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Studies on the epidemiological parameters of Angular leaf spot (ALS) of French bean caused by *Phaeoisariopsis griseola*

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Abstract
Angular leaf spot of French bean caused by the fungus *Phaeoisariopsis griseola*, is one of the most important disease worldwide. The epidemiology of the pathogen *P. griseola*, was studied *in vitro* and *in vivo* at Department of Plant pathology, UHF, Nauni. The epidemiological parameters such as temperature, relative humidity and rainfall greatly influence the development of disease. Meteorological data on epidemiological parameters were recorded for the intervening period. Simple and multiple correlations were worked out separately to establish the relative contribution of these factors in the spread of the disease.

Keywords: French bean, angular leaf spot, *Phaeoisariopsis griseola*, epidemiological parameters

Introduction
French bean (*Phaseolus vulgaris* L.) has been cultivated in different countries of the world. It is originated in Mexico and Central America (Smart, 1976) [1] and belongs to the family Leguminosae and sub family papilionaceae. It is an annual herbaceous plant and widely cultivated throughout the world. It is grown for its pods and seeds which are used as vegetable and pulses. Beans are rich in total protein, and thus serve to balance human diets based on cereal grain and starchy crop. Phaseolus beans are among the most important edible pulse in the world. Angular leaf spot (ALS), caused by the fungus *Phaeoisariopsis griseola*, is considered as a serious disease of beans in many regions. According to the Commonwealth Mycological Institute (CMI), the disease occurs in more than 60 countries (Sartorato and Rava, 1994) [2] and, under favourable environmental conditions, yield losses may reach upto 80 per cent (Ponnappa *et al.*, 1976; Gupta and Shyam, 1998; Shukla and Sharma, 2009) [3-4]. The disease affects foliage and pods throughout the growing season and is particularly destructive in areas where warm, moist conditions are accompanied by abundant inoculums from infected plant residues and contaminated seed. Fungicides are used for the control of ALS but it is risky to wait for the first disease symptoms to initiate spray applications. On the other hand, use of fungicides may represent increased, unnecessary costs and causes environmental pollution. One strategy for optimizing disease management is to predict its occurrence. This can be done by monitoring the weather variables needed for infection. So, some more information is needed to develop a forecast system for such disease. This research was oriented to study the epidemiology parameters that lead to plant infection and to the need for disease control.

Materials and Methods
Effect of different regimes temperature on conidial germination of *Phaeoisariopsis griseola*
Optimum temperature for conidial germination of *Phaeoisariopsis griseola* was measured by placing 3 drops of freshly prepared conidial suspension (2×10^4 conidia m^{-l}) in sterilized cavity slides with the help of a hypordermic syringe. The slides were placed on a glass rod triangle kept in petridish (90mm) containing sterilized distilled water at the bottom and moistened cotton lining on the inner surface of the upper lid and incubated at different temperature i.e. 15, 20, 25, 30 and 35^0C, respectively. In all, 100 conidia were examined microscopically from each slide (replicated thrice) selecting at random in different microscopically fields (totaling 300 conidia) to ascertain conidial germination.

Effect of different relative humidity levels on angular leaf spot disease development

Effect of various relative humidity levels on disease development, was done by raising bean seedlings of cv. Lakshmi in plastic pots (10 cm dia.). Two plant per pot is maintained. Twelve day old seedlings were inoculated with conidial suspensions and subsequently kept in humidity and temperature control cabinet at 25±1^{0}C. The humidity levels viz., 100.0, 95.0 90.0, 85.0 and 80.0 per cent were maintained. The inoculated plants were observed for disease symptom including the development of number of lesions on the leaves at 3 day interval after 6 day of inoculation.

Effect of different leaf wetness durations on angular leaf spot disease development

In order to find out the optimum leaf wetness duration for infection by *P. griseola*, the potted French bean plants (25 days old) of cv. "Lakshmi" were inoculated by spraying conidial suspension on both surfaces of the leaf and immediately transferred to a humid chamber. Distilled water sprays were given frequently given so that leaves remained completely wet. After 3, 6, 9, 12, 18, 24, and 48 h of wetness, the plants were removed from moist chamber and leaves were dried in front of a fan before transferring the plants to a net house to wait for symptom development. All the treatments were replicated three times. The data on number of lesions per leaf and incubation were recorded.

Influence of meteorological factors on angular leaf spot disease development

To study the role of meteorological factors on disease development, seeds of French bean cv. Lakshmi were sown in replicated plots during the last week of May at the experimental farm of Department of Plant pathology, Nauni during 2012, season. The severity of angular leaf spot was recorded at 7 day interval commencing from first week of July and continued up to last week of September. Simultaneously, meteorological data on temperature, relative humidity and cumulative rainfall were also recorded for the intervening period. Simple and multiple correlations were worked out separately to establish the relative contribution of these factors in the spread of the disease. Meteorological data were collected from the university meteorological station.

Results and Discussion

Effect of different temperature regimes on conidial germination of *Phaeoisariopsis griseola* under *in vitro* conditions

Data (Table 1) revealed significantly varied response of conidial germination of the test pathogen at different temperature regimes. The conidial germination was significantly high (70.5%) at 25^{0}C followed by that at 20 and 30^{0}C. The conidial germination being considerably less at 15^{0}C and was minimum (43.3%) at 35^{0}C.

Table 1: Effect of different temperature regimes on conidial germination of *Phaeoisariopsis griseola* under *in vitro* conditions

Temperature (^{0}C)	Conidial germination (%)
15	47.5
20	64.6
25	70.5
30	60.1
35	43.3
C.D$_{0.05}$	3.732

Effect of different relative humidity levels on angular leaf spot disease development

Data (Table 2) indicated a significant increase in number of lesions/leaf with the increasing level of humidity levels from 80 per cent to 90 per cent. However, the number of lesions/leaf was considerably less at 100 per cent humidity level. The humidity level of 95% was found most favourable for the disease development exhibiting 54.7 lesions/leaf. The disease response being somewhat less at 90 per cent (42.1), 100 per cent (41.2) and was minimum at 80 % (30.2). Cardona-Alvarez and Walker (1956) [7], Ploper (1980) [8], Sartorato (1988) [9] also reported the higher disease progress of angular leaf spot on French bean at relative humidity levels exceeding 90 per cent.

Table 2: Effect of different relative humidity levels on angular leaf spot disease development under pot culture experiment

Relative humidity (%)	No. of lesions/ leaf
80	30.2
85	38.1
90	42.1
95	54.7
100	41.2
C.D$_{0.05}$	7.0447

Effect of different leaf wetness durations on angular leaf spot disease development under pot culture experiment

The results (Table 3) of the study revealed that angular leaf spot development differed significantly at different leaf wetness levels. Amongst the different leaf wetness period studies, leaf wetness period of 24 h was found optimum exhibiting 53.4 lesion/leaf followed by that of 48 h and 18 h respectively. Least disease development was reflected at 3 h wetness period. The disease manifest itself after 12 days of incubation in potted bean plants provided with 3 and 6 h of leaf wetness periods. The further increase in leaf wetness period beyond 6 h resulted in the appearance of angular leaf spot disease within 9 day of incubation. These results also corroborate the findings of various workers (Sartorato, 1988; Stenglein *et al*., 2003) [9-10] who reported that the prevalence of rainy conditions and frequent irrigation, higher relative humidity levels and temperature to be the most conducive for severe outbreak of the angular leaf spot disease.

Table 3: Effect of different leaf wetness durations on angular leaf spot disease development under pot culture experiment

Leaf wetness (h)	No. of lesions/ leaf	Incubation period (days)
3	8.8	12
6	21.5	12
9	32.8	9
12	37.2	9
18	44.2	9
24	53.4	9
48	47.6	9
C.D$_{0.05}$	4.2328	

Effect of different meteorological factors on the progression of angular leaf spot of French bean during 2012-2013

The data (Table 4) on weather parameters and disease development indicated that the disease appeared in the second week of the July and attained the severe form during month of August. High relative humidity, coupled with wet climate and temperature range of 23 ± 2^{0}C prevailing during the cropping season were found to favour the disease. The disease

progression was comparatively less during the month of September. These results also corroborate the findings of various workers (Sartorato, 1988; Stenglein *et al*., 2003) [9-10] who reported that the prevalence of rainy conditions and frequent irrigation, higher relative humidity levels and temperature to be the most conducive for severe outbreak of the angular leaf spot disease.

a) Simple Correlations

Combined analysis of simple correlation coefficients worked out between angular leaf spot severity and meteorological factors are presented in Table 4. The data revealed that simple correlation coefficients between angular leaf spot disease severity and meteorological parameters (mean air temperature, average relative humidity and rainfall) were positively correlated. The relative humidity has much more pronounced effect on the angular leaf spot disease development as compare to the mean air temperature and rainfall.

Correlation pairs	Correlation coefficient
Disease severity x Temperature	+ 0.2826
Disease severity x Relative humidity	+ 0.5070
Disease severity x Cumulative rainfall	+ 0.0847

b) Multiple Correlation

The coefficient of multiple correlation (R^2) was calculated to measure the contribution of linear function of independent variables, such as mean air temperature (X_1), average relative humidity (X_2) and cumulative rainfall (X_3) on dependent variable i.e. per cent disease severity (Y). It was found to be 0.8827 per cent, between disease severity and the independent variables which indicated the significant effect of all the meteorological parameters contributing 88.27 per cent towards the development of angular leaf spot of French bean.

Table 4: Effect of different meteorological factors on the progression of angular leaf spot of French bean during 2012 season

Duration	Mean air temperature (%)	Average relative humidity (%)	Cumulative rainfall (mm)	(%) Increase in disease severity
3July-9July	23.55	74.0	5.5	0
10July-16July	23.65	84.0	143.1	0.9
17July-23July	22.65	80.0	80.0	3.5
24July-30July	24.4	84.0	30.0	8.1
31July-6August	24.04	75.0	4.0	6
7August-13August	24.05	82.0	15.5	10.5
14August-20August	22.1	84	67.1	12.6
21August-27August	23.9	79	83.2	11.2
28August-3Sept	23.7	88	20.0	6.2
4Sept-10Sept	23.35	78	6.0	4.1
11Sept-17Sept	23	82	22.4	7.9
18Sept-24Sept	21.55	68	1.6	2.6
25Sept-1Oct	21	69	Nil	1.5

Multiple correlation among angular leaf spot disease severity and meteorological factors and regression equation

R^2	Multiple coefficient of determination (%)	Regression equation
0.8827	88.27	$Y = -4.952 + 1.704X_1 - 0.769 X_2 + 0.245 X_3$ (0.359) (0.451) (0.335) Where, Y = Disease severity (%) X_1= Mean air temperature (ºC) X_2= Average relative humidity (%) X_3= Cumulative rainfall (mm)

Figures in parentheses are respective standard errors of regression coefficients

*Significant at 5 per cent level of significance

There is positive correlation between angular leaf spot disease severity and meteorological parameters (mean air temperature, relative humidity and rainfall) was observed. The relative humidity has much more pronounced effect on the angular leaf spot disease development as compare to the mean air temperature and rainfall. The present study further revealed the high multiple correlation coefficient (0.8827) between disease severity and group of independent variables indicating approximately 90 per cent contribution of all the meteorological parameters towards the development of angular leaf spot of French bean.

Statistical Analysis

The data recorded from various laboratory and field experiments were subjected to statistical analysis. The differences exhibited by treatments in various experiments were tested for their significance at 5 per cent using standard procedure as described (Gomez and Gomez, 1983) [6].

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