on the lower surface of *P. malus* and *Pr. persica* leaves.

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TABLE (4) - Occurrence (+) of the isolated genera of Fungi from the phylloplane of *P. malus*; *Pr. domestica*; *Pr. armeniaca* and *Pr. persica*.

Isolated genera	Foliage leaf surfaces of							
	P. malus		*Pr. domestica*		*Pr. armeniaca*		*Pr. Persica*	
	U	*L*	*U*	*L*	*U*	*L*	*U*	*L*
Alternaria	+	+	+	+	+	+	+	+
Curvularia	+	+	+	+	+	+	+	+
Helminthosporium	+	+	+	+	+	+	+	+
Spondylocladium	+	+	+	+	+	+	+	+
Stemphylium							+	
Cladosporium							+	
Chaetamium	+							
Hormodendrum				+		+		
Cephalosporium	+							
Scopulatariopsis		+						+
Trichoderma	+	+			+	+		
Trichosporium			+	+				
paecilomyces				+				+
Penicillium				+	+	+		
Geotrichum	+	+			+	+		
Fusarium	+	+	+	+	+	+		
Chromogenic yeasts	+	+	+	+	+	+	+	+
Non-chromogenic yeasts	+	+	+	+	+	+	+	+

DISCUSSION

Attempts have been made in different parts of the world to characterize the microflora of different phylloplanes using a variety of methods. The dilution plate culture technique and the impression technique were used in the present investigation. The former technique gave more counts than the latter one, however, a limitation which is the inability to reveal the topology of microbes on various sites of theleaf has faced the first technique Using the impression technique , this investigation shows a developmental pattern of yeasts and fungi in a period of few months. Yeasts were found to be sticking to the veins in the centre of the leaf whereas filamentous fungi colonized the vein free area. The sticking of yeasts to the veins could find interpretation in the increase in moisture in the centre of the leaf as shown by Burrage 1971 and would suggest a role for leaf exudates as given by Tukey 1971.

Plant leaves, like roots, secrete substances from their surfaces. Deposition of spores and microorganisms on the outer surfaces of plant leaves is certainly the source of the microbial populations on these surfaces, but this must not minimize "selection" to develop an "autoecological" niche with distinct features. Selection would vary with variation of the microloci on the leaf surface. So one would expect selection related to the topography of the leaf surface. Topography might be associated with biological activity to orient the effects. Upper exposed - surface of leaf would differ in biosis from lower rather sheltered surface. The same would happen in vein and

vein-free microloci.

The type of the plant and the age of the leaf are undoubtedly effective on the quantity and quality of phylloplane fungi. Specificity of the fungal flora in this investigation may be related to plant type e.g. *Trichosporium* found on both surfaces of *Pr. domestica* leaf; to one surface of *Pr. domestica & Pr. armeniaca*, *Scopulariopsis* on lower surface of *P. malus & Pr. Persica* and *Paecilomyces* on lower surface of *Pr. persica* and *Pr. domestica*, *Stemphylium & Cladosphorium* were specific to upper surface of *Pr. Persica* and *Chaetomium & Cephalosporium* were restricted to upper surface of *P. malus* or specificity may be related to site on the leaf surface as was shown by the vein and vein-free area, the former colonized by the yeasts and the latter by filamentous fungi. Specificity to leaf surface microbes was reported by Sinh., (1971).

REFERENCES

Ali, M.I.(1978): Non-parasitic. Fungal Flora on the-surfaces of living leaves. Proc. Saudi Biol. soc. 2:53 - 60.

Barnett, H.L. and I. Hunter (1972): IIIustrated Genera of Imperfect Fungi. Burgess Publishing Company, Minneapolis, pp. 241.

Burrage, S.W. (1971): The microclimate at the leaf surface. In Ecology of leaf surface microorganisms. Edited by T.F. Preece and C.H. Dickinson. London Academic Preass.

Dickinson, C.H. (1967): Fungal Colonization of *Pisum sativum*. Canad. J. Bot., 45:915 - 927.

Dickinson, C.H. (1971): Cultural Studies of leaf saprophytes. In Ecology of leaf surface microorganisms, ed. T.F. Preece and C.H. Dickinson, pp. 124 - 137, London Academic Press.

DiMinna, M.E. (1959): Yeasts From leaves of pasture plants. New Zeland J. of Agric. Research, 2: 394 - 405.

Eicker, A. (1976): Non-parasitic mycoflora of the phylloplane and litter of *Panicum coloratum coloratum*. Trans Brans br. Mycol. Sec. 7, 67: 275 - 281.

El-Naggar M.R. and H.A. Emara (1981): Studies on the phylloplane bacteria and fungi of the semiparasite *Loranthus* sp Proc. Saudi Biol. Soc.

Hiltner, (1904), C.F. Preece and Dickinson (1971).

Gilman, J.C. (1966): Nanual of Aoil Fungi, Iowa State College Press, 3 rd edition.

Last, E.T. (1955): Seasonal incidence of *Sporobolomyces* on cereal leaves. Trans. Br. Mycol. Soc. 38: 221 - 239.

Last, F.T. and F.C. Deighton (1965): The non-parasitic Mycoflora on the surface of living leaves. Trans. Br. Mycol. Soc., 48:83 - 99.

Leben, C. (1961): Microorganisms on cucumber seedlings. Phytopathology, 51: 553 - 557.

Lindsey, B.L. and J.F. Paugh (1976): Succession of micro fungi on attached leaves of *Hipophae rhamnoides*. Trans. Br. Mycol. Soc., 67: 61 - 67.

Ruinen, J. (1961): The phyllosphere. I: An ecologically neglected milieu. Plant and Soil. 15: 81 - 109.

Sinha, S. (1971): The microflora of leaves of *Capsicum annuum* (L.) Watt E.D., Solanum melongena L., *Solanum melongena* L., *Solanum tuberosum* L. and *Lycopersicum esculentum* Mill. In Ecology of leaf surface microorganisms., pp. 175 - 189, (ed. T.F. Preece and C.H. Dickinson), London Academic Press.

Tukey, H.B. (1971): In: Ecology of leaf-surface microorganisms, pp. 67 -80 (ed. T.F. Preece and C.H. Dickinson), London Academic fress.