

FILAMENTOUS FUNGI INFECTING FRUITS AND LEAVES OF *CAPSICUM ANNUM* L. IN LOWER SINDH

Faisal Hussain^{1*}, S. Shahid Shaukat², Muhammad Abid¹, Farzana Usman¹ and Muhammad Akbar¹

¹Dr. A.G. Aerobiology and Plant Pathology Lab., Department of Botany, Federal Urdu University of Art, Science & Technology, Gulshan e Iqbal Campus Karachi, Karachi, Pakistan.

²Department of Environmental Sciences, University of Karachi, Karachi-75270, Pakistan.

*Correspondence author E-mail: faisal.botanist2011@gmail.com

ABSTRACT

In Pakistan, the production of chilli (*Capsicum annum* L.) crop usually grows under stressed conditions due to a number of pathogens and mostly fruit and foliar fungal diseases are regarded as responsible for reduced production. For this purpose, eight different localities of lower Sindh including Hyderabad, Tando Allahyar, Mirpurkhas, Umerkot, Kunri, Samaro, Kot Ghulam Muhammad and Digri areas were selected for survey of fungal infection as they are the major chilli producing areas of Pakistan. One hundred sixty samples of infected fruits and foliar parts with fruit as well as without fruit were collected. The stored fruits were also collected from the markets and godowns of the major storing units of chilli fruit. Eleven fungi including *Alternaria alternata*, *A. solani*, *Aspergillus flavus*, *A. niger*, *Botrytis cinerea*, *Cercospora capsici*, *Colletotrichum capsici*, *Leveillula taurica* and *Verticillium* spp. were isolated and identified from the fruits and foliar parts of chilli. The diversity of the fungal assemblages was estimated using diversity indices.

Key-words: Chilli, fruits, leaves, fungi, Sindh,

INTRODUCTION

Chilli is among the world's most popular vegetable belonging to family Solanaceae (Berke, 2002). Seventy percent of chilli is produced in Asia (Venkataiah *et al.*, 2003). In Pakistan, during 2004-05 the total cropped area under chili cultivation was 48700 hectares, producing 90500 tons with an average national yield of 1.9 tons per hectare. Sindh produces 85% of the chili crop followed by Punjab with 11% (Anon., 2004-05). Two species of chillies are cultivated in Pakistan viz. *Capsicum annum* and *C. frutescence* but the former is most common. Lower regions of Sindh province including Kunri, Umerkot, Mirpurkhas, Naon Koat and some other towns are main chilli producing area by in Pakistan. Kunri a small town of District Umerkot is considered the home of red chillies. It contributes around 85% of Pakistan red chilli production and is known as one of the largest production centers for red chillies in Asia (Hussain and Abid, 2011). Several abiotic and biotic stresses affect the productivity of chilli crop worldwide. In addition to fungal, bacterial nematodes diseases and viral diseases are also responsible significant production constraint affecting both yield and quality and are difficult to control (Nono-womdim, 2001). The pathogens attack roots, stems, leaves and fruits of pepper plants and cause 70 to 100% yield loss (Liu and Lu, 2003).

Among the pathogenic fungi that cause heavy losses to chilli crop, particularly those causing foliar diseases are responsible for significant decline in the chilli growing areas of Sindh Province. Fungi isolated from fruits and foliar parts of chilli were *Alternaria alternata*, *A. solani*, *Aspergillus flavus*, *A. niger*, *Botrytis cinerea*, *Cercospora capsici*, *Colletotrichum capsici*, *Leveillula taurica*, *Phytophthora capsici*, and *Verticillium* spp. The objectives of the present study were: 1) to survey the various filamentous fungi infecting (or just occurring as epiphyte) on the fruits or leave (phylloplane), 2) to compare the fungal composition of the assemblages on fruits and leaves of chilli in eight different localities of lower Sindh, and 3) to measure the diversity of the fungal assemblages.

MATERIAL AND METHODS

Collection and isolation of the Pathogens:

The foliar parts of chilli including fruits, leaves and stems showing wilting were collected from the lower regions of Sindh province including Hyderabad, Tando Allahyar, Mirpurkhas, Umerkot, Kunri, Samaro, Kot Ghulam Muhammad and Digri from June 2010 to August 2012. The infected samples were cut into small pieces up to 1.5 to 2 cm and surfaces were sterilized by 1 % Ca (OCI)₂ for 1 min and these pieces were transferred on Potato Dextrose agar (PDA) medium and Czapek's agar medium containing anti-bacterial (Penicillin and Streptomycin) drops. The Petri dishes were incubated for 3 days at 28°C. Infection percentage was calculated with the help of following formula:

Identification of fungi:

Isolated fungi were identified using standard references (Ellis 1971;1976; Barnett & Hunter 1972; Nelson *et al.*, 1983; Domsch *et al.*, 1987; Singh *et al.*, 1991; Sutton, 1980).

$$\text{Infection \%} = \frac{\text{Number of plants infected by a pathogen}}{\text{Total number of plants}} \times 100$$

Statistical analysis:

Data were subjected to one-way analysis of variance (ANOVA) using the statistical software BIOSTAT developed by one of us (S.S.S) in C++. The post-hoc tests included Fisher's least significant test (LSD) at P=0.05 and Duncan's multiple range test (also at p=0.05). The methods employed are those described in Zar (2008). Cluster analysis was performed by Ward's agglomerative technique using MINITAB release 11.12.

A number of diversity indices have been proposed to measure diversity (Magurran, 2004). Diversity indices represent a useful means for quantifying community diversity and have been instrumental in revealing the microorganism diversity associated with the phylloplane communities (Thomas and Shattock, 1986). Several diversity indices were employed to compare the mycobiota assemblage of different localities. Various diversity measures estimate different aspect of community structure. The general species diversity of the fungal communities was measured by the generally accepted Shannon–Wiener information theory function:

$$H' = - \sum P_i \log P_i \quad i=1 \dots S$$

Where H' is the general species diversity and P_i the proportion of total number of CFU for fungal species belonging to the i th species and S equals the total number of species in the assemblage (Shannon and Weaver, 1963). . The general diversity incorporates two components of diversity: species richness, which expresses the number of species S as a function (ratio) of the total number of individuals N ; and equitability that measures the evenness of allotment of individuals among the species (Magurran, 2004). The equitability component of diversity was measured in accordance with Pielou (1975):

$$J' = H' / H'_{\max} = H' / \log S$$

The equitability index J' is the ratio between observed H' and maximal diversity H'_{\max} . The species richness (d_1) was computed in accordance with Menhinick (1964) as follows:

$$d_1 = S / \sqrt{N}$$

Where S equals the number of species and N equals the total number of CFUs.

Dominance concentration (complement of diversity) was measured by using Simpson's index (Southwood and Henderson, 2000) as:

$$D = \sum \{ [n_i (n_i - 1)] / [N(N-1)] \} \quad i = 1 \dots S$$

in which n_i number of CFU for a fungal species.

For the computation of diversity indices and the dominance concentration, a program package was developed by one of us (S.S.S.) in C++ and is available at a nominal cost.

RESULTS AND DISCUSSION***Fungi associated with fruits:***

One hundred and sixty samples were obtained from eight different localities. The infected pre and post-harvest fruit samples were also collected. Five fungi were isolated from these samples of fruits. Result of the present study showed that isolates *Aspergillus flavus*, *A. niger* and *Colletotrichum capsici* were the pre-dominant ones in that order with mean values of 61.6, 48.5 and 47.2% respectively than the other species included *Alternaria solani* and *Alternaria alternata* (Table. 1.). All five fungi are known to cause pathogenic activity, i.e., they either cause necrosis or spots/patches on the fruits.

Table 2 shows the results of ANOVA for the percentage infection on chilli fruits in various localities. All five fungal species including *Alternaria solani*, *A. alternata*, *Aspergillus flavus*, *A. niger* and *Colletotrichum capsici* showed highly significant differences among localities.

Fig.1 shows the dendrogram derived for the agglomerative clustering of the percentage infection of fruits in various localities. Basically two groups can easily be recognized, one group of six localities and the other group of two localities (Kunri and Samaro). The two localities Kunri and Samaro are closely located to one another. This could be the reason for fungal similarity. In group one also the members of subgroups are generally located in the neighborhood of each other and are similar in fungal composition.

Table 1. Mean and Standard error of infection percentage of different fungi isolated from chill fruit at various localities of southern Sindh.

Isolated Fungi	Mean percentage and standard error of Fungi								Grand Mean
	HYD	TAND	MPK	KGM	DIGR	UME	KUN	SAM	
<i>Alternaria solani</i>	31±2.19	29±2.66	41±1.34	37±2.25	28±3.6	39±2.32	63±2.21	41±1.90	38.62±3.94
<i>A. alternata</i>	28±1.94	37±1.19	39±1.64	20±1.36	17±1.34	29±1.39	26±1.84	29±1.82	28.12±2.64
<i>Aspergillus flavus</i>	57±5.15	43±1.57	67±2.26	52±2.80	62±1.37	69±1.41	78±2.07	65±2.92	61.62±3.83
<i>A. niger</i>	43±1.29	32±1.30	39±1.04	38±2.08	45±1.71	51±1.79	71±2.14	69±2.03	48.5±5.08
<i>Colletotrichum capsici</i>	35±1.57	56±2.24	53±1.61	42±1.92	34±1.80	45±1.80	61±2.19	52±2.19	47.25±3.48

HYD= Hyderabad, TAND= Tando Allahyar, MPK= Mirpurkhas, KGM= Kot Ghulam Muhammad, DIGR= Digri, UME= Umerkot, KUN= Kunri, SAM= Samaro

Table 2. F ratios derived from ANOVA for infection % of fruits by fungal species in various localities of southern Sindh.

Species	F	P	LSD _{0.05}
<i>Alternaria solani</i>	25.96	.001***	6.174
<i>A. aletrnata</i>	22.13	.001***	4.48
<i>Aspergillus flavus</i>	16.01	.001***	7.64
<i>A. niger</i>	69.66	.001***	4.85
<i>Colletotrichum capsici</i>	25.96	.001***	5.45

F= F-ratio was obtained from ANOVA tables, LSD=Least significant difference at P=0.05

Similarity

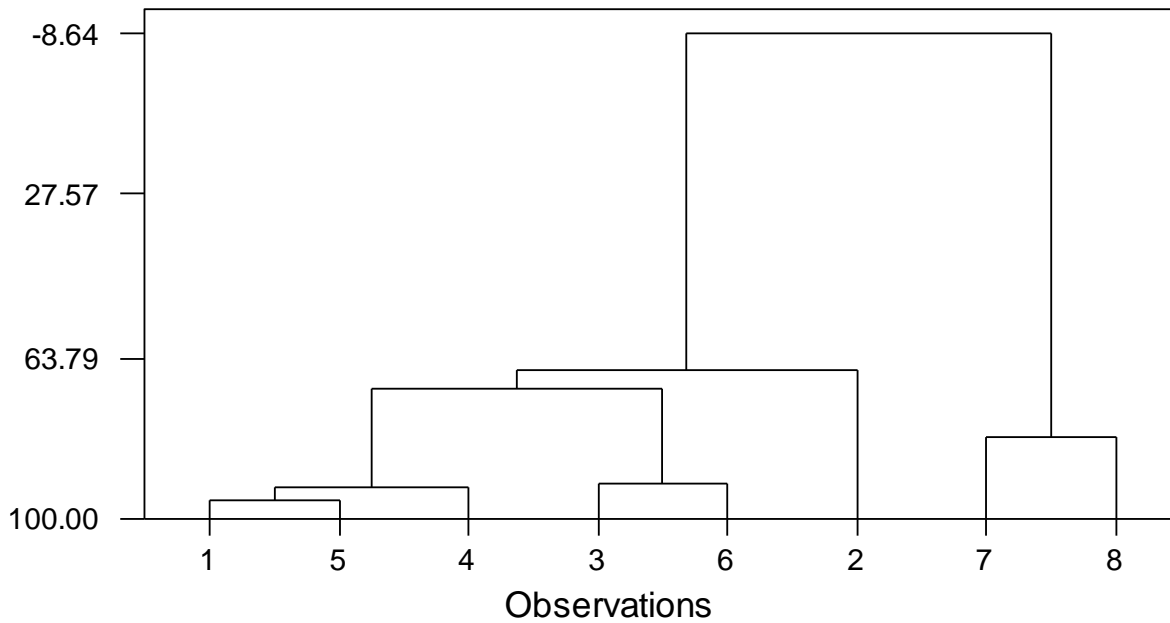


Fig.1. Dendrogram of eight different localities based on the prevalence of fungi on the fruit surface of chilli. The numbers at the base refer to the localities as follows: 1= Hyderabad, 2= Tando Allahyar, 3= Mirpurkhas, 4= Kot Ghulam Muhammad, 5= Digri, 6= Umerkot, 7= Kunri, 8= Samaro

Fungi associated with foliar parts:

Six fungi were also isolated from the leaves of chilli. Among these *Leveillula taurica*, *Alternaria solani*, and *Cercospora capsici* were the pre-dominant ones in that order with mean values of 16.5, 15.2 and 13.2% respectively while the other species included *Verticillium* sp., *Botrytis cinerea*, and *Phytophthora capsici* (Table. 3.).

Table. 3. Mean and Standard error of different fungi isolated from leaves and stem.

Isolated Fungi	Mean percentage and standard error of Fungi								Grand Mean
	HYD	TAND	MPK	KGM	DIGR	UME	KUN	SAM	
<i>Alternaria solani</i>	12.5±1. 3	13.4±1. 4	11.1±2 4	15.3±1. 4	16.7±1. 3	16.7±1. 7	21.6±1. 3	18.1±1. 6	15.2±1. 1
<i>Botrytis cinerea</i>	6.6±0.6	9.5±0.6	12.4±0. 6	7.4±0.4	9.2±0.5	10.9±0. 5	13.9±0. 5	11.3±0. 8	9.6±0.8 4
<i>Cercospora capsici</i>	9.9±0.5	12.7±0. 6	12.5±0. 9	15.2±0. 5	15.9±0. 4	9.5±0.8	19.8±0. 6	15.9±0. 6	13.2±1. 2
<i>Leveillula taurica</i>	11.5±0. 7	15±0.6	16.1±0. 6	16.8±0. 4	19.7±0. 8	19.8±1. 3	20.8±1. 3	16.4±0. 6	16.5±1. 0
<i>Phytophthora capsici</i>	6.1±0.6	12.7±1. 2	12.2±0. 4	5.6±0.6	8.9±0.7	11.1±0. 3	12.3±0. 5	10±0.3	9.5±1
<i>Verticillium</i> sp.	9.4±0.6	9.6±0.5	15±0.8	7.8±0.6	11.1±0. 3	13.8±0. 5	15.1±0. 8	10.7±0. 8	11.1±1. 0

HYD= Hyderabad, TAND= Tando Allahyar, MPK= Mirpurkhas, KGM= Kot Ghulam Muhammad, DIGR= Digri, UME= Umerkot, KUN= Kunri, SAM= Samaro

Table 4. F values derived from ANOVA for infection % of leaves by fungal species in various localities.

Species	F	P	LSD ₀₋₀₅
<i>Alternaria solani</i>	4.75	.0002***	4.35
<i>Botrytis cinerea</i>	16.11	.000***	1.73
<i>Cercospora capsici</i>	16.82	.000***	1.89
<i>Leveillula taurica</i>	11.51	.000***	2.53
<i>Phytophthora capsici</i>	16.46	.000***	1.93
<i>Verticillium</i> sp.	0.67	.691ns	14.2

F= F-ratio was obtained from ANOVA tables, LSD=Least significant difference at P=0.05

Table 4 shows the results of ANOVA for the fungal infection percentage on chilli leaves (phylloplane) in various localities. Five fungal species including *Alternaria solani*, *Botrytis cinerea*, *Cercospora capsici*, *Leveillula taurica* and *Phytophthora capsici* showed highly significant differences among localities. Whereas, *Verticillium* spp. did not show significant difference among localities. All six species are pathogenic on chilli plants.

Fig.2 shows the dendrogram derived for the agglomerative clustering of the percentage infection of leaves in various localities. Similarly, two groups can be easily recognized, one group of seven localities and the other group of only one locality (Kunri). The Kunri group is an isolated group as it is remarkably different from the rest of the localities. In the large group the fungal assemblages are considerably similar probably due to nearness of localities and similar climatic and weather conditions.

Diversity of mycobiota:

Table 5 shows the diversity measures for the fruit mycobiota in various localities. Although most diversity indices show closely similar values yet there are subtle differences. General species diversity (H) of Mirpurkhas fruits showed highest H value while Digri gave lowest species diversity (H). Equitability (J) was found highest for Mirpurkhas while lowest for Digri. Dominance showed opposite trend to diversity.

Similarity

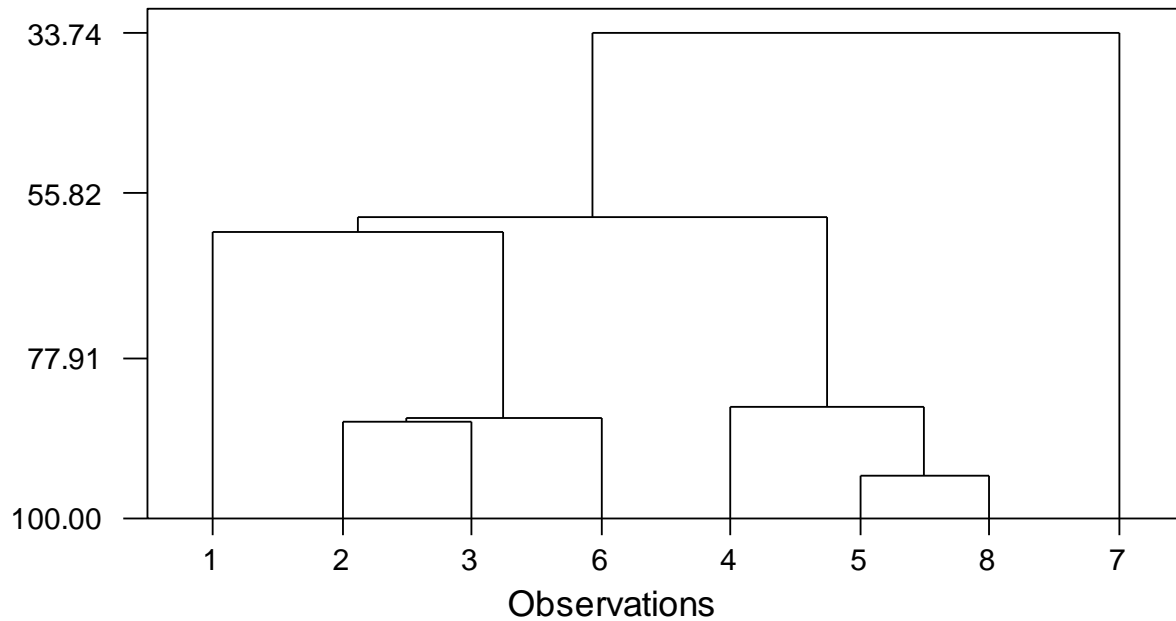


Fig.2. Dendrogram of eight different localities based on the prevalence of fungi on the leaf surface of chilli. The numbers at the base refer to localities as follows: 1= Hyderabad, 2= Tando Allahyar, 3= Mirpurkhas, 4= Kot Ghulam Muhammad, 5= Digri, 6= Umerkot, 7= Kunri, 8= Samaro

Table 5. Diversity measures for the fungi occurring on chilli fruits in various localities of southern Sindh. Species diversity=H, equitablity=J, species richnes=d₁, dominance=D

Localities	H	J	d ₁	D
Hyderabad	1.575	0.978	0.358	0.210
Tando Allahyar	1.581	0.982	0.356	0.207
Mirpurkhas	1.584	0.984	0.323	0.207
Kot Ghulam Muhammd	1.568	0.974	0.363	0.210
Digri	1.523	0.946	0.366	0.229
Umerkot	1.569	0.974	0.327	0.213
Kunri	1.556	0.967	0.289	0.215
Samaro	1.565	0.972	0.312	0.213

Table 6. Diversity measures for fungi occurring on the chilli leaves in various localities of southern Sindh. Species diversity=H, equitablity=J, species richness=d₁, dominance=D.

Localities	H	J	d ₁	D
Hyderabad	1.758	0.981	0.824	0.161
Tando Allahyar	1.774	0.990	0.717	0.160
Mirpurkhas	1.782	0.994	0.679	0.159
Kot Ghulam Muhammd	1.699	0.948	0.744	0.183
Digri	1.744	0.973	0.679	0.171
Umerkot	1.756	0.980	0.679	0.186
Kunri	1.769	0.987	0.600	0.165
Samaro	1.764	0.984	0.670	0.165

Table 6 presents diversity measures for percentage infection of fungal species on the phylloplane of chilli in southern Sindh. General species diversity (H) was found to be maximum for Mirpurkhas and minimum for Kot Ghulam Muhammad. Equitablity (J) showed the same result as general diversity. Species richness (d₁) was highest for Hyderabad and lowest for Kunri. Dominance was highest for Umerkot while lowest for Mirpurkhas.

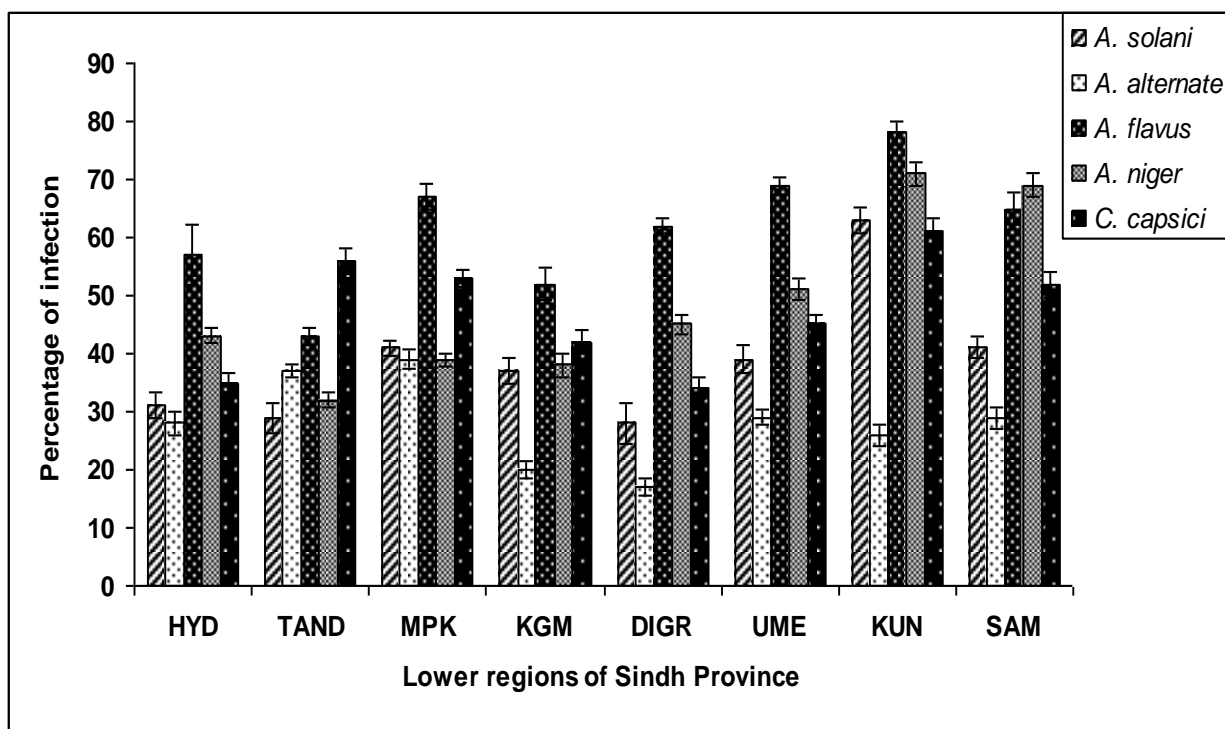
If compared on the basis of regions, the occurrences of the three fungi, namely *Aspergillus flavus*, *A. niger* and *Colletotrichum capsici* on fruit (that cause spoilage of fruit particularly at post-harvest) were maximum in samples from Kunri (78%), Umerkot and Samaro (69%) and Mirpurkhas (67%) respectively and the minimum (17%) from Digri region (Fig. 3) while the occurrences of three fungi in leaves were maximum in samples from Kunri (21%), Umerkot and Digri (19%) and Samaro (18%) respectively and the minimum (7%) from Kot Ghulam Muhammad region (Fig. 4).

Hashmi (1990), Mushtaq and Hashmi (1997), Nahar *et al.*, (2004), Ahmed *et al.*, (1990) and Hafeez, (1986) also mentioned that fruit and foliar fungi such as; *Alternaria alternata*, *A. solani*, *Aspergillus flavus*, *A. niger*, *Botrytis cinerea*, *Cercospora capsici*, *Colletotrichum capsici*, *Leveillula taurica*, *Phytophthora capsici* and *Verticillium* wilt are common in different localities of Pakistan and cause diseases of chilli plants thereby reducing crop yield.

In present study, it is indicated that fruit and foliar parts are commonly infected by different fungi and that eventually result in heavy losses of chilli yield in the chilli growing areas of Pakistan. But some fungi are predominant than others. Fruits are significantly infected by various fungi but *Aspergillus flavus*, *A. niger* and *Colletotrichum capsici* are predominant and more significant than other pathogens. Similarly, *Leveillula taurica*, *Alternaria solani* and *Cercospora capsici* are recorded as predominant species causing foliar diseases.

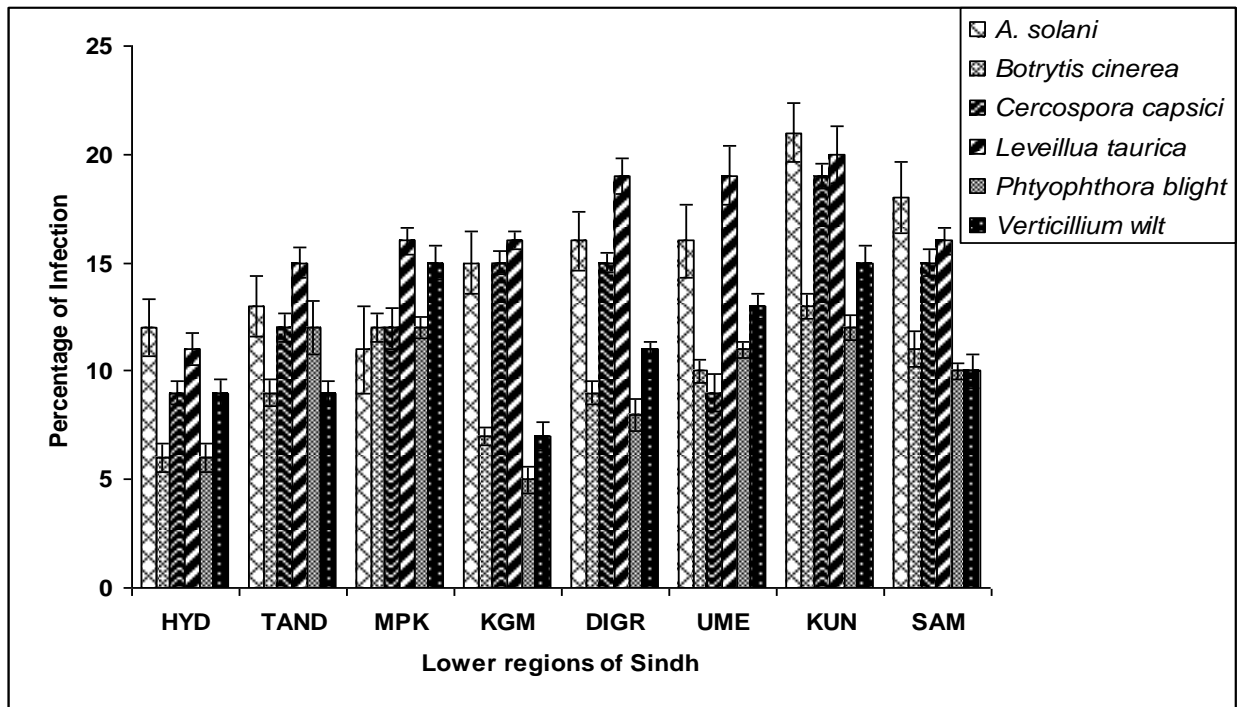
It is observed that *Aspergillus flavus*, *A. niger* and *Colletotrichum capsici* extensively and intensively infecting the fruit of chilli crop. The infection rapidly increased due to many factors such as, infection through godowns that store chillies, presence of moisture and temperature regimes in the godowns, small storage dumps, and poor practices of drying chillies openly near chilli fields. In addition to these increased levels of infection may be caused by winds, gales and dust storms as well as by mechanical vectors.

It is interesting to note that in Karachi, located in southern Sindh, studies on airborne mycobiota (Afzal *et al.*, 2004 and Rao *et al.*, 2009) have demonstrated that the aerospora is dominated by *Aspergillus niger*, *A. flavus* and *Alternaria solani*. Thus the atmospheric mycobiota tends to correspond with the chilli phylloplane and fruit-surface fungal dominance.



HYD= Hyderabad, TAND= Tando Allahyar, MPK= Mirpurkhas, KGM= Kot Ghulam Muhammad, DIGR= Digri, UME= Umerkot, KUN= Kunri, SAM= Samaro

Fig.3. Mean and standard error (S.E) of different fungi isolated from the contaminated fruits.



HYD= Hyderabad, TAND= Tando Allahyar, MPK= Mirpurkhas, KGM= Kot Ghulam Muhammad, DIGR= Digri, UME= Umerkot, KUN= Kunri, SAM= Samaro

Fig. 4. Mean and standard error (S.E) of different fungi isolated from the infected leaves and stem

REFERENCES

- Afzal, M., F. S. Mehdi and Z.S. Siddiqui (2004). Effect of relative humidity and temperature on airborne fungal allergens of Karachi City. *Pak. J. Biol., Sci.* 7: 159-162.
- Ahmed, S., M. Ansar and A. Iqbal (1989). Root and collar-rot of chillies caused by *Phytophthora capsici* (van Breda As Haan) Waterhouse. A new record for *Pak. J. Agr. Res.*, 27: 155-156.
- Anonymous, (2004-05). *Agricultural Statistics of Pakistan*, Government of Pakistan, Ministry of Food, Agriculture and Livestock, Economic Wing, Islamabad. 76p.
- Barnett, H.L. and B.B. Hunter (1972). *Illustrated Genera of Imperfect Fungi*. Burgess Publishing Co., Minneapolis, Minnesota, 241p.
- Berke, T. (2002). The Asian vegetable Research Development Center, Pepper Project. In: *Proceeding of the 16th international pepper conference Tampico*. Tamaulipas, Mexico. November 10-12.
- Domsch, K.H., W. Gams and T.H. Anderson (1980). *Compendium of Soil Fungi*. Volume I. Eching, IHW-Verlag. 860p.
- Ellis, E.B. (1976). *More Dematiaceous Hyphomycetes*. Commonwealth Mycological Institute, Kew, UK: 507p.
- Ellis, M.B. (1971). *Dematiaceous Hyphomycetes*. CMI., Kew Surrey, England. 608p.
- Hafeez, A. (1986). *Plant Diseases*. Khurseed Printers (Pvt) Ltd. Islamabad PARC, 552p.
- Hashmi, M.H. and U. Thrane (1990). Mycotoxins and other secondary metabolites in species of *Fusarium* isolated from seeds of capsicum, coriander and fenugreek. *Pak. J. Bot.*, 22:106-116.
- Hussain, F. and M. Abid (2011). Pest and diseases of chilli crop in Pakistan: A review. *Int. J. Biol. Biotech.*, 8: 325-332.
- Liu, R.Z. and J. Lu. (2003). Inhibition of *Trichoderma harzianum* against the soil born fungal diseases of *Capsicum*. *J. of Zhongkai Agrotech. Colle.*, 16: 6-11.
- Magurran, A.E. (2004). *Measuring Biological Diversity*. Blackwell Scientific, Oxford.
- Menhinick, E.F. (1964). A comparison of some species-individuals diversity indices applied to samples of field insects. *Ecology*, 45: 859-861.

- Mushtaq, M. and M. H. Hashmi (1997). Fungi associated with wilt disease of *Capsicum* in Sindh, Pakistan. *Pak.J. Bot.*, 29: 217-222.
- Nahar Sharfun, M. Mushtaq and I. H. Pathan (2004). Seed-borne mycoflora of *Capsicum annum* imported from India. *Pak. J. Bot.*, 36: 191-197.
- Nelson, P.E., T.A. Toussoun, and W.F.O. Marasas (1983). *Fusarium species: an illustrated manual for identification*, The Pennsylvania State Uni. Press, 193p.
- Nono-Womdim, R. (2001). An overview of major virus diseases of vegetable crops in Africa and some aspects of their control. *Plant virology in sub Saharan Africa*. 213-230p.
- Pielou, E.C. (1975). *Ecological Diversity*. Wiley, New York. 165p.
- Rao, T.A., A.H. Sheikh and M. Ahmed (2009). Airborne fungal flora of Karachi. *Pak. J. Bot.* 41: 1421-1428.
- Shannon, C.E. and W. Weaver (1963). *The Mathematical Theory of Communication*. University of Illinois Press, Chicago.
- Singh, K., J.C. Frisvad, U. Thrane and S.B. Mathur (1991). *An Illustrated Manual of Identification of Some Seed-borne Aspergilli, Fusaria, Penicillia and their Mycotoxins*. Danish Govt. Inst. Seed Path. for Dev. Count., Ryvangs Allc 78, DK-2900 Hellerup, Denmark, 133p.
- Southwood, T.R.E. and P.A. Henderson (2000). *Ecological Methods*. 3rd Ed. Blackwell Scientific, Oxford. 575p.
- Sutton, B.C. (1980). *The Coelomycetes* (CAB, IMI) Kew, Surrey, U.K.. 696p.
- Thomas, M.R. and R.C. Shattock (1986). Filamentous fungal associations in the phylloplane of *Lolium perenne*. *Trans. Br. Mycol. Soc.*, 87: 255-268.
- Venkataiah, P., T. Christopher and K. Subhash (2003). Thidiazuron induced high frequency adventitious shoot formation and plant regeneration in *Capsicum annum* L. *J. Pl. Biotech.*, 5:245-250.
- Zar, J.H. (2008). *Biostatistical Analysis*. 5th ed. Prentice-Hall Englewood Cliffs, New Jersey, USA.

(Accepted for publication December 2012)