Mycopath (2014) 12(1): 25-30 **Research Article**

Effect of metrological factors on incidence of aeromycoflora of potato field

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Abstract

Aeromycoflora of potato field was isolated using gravimetric Petri Plate method using 3 different growth media i.e. 2% potato dextrose agar, 2% nutrient agar and 2% water agar. A total of 14 fungi belonging to 9 genera (*Aspergillus, Fusarium, Alternaria, Drechslera, Cladosporium, Curvularia, Botrytis, Penicillium and Rhizopus*) were identified. The most dominant fungus was *Cladosporium* sp. (10.67%), followed by *Alternaria alternata* (10.30%), *A. solani* (7.80%), *Aspergillus niger* (7.27%), *Penicillium* sp. (7.12%) and *Fusarium oxysporum* (7.02%). Early blight disease was predominantly observed on potato crop during course of investigation. *A. solani*, the causal agent of early blight was isolated from diseased potato plants as well as from the air. The results of pathogenicity tests on potato plants were found positive as well. Metrological parameters (temperature, rainfall and wind speed) were positively correlated with prevalence of isolated fungi as well as with *A. solani*. Moreover, aeromycoflora was found maximum during March, April and May as compared to December and January. The present investigation on air borne mycoflora will be helpful in disease forecasting, epidemiology and timely disease management especially in case of early blight in potato field.

Introduction

Potato *(Solanum tuberosum* L. Family: Solanaceae) ranks fourth on production basis in the world (Rauscher *et al.,* 2006) and fifth in Pakistan. Tuber is an excellent source of carbohydrates, protein, and vitamins (Ducreux *et al.*, 2005). Potatoes are grown in irrigated areas of the central and northern plains of Punjab, including Sahiwal, Okara, Sialkot, Kasur, Lahore and Jhang. It is also cultivated at low scale in Peshawar, Swat, Dir, Kaghan, Kalat and Pishin (Malik, 1995). In Pakistan, 18 different diseases of potato have been documented and 13 diseases are considered as the most important. These diseases are uniquely different in different potato growing zones (Ahmad *et al.*, 1991). Wilt, stem rot, soft rot, early and late blight, powdery and common scab, black scurf, potato cyst nematode and root knot nematode are commonly occurring diseases of potato in Pakistan (Ahmed, 1998).

Among above mentioned diseases, majority of the mycopathogenic spores are air borne. Their aerodynamics differs from soil borne pathogens. In between soil and air borne mycoflora, the main difference lies in survival and viability of spores and their dispersion. In the dissemination of many plant diseases, airborne fungal spores are of extreme importance (McCartney, 1991). They cause serious agricultural problems and sometime result in epidemics (Hirst, 1991). Due to inadequate agricultural facilities the poor agriculturists find it difficult to control such plant diseases once they are established. In Pakistan, this aspect of pathological investigation is more valuable and helpful for the agricultural purpose. Study of aeromycoflora is an essential part of disease epidemiology and management. The source of infection, survival and viability is affected by environmental and biotic factors. A large number of plant diseases like rust, powdery mildew, leaf spots, anthracnose and blight are air-borne (Rowan *et al.,* 1999). Airborne fungi responsible for plant diseases occur in the atmosphere a few days in advance of the actual appearance of the disease (Kumar, 1982). It has been demonstrated by *in vivo* studies on phytopathogens that high moisture and moderate temperatures favor the growth of fungi, while the spore germination is influenced by low relative humidity and high temperatures (Rowan *et al.*, 1999).

Atmospheric fungi are generally studied on seasonal basis for the purpose of plant disease forecasting. Investigations on aerobiology are, therefore absolutely essential to understand the disease epidemiology and control (Ponti and Cavani, 1992) and helpful in establishing the forecasting probabilities of diseases and also the dissemination of pathogenic microbes.

Aerobiology of potato bears similar significance along with epidemiology, population biology and disease prediction systems for the management of foliar pathogens (Cook, 2000).

In order to understand the dissemination and spread of the microbes (specially the pathogenic ones) in the atmosphere of potato growing areas, the study of aeromycoflora is too much important. Suitable means of disease control may be tried or the establishment of the pathogens on the host may itself be checked after adequate information is achieved regarding the time and mode of the spread of the pathogens in air. This has been done in different parts of the world but still not in Pakistan. Keeping in view the above discussed disease problems regarding potato production in Pakistan; the study was proposed with the objectives of Collection, Identification of aeromycoflora and fungal disease samples from the potato field. Correlation of concentration of aeromycoflora with disease incidence and with environmental factors is also a part of this study. The present research will help in formulating the appropriate integrated approach for the fungal foliar diseases of potato in due course of time

Materials and Methods

This work consisted of field and laboratory investigations. Field work was done at farmer's potato fields at Taxila and laboratory work in the Department of Plant Pathology, Arid Agriculture University, Rawalpindi.

Isolation and identification of air-borne mycoflora

Potato fields were selected at random at three different sites of Taxila. Aeromycoflora was collected by gravimetric Petri plate technique (Uddin, 2004). Three different media i.e. 2% potato dextrose agar, 2% nutrient agar and 2% water agar were used. The streptomycin @ 4.0 g L^{-1} was used as anti-bacterial agent. Petri plates with three replications for each media were exposed in open atmosphere of potato fields for three minutes between 10.00 a.m. and 11.00 a.m. at about 1 m height at weekly intervals for 32 weeks. After exposure the Petri plates were tightly sealed with Para film and brought to the laboratory. These plates were incubated at 25-30 °C for seven days (Talley *et al*., 2002). The fungal colonies appeared in plates were observed under stereoscope. New plates were inoculated with individual fungal colony by loop method to obtain pure culture.

All the fungi isolated from air were identified on the bases of colony characters (color, texture, and size), microscopic features (size, shape, color and growth pattern of spores and hypha) using authentic literature (Larone, 2002; Schell *et al.,* 2003; Sutton *et al.,* 1998).

Pathogenicity test

During entire season three crops of potato were sown in Taxilla i.e. October to December, January to March and March to May. During this period late blight in first potato crop in and early blight in second and third crop was observed. Therefore, pathogenicity test was performed with only *A. solani* using three available potato verities (Desiree, Cardinal and Faisalabad white) in Taxila. Potato tubers were planted with three replications and one control for each variety into 25 cm earthen pots containing mixture of field soil, sand and manure (1:1:1). The plants at 4-5 leaf stage were inoculated with each of spore suspension of *A. solani* and maintained in the disease free place. Symptoms development was recorded after 2-3 weeks of inoculation. Control plants were sprayed with water instead of spore suspension.

Potato plants naturally manifesting foliar infection were collected from fields in sterilized plastic bags and stored in a refrigerator at 4 °C. The infected leaves were surface sterilized with 2% Clorox mixture, cut into small pieces, inoculated on 2% PDA and incubated at 25-28 °C for a week. The fungal identification was carried as mentioned above.

Preservation of isolated fungi

Fungal cultures were maintained and preserved on PDA in mineral oil, and in sterilized distilled water in MeCauteny vials (Elis, 1976). These cultures were stored at 4 °C for reference to be used in further research.

Meteorological data

Meteorological data of maximum, minimum and mean temperature, relative humidity, rain fall, wind speed and wind direction was recorded at Taxila during the whole potato season. Weekly means were calculated corresponding to weekly exposure of Petri dishes. It was correlated with the average number of colonies of airborne mycoflora.

Statistical data

The data was analyzed according to Spearman's correlation by using SPSS software.

Results and Discussion

A total of 14 fungal species belonging to 9 genera were isolated from the atmosphere of Taxila. Among them, the most dominant types were *Cladosporium* sp. (10.67%) and *A. alternata* (10.30%) followed by *A. solani* (7.80%), *Aspergillus niger* (7.27 %), *Penicellium* spp. (7.12%) and *Fusarium oxysporum* (7.02%) (Table 1). A number of scientists like Agarwal and Shivpuri (1969), Beaumont *et al.* (1985), Savino and Caretta (1992) and Takahashi (1997) recorded the *Cladosporium* as most common genus in air followed by *Alternaria, Aspergillus, Penicillium* and *Fusarium* genera. In the same way *Alternaria, Penicillium, Aspergillus* and *Fusarium* genera were found to be the dominant types in some studies described by Khandelwal (1991). The concentration of aeromycoflora in an atmosphere is largely affected by various metrological factors like temperature, rainfall, relative humidity and wind speed (Bandyopadhyay *et al.,* 1991; Pasanen, 1992; Di-Giorgio *et al.,* 1996). The concentration most of the collected aeromycoflora was found maximum in March, April and May. It might be because of favorable environmental conditions like prevailing temperature 20-30 °C with sufficient rainfall and higher relative humidity (Table 2). Similarly the lower concentrations were observed in December and January (Fig. 2), with average temperature ranging from 12-16 °C along with minimum to no rainfall and with sufficient humidity (Table 2).

The correlation with all other metrological factors (temperature, rainfall and wind speed) was found positive with isolated aeromycoflora except *A. flavus* (Table 3). It had been reported by many researchers (Agarwal and Shivpuri 1969; Di-Giorgio *et al.,* 1996) that variety and concentrations of airborne fungi is varied due to several meteorological factors (wind speed, relative humidity and temperature). Pasanen *et al.* (1991) recorded that 1.0 ms^{-1} is the minimum air velocity at which *Cladosporium* sp. can release its spores. Whereas, huge number of spores were observed at air velocity of 0.5 ms^{-1} in case of *Aspergillus* sp. and *Penicillium* sp. However, dissemination of fungal spores into air is very important. In case of *Alternaria* sp. the results were opposite. Hasnain *et al.* (1995) found that *Aspergillus* had no significant correlation with any meteorological parameter.

In comparison of three media i.e. Potato Dextrose Agar (PDA), Nutrient Agar (NA) and Water Agar (WA), the maximum number of colonies were isolated on Nutrient Agar (531 colonies, 35%) followed by PDA (486 colonies, 33%) and WA (472 colonies, 32%). Differential composition of growth media favored diversity of mycoflora.

Interestingly, early blight disease was predominantly observed on potato crop during three growth seasons i.e. October to December, January to March and March to May. *A. solani*, causing early blight was isolated from diseased samples as well as from the air. The results of pathogenicity tests on potato plants were found positive as well. The presence of *A. solani* in air showed that the disease was airborne as reported by Malik and Khan (1967). The prevalence of *A. solani* spore in the atmosphere of Taxila was least during October to January in the absence of disease in the field (Fig. 1). It could be due to non-availability of prerequisite growth conditions (optimum temperature: 25-30 ^{o}C with high relative humidity and a suitable wind speed) for sporulation and dispersion of the fungus during that period. During March to May, disease was observed in mid-April (Fig. 1) and disease acquired peak during May (mature stage). Therefore, a gradual increased in spore concentration was observed with the onset of disease during March to May (Fig. 1), when all metrological factors and plant stages (flowering to maturity) were in favor of disease development (Table 2). Likewise, during January to March, the disease was started by the end of February with increase in spore number and reached its peak by the end of March (Fig. 1), when plants were fully matured. A rise in the concentration of *A. solani* in atmosphere in January and disease appearance in the field in February was in line with the findings of Kumar (1982). He observed pathogenic aeromycoflora in the atmosphere a few days in advance of the disease appearance.

Therefore, it was noticed that early blight infected the potato crop during January to March and March to May, so more losses occur by early blight as compared to late blight that infected only one crop (October to December).

The study indicated the importance of aeromycoflora especially *A. solani* with respect to disease forecasting, epidemiology and impact of spore concentration in air on disease development in potato. Aeromycobiology is new research area in our country and this study is a key initiative for further investigation on air-borne diseases of other important crops.

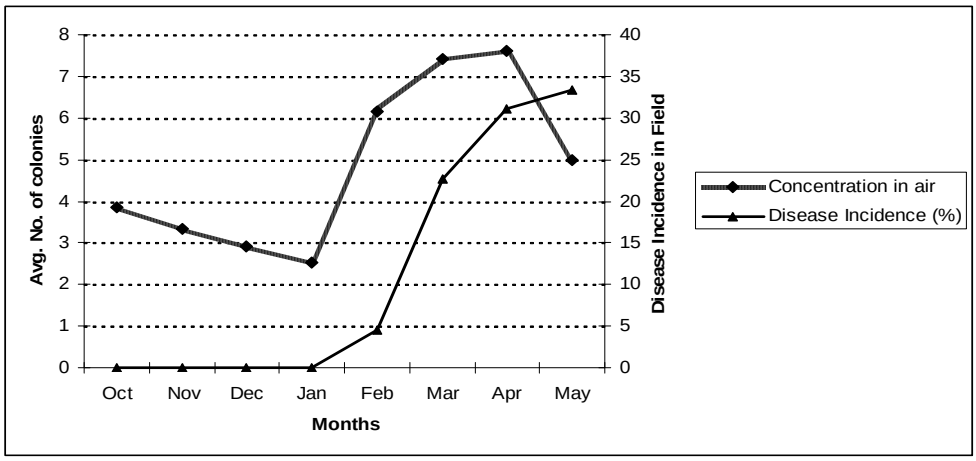

Fig 1. Disease incidence (%) of early blight in field and concentration in air during different months.

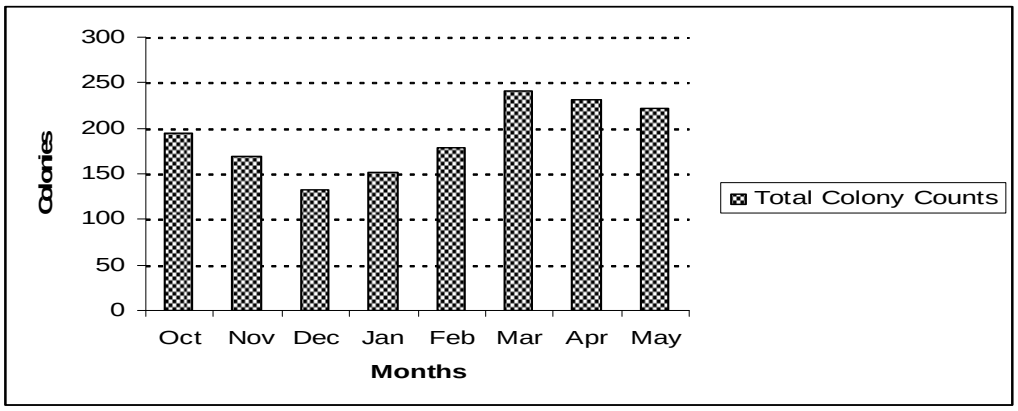

Fig 2. Number of fungal colonies in different months collected from air during whole research period.

Table 1: Percentage of isolated aeromycoflora on different media.

Name of fungi	Incidence of Mycoflora (%)			Average
	Potato dextrose agar	Nutrient agar	Water agar	
Alternaria alternata	11.31	9.65	9.96	10.30
A. solani	8.93	8.08	6.4	7.80
Alternaria spp.	6.91	6.74	7.79	7.14
Aspergillus flavus	7.01	6.79	6.61	6.80
A. niger	8.06	7.24	6.52	7.27
Aspergillus sp.	1.44	6.15	6.65	4.74
Botrytis sp.	6.72	5.89	6.95	6.52
Cladosporium sp.	11.68	10.1	10.24	10.67
Curvularia sp.	5.94	5.29	6.59	5.94
Drechslera sp.	5.65	6.3	7.20	6.38
Fusarium oxysporum	6.89	7.73	6.44	7.02
Fusarium sp.	6.17	7.49	5.25	6.30
Penicillium sp.	6.99	7.49	6.88	7.12
Rhizopus sp.	6.23	5.02	6.48	5.91

Table 2: Monthly averages of metrological data.

Months	Temperature (°C)	Relative Humidity (%)	Rainfall (cm)	Wind Speed (km h^{-1})
October	23.20	53.86	6.35	3.20
November	16.40	54.40	13.4	1.90
December	12.43	58.60	0.00	1.70
January	11.96	57.43	16.0	3.16
February	13.80	70.90	71.2	3.76
March	21.73	56.26	91.4	3.96
April	27.00	47.40	26.6	4.95
May	29.00	34.26	0.00	4.20

Table 3: Correlation between metrological factors and isolated aeromycoflora.

Fungi	Temperature (°C)	Relative Humidity (%)	Wind Speed (km h^{-1})	Rainfall (mm)
Alternaria alternata	0.595	-0.119	0.758	0.726
Alternaria solani	0.557	-0.072	0.804	0.705
Alternaria sp.	0.918	-0.519	0.809	0.245
Aspergillus flavus	-0.266	0.291	-0.077	0.521
Aspergillus niger	0.505	0.067	0.550	0.548
Aspergillus sp.	0.964	-0.771	0.762	0.068
Botrytis sp.	0.915	-0.758	0.702	0.105
Cladosporium sp.	0.654	-0.218	0.522	0.577
Curvularia sp.	0.517	-0.254	0.588	0.272
Drechslera sp.	0.510	0.010	0.697	0.784
Fusarium oxysporum	0.698	-0.665	0.509	0.165
Fusarium sp.	0.805	-0.648	0.355	0.099
Penicillium sp.	0.708	-0.629	0.845	0.164
Rhizopus sp.	0.873	-0.600	0.814	0.069

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