Ent. Phyt. Appliq.
Vol. 54, No. 1 &2, 1987

LEAF SURFACE MYCOFLORA OF DISEASED AND NON - DISEASED PEA

(*Pisum sativum* L.)[1]

NAMEIRAKPAM IRABANTA SINGH[2] & 3 MISS PANGABAM MEMCHA DEVI[3]

SUMMARY

The investigation on leaf surface mycoflora (Phylloplane & phyllosphere) of diseased and non - diseased pea (*Pisum sativum* L.) leaves were carried out by using leaf washing technique for one complete crop season (October 1984 to March 1985) Analysis of the diseased and non - diseased leave surfaces revealed 23 fungal types out which 15 belongs to Deuteromycetes, 3 to Ascomycetes, 2 to Phycomycetes, 2 sterile mycelia and one to Actinomycetes. *Cladosporium* sp; *Alternaria* sp. were dominant types on diseased leaves while *Cladosporium* sp; *Alternaria* sp. *Mucor* sp. *Penicilium* sp. were dominant types in case of non - diseased leaves. It was also observed that the total population of the leaf surface mycoflora in diseased (341) was higher than that of non - diseased (297). The present investigation would be useful for further study of antagonistic and stimulation relationship between leaf surface microorganisms and its pathogens *Uromyces fabae* Pers (de Bery) etc.

---

1 - Received for publication, February 10, 1986.

2 - For correspondence.

3 - Plant pathology & Aerobiology Laboratory, Department of Life Sciences, Manipur University, Canchipur, Imphal - 795003, INDIA.

INTRODUCTION

Leaf surfaces are covered by a large and varied population of micro - organisms (Dickinson 1976; Sharma Gupta and Dixit 1984). Various microbes, both parasites and saprophytes, are colonized on the leaf -surface due to the richness of the nutrients (Mishra and Srivastava 1970) . Some of these colonizers are responsible for disease development (Last 1955, Kerling 1958, Sinha 1971). The age of the plant, season, maturity of leave and climatic factors play an important role in controlling the composition of surface mycoflora (Sharma, Gupta, Dixit 1971). Fungal colonization of aerial parts of terrestrial and crop plants were studied by various workers (Memcha Devi, 1984). The importance of microorganisms prosent on leaf surface indicates a cloeser lation between surface microbes and those microbes which are associated with foliar diseases and need to study it for increasing crop productivity by controlling the diseases (Purkayastha and Bhattacharya, 1982; Mishra and Srivastava, 1970).

The aim of the present investigation has been, therefore, to isolate the fungal population of diseased and non - diseased pea leaves by using leaf washing technique. Further, the qualitative and quantitative analysis of diseased and non - diseased surface mycoflora of pea (*Pisum sativum* L.) in relation to climatic conditions are also taken into consideration.

MATERIALS AND METHODS

The diseased and non - diseased sea (*Pisum sativum* L.) cultivar Makhyat Mubi (Local variety) leaves were collected at random from a field grown in Imphal (24. 44, N. Latitude, 93. 58, E. Longitude), India measuring an area of one hectare at monthly interval from Octoboer 1984 to March 1985 . The leaf surface mycoflora was studied following leaf washing technique of Voznyakovaskya and Kudyakov (1960). The mycoflora were divided into two categories, viz. *Phyllosphere* and *Phylloplane* for both diseased and non - diseased leaves.

The field infections of the pea crop were noticed when the plants are

28 days old. Thirty leaves were collected at random from the field described above for both types of experiments. After cutting into pieces, equal amounts of leaves for both types (diseased and non - diseased) were introduced separately into 50 ml of sterile water contained in a 250 ml capacity conical flask. They were shaken vigorously by hand for 25 minutes. 5 ml each of such spore suspensions was inoculated separately into 3(three) petridishes (9 cm. in dia.) containing Czapek's Dox agar medium for isolation of diseased and non - diseased phyllosphere fungi.

The leaf pieces were again washed for 5 times with sterilized water. Then pieces (separately for diseased and non - diseased leaves)were introduced directly into 3 (three) petridishes (9 cm in diam) containing Czapek's Dox agar medium for isolation of diseased and non - diseased phylloplane fungi.

All such petridishes were incubated at 27 °C ± 1°C for 7 days . The colonies thus developed were counted and identified with the help of published literatures.

RESULTS

The fungi isolated from the phyllosphere and phylloplane regions of diseased and non - diseased pea leaves are listed in Table I & II.

The seasonal variations in the total population of the surface mycoflora inhabiting diseased and non - diseased pea leaves revealed that the highest count (59) of diseased phyllosphere population was recorded in October 1984 while the lowest (11) was recorded in March, 1985. The highest count (62) of non - diseased phyllosphere population was recorded in December, 1984, while the lowest (11) was in March 1985 (Table I).

The highest count (52) of diseased phylloplane population was recorded in October 1984, while the lowest (11) was recorded in January, 1985 . The highest count (34) of non - diseased phylloplane population was recorded in October, 1984 while the lowest (9) was in February, 1985 (Table I).

The total number of mean fungal types both in diseased and non - di seased pea leaves were 23 (Table III).

Becker and Manning (1983) determined the phylloplane mycoflora of two scab immune (liberty and Nova Easy gro) and one scab - susceptible (lmperial Mc Intosh) apple (*Malus domestica* Berkh) cultivars. Fungi isolated from the leaves at monthly intervals unit harvest by leaf washing method revealed that phylloplane microflora for all these three cultivars were found quantitatively and qualitatively similar.

Mishra and Srivastava (1971) reported that leaf surface mycoflora of Virus (VMM) infected and non - infected *Petunia hybrida* are different. They recorded maximum number in the diseased leaves than the non - diseased leaves. They suggested that more fungal population in diseased leaves was due to mutual antagonism between virus and fungal population . In the present investigation also, more surface mycoflora were recorded in the diseased (phylloplane and phyllosphere) leaves than the non - diseased (phylloplane and phyllosphere) pea leaves (Table IV).

A contradicting result was reported by Mishra and Kanaujia (1971) on *Lycopersicum asculantum* where they found lesser number of fungi on virus infected leaves than healthy ones. The reason might be due to cobalt treatment on the diseased leaves, the type of virus and test plant.

Pace and Campbel (1974) stated that population level was an important factor in disease incidence as it was directly related with the active growth of the saprophytes on leaf surface which might compete for nutrient or produced inhabiting substances against the growth of the pathogen.

According to Upadhya & Dwivedi (1977) fungi having the highest population level, least effective in inhibition of spore germination in vitro have considerable effect on percentage inhibition of disease incidence, while Gregory (1973) states «the occurrence of many fungi on aerial surfaces may be directly related to inoculation from the atmosphere».

In the present investigation, the total number of fungal population was more in the diseased leaves (phylloplane and phyllosphere) than the non - diseased leaves (phylloplane an dphyllosphere) (Table III). At this stage

no conclusion can be drawn in respect antagonistic nature of *Uromyces fabae* and other surface mycoflora.

Antagonistic interaction between phylloplane microbes of *Venturia inacqualis* was reported by Andrews and Bergee (1981). They reported that the promising antagonistic were *Chaetomium globosum*, *Aureobasidium pullulans*, *Trichoderma viride* and an actinomycete. These fungi reduced lesion size and number, and conidial numbers apparently by nutrient competition and antibiosis.

Further infection of onion leaves by *Alternaria* sp. was reduced approximately 50% in the presence of *Aureobasidium pullulans* and *Sporobolomyces roseus* (Fokkema and Lorbeer 1974, Last and Warren 1972).

The result of the present study, however, are not in agreement with their findings. Here total fungal population in diseased leaf surface was higher both in phylloplane and phyllosphere than the non - diseased leaf surface . The only possible explanation might be in case of pea rust (*Uromyces fabae*) pathogen and surface antagonistic interaction do not come into the picturc in the natural condition.

However, the present finding was in agreement with that of Garg and Sharma (1983). They reported that the surface mycoflora of non - infected and rust infected leaves of Triticale showed higher fungal population in rust infected leaves than non - infected leaves. They also isolated more species of fungi from the infected leaves.

ACKNOWLEDGEMENTS

The authors are thankful to the Head of the Department of Life Sciences, Manipur University, Canchipur for providing laboratory facilities. Thanks are also due to the Department of Science and Technology, Govt. of India for financial assistance to N I S under Young Scientists, Scheme.

Table I. Seasonal variations in the total population (colonies) of the surface mycoflora inhabiting diseased and non - diseased leaves of pea (*Pisum sativum* L.)

Techniques	Type of leaves		Fungal populations						Total population
			Oct.	Nov.	Dec.	Jan.	Feb.	March	
			1984				1985		
Leaf washing	Non - diseased	phyllosphers	41	21	62	30	30	11	195
		phylloplane	34	13	17	16	9	13	102
	Diseased	phyllosphere	59	12	46	40	16	11	204
		phylloplane	52	20	26	11	15	13	137

TABLE II. DISTRIBUTION OF DISEASED AND NON-DISEASED LEAF SURFACE FUNGI AND THEIR PERCENTAGE OF ABUNDANCE (BY LEAF WASHING TECHNIQUE)

me of the fungus	DISEASED															NON-DISEASED														
	Phylloplane								Phyllosphere								Phylloplane								Phyllosphere							
	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	To-tal	% of abun-dance	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	To-tal	% of abun-dance	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	To tal	% of abun-dance	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	To-tal	% of abun-dance
Actinomycetes sp.	2	-	-	-	-	-	2	0.96	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.alternata	-	-	-	-	-	-	-	-	-	-	-	2	1	1	4	1.9	-	-	-	1	-	1	2	1.9	-	-	-	-	-	-	-	-
Alternaria sp.	-	-	4	4	3	4	15	7.18	-	-	-	6	1	4	117	5.4	-	-	-	3	2	5	10	9.8	-	-	-	-	4	1	5	2.51
Aschochyta sp.	-	-	-	-	-	3	3	1.44	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ascomycetes ?	-	-	1	-	-	2	3	1.44	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aspergillus sp.	-	-	2	-	1	-	3	1.44	-	-	-	-	-	-	-	--	2	1	1	-	-	4	4	3.9	-	-	4	-	-	2	6	3.02
Chaetomium sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	1	-	3	1.51
Cladosporium sp.	40	15	15	4	-	-	74	35.41	46	10	40	20	30	-146		71.1	24	6	10	7	4	4	55	53.9	30	15	50	30	20	2	147	73.87
Dreschlera sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	2	1.9	-	-	-	-	-	-	-	-
Epicoccum sp.	-	-	2	-	5	2	9	4.31	-	-	-	1	1	2	4	1.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fusarium sp.	4	-	-	-	-	-	4	1.91	6	1	-	-	-	-	7	3.4	1	-	-	1	-	2	4	3.9	3	-	-	1	-	5	5	2.51
Harpographium sp.	-	2	-	1	-	-	3	1.44	-	-	-	-	-	-	-	-	-	-	2	-	-	-	2	1.9	-	-	-	2	2	4	4	2.01
Leptospherulina sp.	-	-	-	-	2	-	-	0.96	-	-	1	-	2	-	3	1.4	-	-	-	1	-	1	2	1.9	1	-	2	-	-	-	3	1.51
Mucor sp.	-	-	2	-	-	1	3	1.44	2	-	1	5	-	1	9	4.4	3	-	-	1	1	-	5	4.9	2	-	-	-	1	2	5	2.51
P. clavatum	-	-	-	-	-	-	-	-	1	-	2	2	-	-	5	2.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Penicilium sp.	1	2	-	-	1	-	4	1.91	1	-	-	2	-	1	4	1.9	2	-	2	1	1	-	6	5.8	2	-	1	-	2	-	5	2.51
Phoma sp.	-	-	-	2	-	-	2	0.96	-	1	-	1	-	1	3	1.48	-	-	2	-	1	-	3	2.9	-	-	-	-	-	-	-	-
Phytopthora sp.	3	-	-	-	-	-	3	1.44	1	-	-	-	-	-	1	0.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rhizopus sp.	2	-	-	-	-	-	2	0.86	1	-	-	-	-	-	1	0.4	-	-	-	-	-	-	-	-	-	3	-	1	-	1	5	2.51
Sterile mycelia(W)	-	1	-	-	2	1	4	1.91	-	-	1	-	1	1	3	1.4	-	3	-	-	-	-	3	2.9	-	3	-	-	-	-	3	1.51
Sterile mycelia(Y)	-	-	-	-	-	-	-	-	1	-	-	1	-	-	2	0.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Torula sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	2	1	4	2.01

Table III. Total number of mean fungal types in diseased and non - diseased leaves of pea (*Pisum sativum* L.) by leaf washing technique

Total number of mean fungal types	Diseased		Non - diseased	
	phylloplane	phyllosphere	phylloplane	phyllosphere
23	16	14	13	13

Table IV. Surface mycoflora of diseased and non - diseased Pea (*Pisum sativum* L.) leaves (by leaf washing technique)

Fungal types	Diseased		Non - diseased	
	phyllo-plane	phyllo-sphere	phyllo - plane	phyllo - sphere
Cladosporium sp.	++	++	++	++
Fusarium sp.	+	+	+	+
Mucor sp.	+	+	+	+
Phythophthora sp.	+	+	+	—
Penicilium sp.	+	+	++	+
Rhizopus sp.	+	+	+	+
Alternaria sp.	++	++	++	++
Epicoccum sp.	+	+	—	—
Leptospherulina sp.	+	+	+	+
Sterile mycelia(Y)	—	+	—	—
Aspergillus sp.	+	—	+	+
Phoma sp.	+	+	+	—
Alternaria alternata	—	+	—	—
Sterile mycelia (W)	+	+	+	+
Aschochyta sp.	+	—	—	—
Dreschlera sp.	—	—	+	—
Ascomycetes ?	+	—	—	—
Penicilium clavatum	—	+	—	—
Hergraphium sp.	+	—	+	+
Actinomycetes sp.	+	—	—	—
Geotichum sp.	—	—	+	+
Chaetomium sp.	—	—	—	+
Torula sp.	—	—	—	+

REFERENCES

BECKER, M. C. and MANNING, W. J., (1983). Phylloplane microflora of apple cultivars immune and susceptible to *Venturia inacqualis*, Phytopath. Vol. 73 (9) 1342 Abstr.

DICKINSON, C. H., (1976). Fungi on aerial surfaces of higher plants in Microbiology of Aerial Plant Surfaces Ed. C. H. Dickinson & T. F . Preece Academic Press, London, New York, 669.

FOKKEMA, N. J. and LORBEER, J. W., (1974). Interaction between *Alternaria porri* and the saprophytic mycoflora of onion leaves. Phytopath. 64, 1128 - 1133.

GARB, A. P. and SHARMA, P. D., (1983). Mycoflora of non - infected and rust infected of Triticale. Indian Phytopath. 36 (4); 672 - 678.

KERLING, L. C. P., (1958). De microflora ophet blad van Beta vulgaris, Tijd cher Plziekt 64 : 402 - 410.

LAST, F. J., (1955). Seasonal incidence of sporobolomyces on cereal leaves. Trans. Brit. Mycol. Soc. 38 : 221 - 239.

LAST, F. T. and WARREN, R. C., (1972). Non- parasitic microbes colonizing green leaves their form, function. Endeavour 31 : 143 - 150.

MEMCHA DEVI, P., (1984). Studies on the fungal air - spora and leaf surface mycoflora of pea (*Pisum sativum* L.) and their relationship with occurrence and intensity of some fungal diseases. M. Sc. Dissertation, Manipur University, 112 p.

MISHRA, R. R. and SRIVASTAVA, V. B., (1971). Leaf surface fungi of *Oryza sativa* Linn. Myco - Pathologia et. Mycologia appl. Vol. 3 : 289 - 294.

MISHRA, R. R. and KANAWJIA, R. S., (1971). Studies on Phyllosphere fungi - III Leaf surface fungi of healthy and rust infected *Lycepercicum esculentum* in relation to cobalt. Sydowia , Annales Mycologia Ser II . Vol. XXV. Hebt 1 - 6.

MISHRA, R. R. and SRIVASTAVA, V. B., (1971). Microflora of leaf surface in relation to virus infection Proc. Nat. Acad. Sci (India), XLI, 90 - 92.

PACE, A. A. and CAMPBELL, R., (1974). The effect of Saprophytes on infection of leaves *Brassica* sp. by *Alternaria brassicicola*. *Trans, Brit Mycol*, 5 Oc, 63, 193 - 196.

PURKAYASTHA, R. P. and BHATTACHARYYA, B. (1982). Phylloplane environment monitoring microbes, Science and Culture Vol. 48 (6) : 193 - 200.

SHARMA, A. K., GUPTA, J. S. and DIXIT, R. B., (1984). Aquantitative and qualitative analysis of phylloplane microflora of yellow sarson and Taramira in relation to micro - climatic factors. Ind. Phytopath. 37 (1) : 107 - 110.

SINHA, S., (1971). Census of microflora of *Capsicum annum* L. watt *Solanum melongena* L. S. *tuberosum* L. and *Lycopersicum esculentum* Mill. Proc. Int. Seminar on «Leaf surface Ecology» New Castle England, Abstr, pp. 115.

UPADHYAY, R. K. and DWIVEDI, R. S., (1977). Phyllosphere microfungi of *Eucalyptus globulus* in relation to its leaf spot disease. Proc. Indian Nat. Sci. Acad. 43 : 33 - 36.

VOZNYAKOVSKAYA, Y. M. and KHUDYAKOV, Y. P., (1960). Species composition of the epiphytic microflora of living plants. Microbiol . 29 : 97 - 103

WARREN, R. C., (1972). Interference by common leaf saprophytic fungi with the development of *Phoma betae* lesions sugar beet leaves. Annals of Applied Biology, 72 : 137 - 144.