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Study dynamics of phylloplane fungi in relation to Ascochyta blight in chickpea

Ghazala Nasim[1], Munawar Din[2], Rukhsana Bajwa[1], Shahbaz Ali[3], Asad Shabbir[1] and M.B. Ilyas[4]

[1]Department of Mycology & Plant Pathology, University of the Punjab, Lahore; [2]Cathederal School, Behind Jinnah Cricket Stadium, Small Civil Lines, Gujranwala; [3]Govt. Degree College for Boys, Murree; [4]Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan

Abstract

Eight chickpea varieties (3 resistant* and 5 susceptible$^+$) were screened for the abundance and diversity of phylloplane fungi. This study was carried out before and after the foliar spray of the pathogen, *Aschochyta rabiei* on field grown chickpea plants. Samples for study were also collected from the local market in the months of March and April. A total of nine fungal species were encountered in the screening process of six market samples collected at an interval of one week. The number of phylloplane fungi associated with the market samples increased with time. The samples collected in April turned up with maximum diversity of phylloplane fungi. Screening of the experimental plants yielded a total of eight fungal species. Except for two species i.e., *Alternaria alternata* and *Aspergillus niger*, the phylloplane flora of the market samples and the experimental plants was totally different. During the early stages (before the spray) the number of fungi associated with the phylloplane was significantly low as compared to the later stages i.e., after the spray. However, the density of these fungi increased during the later stages in three varieties which were NE 1256*, AUG 970$^+$ and ILC 1256$^+$. While for varieties 184 W*, CM 72$^+$, ICC 630$^+$ and ILC 2548* the pattern was not very clear. In the case of C 679$^+$, the density increased with an increase in the age of the host plant.
Key words: Phylloplane fungi, *Ascochyta* blight, chickpea.

Introduction

Plant leaf surface (phylloplane) is a site of infestation by a variety of organisms, including fungi. The fungi inhabiting surfaces of plant leaves are called as phylloplane fungi. These mostly include saprophytic fungi. These fungi spend a critical period on leaf. Early successful penetration may be due to one or several reasons such as availability of nutrients, susceptibility of the host lack of antagonism etc. (Javaid, 1982).

Microorganism's population of leaf surface (phylloplane) varies in size and diversity depending upon influence of numerous biotic and abiotic factors that affect their growth and survival (Blakeman 1985; Dix & Webster1995). Some fungal species show abundance on phylloplane. This abundance is due to their secondary spore production on the aerial surfaces (Skidmore and Dickinson, 1973; Skidmore, 1976; Jones and Lee, 1974). Secondary spore production occurs when environmental conditions on the host plant are unfavorable for continued growth, penetration or invasion. These conidia thus formed are a big aid in the field disposal of pathogen (Skidmore and Dickison, 1973).

Some times the pathogenic fungi like *Ascochyta rabiei* are amongst the phylloplane fungal flora. Such fungi cause reduction in crop yield (Degenherdt *et al.,* 1974). However there exist a lot of interactions among saprophytic and pathogenic fungal flora of the leaf surface and this may result in a suppression of the pathogenic fungi thus increasing the yield of the host plant (Tsuneda and Koropad, 1978). Mishra and Tewari (1976) reported a remarkable decrease in the number of rust pustules with an increase in leaf mycoflora.

The present study therefore, deals with the study of seasonal pattern of phylloplane fungal flora of chickpea plants collected from local market. An attempt has been made to indicate the difference in colonization pattern before and after the spray of the pathogenic fungus *Ascochyta rabiei*. Furthermore, the study aims to evaluate the effects of the spray of *Ascochyta* suspension on density and diversity of phylloplane fungi of eight different cultivars of chickpea.

Materials and Methods

Chickpea plants were sampled from the fields of the Department of Plant Pathology, University of Agriculture, Faisalabad, where an area of 15x52

feet was allocated for the sowing of thirteen (13) chickpea varieties. These varieties were meant for screening against *Ascochyta* blight during the month of November. Seeds of each variety were sown in specified rows. While a row of test plants (highly susceptible ones) was planted in the end. Out of thirteen varieties eight were selected for regular sampling (Table 1). Five harvests were taken. Two harvests were made before and three after the spray of the pathogen. From the early beginning (when seedlings were 4-6 inch in height) and till the appearance of blight symptoms on test plants, the pathogen suspension was sprayed ten times with an interval of two days. Each time the spawn of *Ascochyta rabiei* growing on boiled chickpea seeds (M.B. Ilyas Pers. Comm.) was washed in water (1kg/20liters) and sprayed with the help of a sprayer. Plant samples were carefully brought back into the Biocontrol Research Lab. Department of Botany, University of the Punjab, Lahore for further study. Samples of chickpea plants collected from local market during March to April were also included in the present study.

List of different chickpea varieties used for analysis

Sr. No.	Variety code Number
1.	184 W*
2.	NE1256*
3.	AUG 970+
4.	ILC 1256+
5.	CM 72+
6.	ICC 6304+
7.	ILC2548*
8.	C 679+

+= Susceptible variety
*= Resistant variety

Isolation of phylloplane fungi

Leaves of sampled chickpea plants were gently washed under tap water to remove dust. Then using a flamed sterilized cork borer discs of 5mm in diameter were cut up randomly and aseptically from leaflets of each of the sampled plants. Three replicates were taken for each sample. Leaflet discs from each sample were shaken in 20ml of distilled sterilized water in a 250ml flask for 10 minutes. These samples were passed through two more changes of sterilized distilled water repeating the same procedure. Each time 5ml of the water from washings were taken and plated on 2% water agar medium plates. Leaf discs were then taken out and gently dried on a sterilized filter paper. These were than planted on 2% water agar plates aseptically.

Medium plates were incubated at room temperature (25±2°C). Fungi appeared after a week of incubation. Semi permanent slides from each of differently looking fungi were prepared in trypan blue. Identification of fungi was carried out following the monographs by Ellis (1971), Barron (1968) and compendium of soil fungi by Demcsh *et al* (1980). Number of colonies of each of the different fungal species appearing from each leaf disc was counted.

Results

Data recorded on isolation of phylloplane fungi from local market samples of chickpea plants during March to April at an interval of one week showed a gradual increase in number of fungal species with time. Number of fungi occupying the leaf surface increased from six to nine in the months of March to April, respectively. These fungal species were *Alternaria alternata* (Fr.) Keissler, *Fulvia fulva* (Cooke) Cifein, *Alternaria cheiranthi* (Lib.) Bolle, *Mucor mucedo, Drechslera australiensis* (Bugvicourt) Subran & Jain ex. M.B. Ellis, *Penicillium* sp., *Aspergillus* sp., van Tiegh, *Aurobasidium pullulan* (de Bary) Arnaud. The number of fungal species showed an increase in density and variable pattern from end of March till crop maturity.

Data pertaining to isolation of phylloplane fungi from experimental chickpea plant at five different harvests (2 before the spray of pathogen *Ascochyta rabiei* and 3 after that) indicated that phylloplane fungal flora significantly increased in latter stages (after spray). At first harvest, three fungi namely *Alternaria alternata, A. brassicicola* and *Helminthosporium solani* were isolated from inoculated discs of plants. *Alternaria alternata* was isolated from leaves of seven varieties out of eight (Fig.1&2). At the end of second harvest, number of phalloplane fungi increased to six. These six fungal species were *A. alternata, A. brassicola, Helminthosporium solani, Aspergillus* sp*., Penicillium* sp., and *Fusarium* sp., *Alternaria alternata* was recorded again from 7 out of 8 varieties taken for study (Figs.1 & 2). *Helminthosporium solani* appeared on four varieties Viz; ILC2548, 184W, ILC1250+, ICC6304+. *Alternaria brassciicola* appeared on four varieties Viz; ILC2548, C679+, 184W, and NE1256 *Aspergillus* sp., was found on variety CM72+, C679+, AUG970+ and ILC1256+.

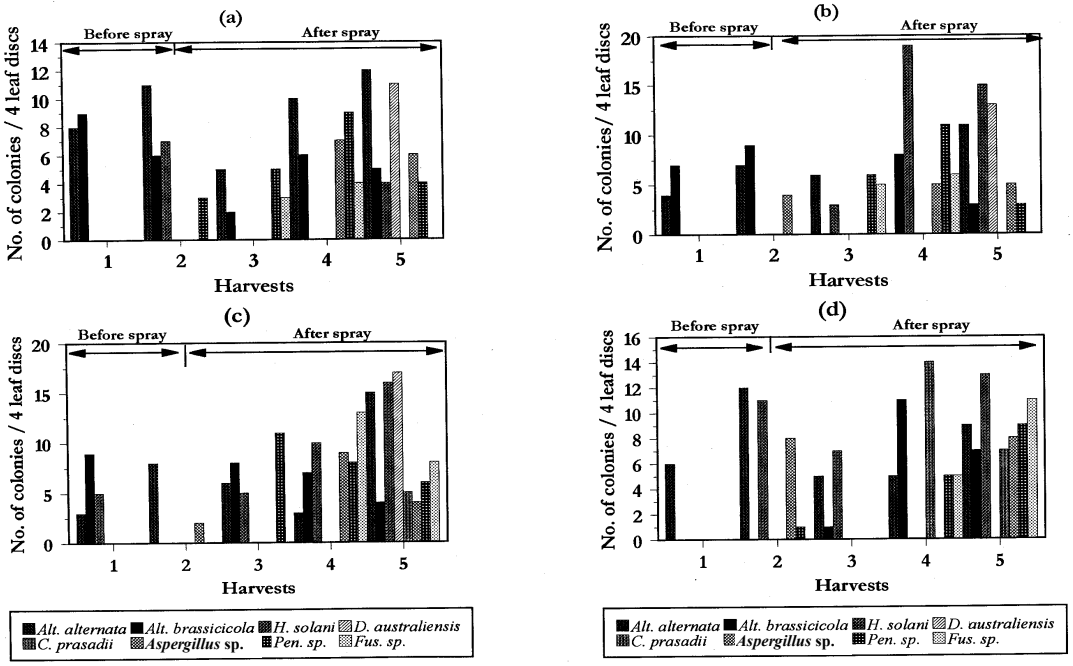

Fig.1: Periodicity of phylloplane fungi on four chickpea varieties. (a) Variety 184W*, (b) Variety NE 1256*, (c) Variety AUG 970+ and (d) Variety ILC 1256+.
(* Stands for susceptible varieties and + for resistant varieties).

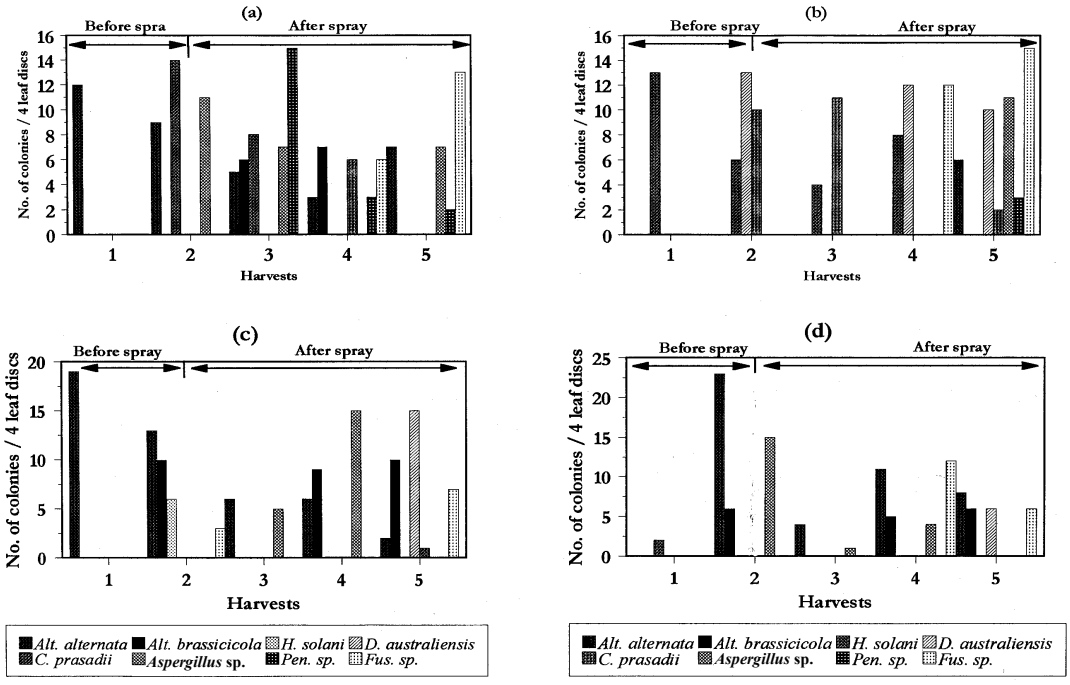

Fig. 2: Periodicity of phylloplane fungi in four other chickpea varieties. (a) CM 72+, (b) ICC 6304+, (c)ILC 2548 *, and (d) C 679 +.

Data recorded at third harvest where treatment in the form of spray showed same number of fungi as in 2nd harvest. *Alternaria alternata* again appeared in all 8 varieties (Figs.1 and 2). *A. brassicicola* was recorded from varieties 184W, ILC1256+Aug970, CM72+, ILC1248 and C679+. *Helminthosporium* sp., from ICC6304+, ILC1256+, Aug 970+and NE1256. while *Aspergillus* sp., was recorded from AUG970+, CM72+, ILC2548*, C694+ (Figs.1 & 2). At the time of fourth harvest 7 species were recorded. These species were *A. alternata, A. brassicicola, Helminthosporium solani, Culnularia prasadii, Aspergillus* sp., *Penicillium* sp., and *Fusarium* sp. (Figs. 1 and 2). *Culvularia prasadii* was recorded for the first time from varieties ILC1256+ and CM72+ during the course of experiment. *Alternaria alternata* was recorded from six out of eight varieties. At this harvest the frequency of occurrence of *A. brassicicola* increased as it was represented in all varieties except C679+ (Figs.1 and 2). *Aspergillus* sp., was isolated from four varieties Viz. AUG 970+, 184W* ,ILC1248* and C679+. *Fusarium* sp. was recorded from 7 varieties CM72+, ILC6304+, C679+, AUG970+, 184W*, ILC1256+ and NE1256*. The number of colonies also increased in case of *Fusarium* sp.

Data recorded from fifth and final harvest revealed a total of 8 different fungal species viz., *A. alternata, A. brassicicola, H. solani, D. australiensis, C. prasadii, Aspergillus* sp., *Penicillium* sp., and *Fusarium* sp. *Alternaria alternata* was reported from all the varieties whereas *Drechslera australiensis, Aspergillus* sp., and *Penicillium* sp., were recorded from six varieties (Fig.1&2). The presence of *H. solani* was increased at this stage being recorded from four varieties Viz; ILC1256+ NE1256*, AUG970+ and 184W*.

Discussion

In the present study eight different chickpea varieties were selected. Five of the chickpea varieties viz; AUG970+, ILC1256+, CM72+, ILC6304+ and C679+ were resistant and three varieties viz; ILC1248* 184W*and NE1258* were susceptible (Dr. M.B. Ilyas, personal communication).

In a survey work to enlist phylloplane mycoflora of chickpea from market samples of chickpea, *Alternaria alternata* was found to be a dominating species. This species was also abundant in isolations from experimental plants. However, the number and diversity of rest of species differed in plants taken from market and grown experimentally. The probable reason for this difference is that it takes comparatively larger time for chickpea plants to reach market and plants may come in contact with some airborne mycoflora other than phylloplane fungi during this course of time. Blakeman (1985) describe the leaf age and external nutrients as critical factors influencing the size and diversity of phylloplane microflora.

The dominance of *Alternaria alternata* may be due to the habit of secondary spore production in this species as reported in case of rice (Skidmore and Dickinson, 1973; Skidmore, 1976). *Alternaria alternata* is amongst the hyperparasites (Bolland, 1973; Sharma and Heather, 1978; Omar, 1978), which plays a limited role in reducing epidermic of disease in plants (Drapaux, 1960; Bier, 1964; Sharma and Heather, 1978). This is supported by present study. Chickpea varieties showing abundance of *Alternaria alternata* and its alien mycoflora resulted in significantly low level of *Ascochyta* blight.

A healthy plant shows significantly high values for density and diversity of phylloplane fungi with an increased level of arbuscule formation as well and appreciably decreases in blight incidence (Nasim *et al*., 2003).

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