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Occurrence of angular leaf spot of common bean in major growing areas of Himachal Pradesh and effect of leaf wetness durations on its development

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Abstract

Angular leaf spot (ALS) caused by the hemibiotrophic fungus, *Pseudocercospora griseola* is one of the most widely distributed and damaging disease of common bean particularly in humid conditions. In Himachal Pradesh, common bean is grown during *Kharif* season and affected by ALS. Information on the occurrence and distribution of disease is important to develop management methods. Therefore, present study was carried out to assess the occurrence and distribution of angular leaf spot disease in major common bean growing areas of Himachal Pradesh and effect of leaf wetness durations on disease development was also evaluated under *in-vivo* conditions. The disease was observed in all the areas with different levels of disease severity ranging from 21.3 to 76.2 per cent. The highest disease severity was recorded in Kullu district *i.e.* 61.28 per cent and lowest disease severity of 43.50 per cent was recorded in Kinnaur district. The effect of leaf wetness durations on disease development studied under *in-vitro* conditions on susceptible variety Jwala showed a progressive increase in lesion number per leaf with the increase in wetness duration up to 24 hours.

Keywords: Angular leaf spot, Severity, leaf wetness, *Pseudocercospora griseola*

Common bean (*Phaseolus vulgaris* L.; 2n= 2x= 22), a member of family Leguminosae is the globally valued relished legume crop including India, for its green pods as well as dry beans (Sharma *et al*. 2021), having its origin in South Mexico and Central America (Vavilov 1979). In Himachal Pradesh, dry bean, locally called as 'Rajmash' and green bean as 'French bean' are among the premier pulse crops being grown in areas which are confined to 900 meters to 3000 meter above mean sea level in Shimla, Chamba, Sirmour, Kullu, Kinnaur, Mandi and Lahual & Spiti districts (Katoch *et al*. 2019). Environmental conditions which favour the growth of common bean plants, also predispose them to the attack of various fungal, bacterial and viral diseases (Dhiman *et al*. 2020). Among fungal diseases, angular leaf spot (ALS) caused by the hemibiotrophic fungus, *Pseudocercospora griseola* (Sacc.) Crous & U. Braun (*Phaeoisariopsis griseola* (Sacc.) Ferraris) is one of the most widely distributed and damaging disease of common bean particularly in humid conditions (Allorent and Savary, 2005). The optimum conditions for infection include 80-90 per cent humidity and a temperature of ≥ 24°C (Librelon *et al*. 2022). According to Alvarez and Schwartz (1979), infection might develop after a period of 24-48 hours of leaf wetness. To allow for effective infection and subsequent synnemata development, high relative humidity, rainfall, or dew periods are required (Lianos, 1957). The global impact of the disease has led to significant agricultural losses worldwide. The fungus infects most aerial plant parts and resulted in yield loss up to 80 per cent (Ponnappa *et al*. 1976; Shukla and Sharma 2006). In India, the disease has resulted in estimated losses ranging from 40 to 70 per cent, including damaged and unmarketable pods (Singh and Saini, 1980). In this study, disease survey was carried to know the prevalence of disease in different regions of Himachal Pradesh and effect of leaf wetness durations on development of disease, so that effective control measures can be adopted to combat the disease.

Materials and Methods

Disease survey

A disease survey was conducted during Kharif 2022 covering different locations of Kangra, Mandi,

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Kullu, Shimla, Chamba and Kinnaur districts of Himachal Pradesh (3 Zones) to record the prevalence and distribution of angular leaf spot of common bean. Observations were made by randomly selecting plants from the farmer's fields of each location with quadrant of 1.0 m^2 area and disease score was given on the basis of 1-9 disease rating scale (CIAT 1987) (Table 1). Further, disease severity was calculated by Townsend and Heuberger's (1943) formula as below

Disease severity index (DSI) was calculated as per following formula:

$$\text{Disease severity (\%)} = \frac{\sum (\text{No. of leaves at each scale} \times \text{Scale value})}{\text{Highest scale value} \times \text{Total no. of leaves}} \times 100$$

Isolation of pathogen and pathogenicity test

Isolation of pathogen (*Pseudocercospora griseola*) was done by picking up synnemata from diseased leaves, on V8 juice agar medium. In this method, a diseased leaf was carefully placed under a stereo microscope and microscope was focused on the synnemata present on the diseased portion of the leaf. Using a sterilized blade, the synnemata were delicately collected. Subsequently, these collected synnemata were transferred onto fresh V8 juice agar plates and the plates were kept in an incubator at temperature of 25±1 ºC. After 7 days of incubation, Petri plates showing small olive green or grey coloured colony of *P. griseola* were selected and maintained for further studies. Rezene *et al*. (2018) isolated the pathogen by picking up synnemata from diseased leaf with small piece of agar.

Following purification, Petri plates carrying the best growth of pathogen were selected. Sterilized distilled water was then added to the selected fungal growths and then surfaces of isolates were scraped with a sterilized spatula to detach the conidia. This suspension was subjected to microscopic examination using a heamocytometer to establish a conidial concentration of 2 x 10^4 conidia/ml. For inoculation, three seeds of the susceptible cultivar (Jwala) were planted in sterilized 15 cm pots containing sterilized soil. The seeds were sterilized using a 2.0 per cent sodium hypochlorite solution. The conidial suspension was applied to the plants at the 3-leaf stage and covered with plastic bags that had small aeration holes. These inoculated plants were then placed in a controlled glasshouse environment with a temperature of 25±3ºC and high humidity was maintained by spraying water seven times for four consecutive days. Daily observations were recorded until the first appearance of symptoms. Infected leaves were removed, examined under a microscope by slide preparations and the pathogen was re-isolated using the afore mentioned method. The morpho-cultural characteristics (colony color, colony growth, dimensions and shape of conidia) of the re-isolated pathogen were compared to the originally isolated pathogen. Isolates were maintained for further studies.

Effect of leaf wetness durations on disease development

In order to find out the most optimum leaf wetness duration for infection by *Pseudocercospora griseola*, potted common bean plants of cultivar Jwala were inoculated by spraying conidial suspension on both surfaces of the leaf. Water was sprayed for different time intervals *i.e*., 3, 6, 9, 12, 18, 24 and 48 hours to maintain leaf wetness. After 48 hours of wetness, the water spray was stopped and the plants were kept in glasshouse to wait for symptom development. All the treatments were replicated three times. The data on number of lesions per leaf and incubation period were recorded.

Effect of intermittent leaf wetness on infection efficiency

To study the influence of intermittent leaf wetness on infection efficiency, 21 days old potted plants of common bean cv. Jwala were subjected to total wetness periods ranging from 12 to 48 hours each alternating

Table 1: Disease scale for common bean against angular leaf spot under field conditions (CIAT 1987)

Scale	Type of lesion
1	No visible disease symptoms (0% infection)
3	Plants with 5 -10% leaf area having lesions
5	Plants with 20% leaf area having lesions and sporulating
7	Plants with up to 60% leaf area having lesions associated with chlorosis and necrosis,
9	Plants with 90% leaf area having lesions associated with early leaf fall and plant death

with 12 hours dryness in each 24-hour period. The plants were inoculated by spraying conidial suspension on both surfaces of the leaf. Wetting of plants was started after 12 hours of inoculation. Water was sprayed on plants to keep them wet for required period. Dry periods were achieved by stopping the water spray. Three pots in each treatment were allocated for lesion counts and average number of lesions in each treatment was worked out.

Results and discussion

Occurrence of angular leaf spot of common bean in major growing areas of Himachal Pradesh

To assess the occurrence and distribution of angular leaf spot disease, major common bean growing areas in six districts of Himachal Pradesh *viz*., Kangra, Mandi, Kullu, Shimla, Chamba and Kinnaur were surveyed during *Kharif* 2022. The data recorded on disease severity is presented in Table 2. The disease was observed in all the areas with different levels of disease severity ranging from 21.3 to 76.2 per cent. Highest disease severity of 76.2 per cent was recorded in district Kullu while lowest disease severity of 21.3 per cent was recorded in district Mandi. However, highest mean disease severity of 61.28 per cent was recorded in district Kullu followed by Shimla (60.85%), Kangra (59.77%), Chamba (58.05%), Mandi (54.12%) and lowest mean disease severity of 43.5 per cent was recorded in district Kinnaur (Table 2).

The findings from the survey conducted in six districts of Himachal Pradesh during the *Kharif* season of 2022 reveal significant insights into the occurrence and distribution of angular leaf spot disease in common bean cultivation. The data presented in Table 2 demonstrates that the disease was pervasive across

Table 2: Prevalence of angular leaf spot of common bean in different districts of Himachal Pradesh

District	Number of Locations	Disease Severity (%)	
		Range	Mean
Mandi	15	21.3-70.2	54.12
Kullu	17	30.5-76.2	61.28
Kangra	8	43.2-73.1	59.77
Kinnaur	6	32.6-51.4	43.50
Chamba	2	51.0-65.1	58.05
Shimla	2	60.4-61.3	60.85

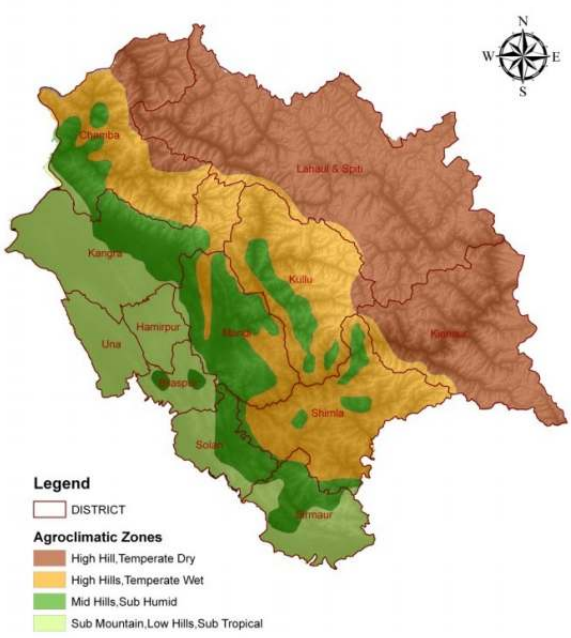

Figure 1 Different agro-climatic zones of Himachal Pradesh

all surveyed areas, with varying degrees of severity. This suggests that angular leaf spot poses a notable threat to bean crops throughout the region.

The significant variation in disease severity observed among the different districts and villages. The highest recorded severity of 76.2% in Kullu district, highlights the potential for significant economic losses in areas heavily impacted by the disease. Conversely, the lowest severity of 21.3% observed in Mandi district, highlights the existence of local factors or practices that may contribute to disease suppression. The geographical distribution of angular leaf spot is influenced by various factors, including climate conditions, bean production practices and the movement of infected plant material. Climatic conditions play a crucial role in the prevalence and severity of the disease. Regions with high humidity, moderate temperatures and frequent rainfall create favorable conditions for the growth and spread of the pathogen. Due to these factors, district Kullu emerges as the most severely affected, followed closely by Shimla. Several other researchers also conduct the disease severity survey of angular leaf spot in their regions. Pamela *et al*. (2014) surveyed different farmers field in Uganda and 21-80 per cent disease severity was recorded. Kijana *et al*. (2017) conducted field survey during two crop seasons, in two main beans growing zones of eastern Democratic Republic of Congo. Angular leaf spot in these fields had an average severity index (PSI) of 49.9%. Percent severity index was significantly different ($P<0.05$) between districts and seasons. The highest severity (PSI=59.7%) was observed in Kabare district, and the lowest in Uvira district (PSI=39.5%). Canpolat and Maden (2021) also surveyed western black sea region of Turkey during autumn and summer periods to assess the occurrence of angular leaf spot of common bean. They revealed that disease severity ranged from 66 to 82 per cent in the autumn period and from 74 to 86 per cent in the summer period, respectively.

These findings highlight the importance of proactive disease management strategies in common bean cultivation. Furthermore, continued surveillance and research efforts are essential to monitor disease dynamics and develop effective control measures in response to evolving challenges posed by plant pathogens.

Isolation and maintenance of *Pseudocercospora griseola* isolate

Isolations were made by picking up synnemata from diseased leaves, on V8 juice agar medium.

After successful isolation of pathogen, pathogenicity of *Pseudocercospora griseola* isolate was proved under *in-vivo* conditions on variety "Jwala" by inoculating pathogen at 3 leaf stage plants. The initial symptoms of the disease appear as grey spots undersides of leaves, delimited by veins and veinlets on trifoliate leaves. Subsequently, characteristic symptom development occurred between 10-14 days after inoculation as grey spots undersides of leaves turn into brown and dark grey to black, synnemata bearing conidia produced on all types of lesions. It had exhibited identical symptoms as observed from collected disease samples. The pathogen was re-isolated from leaves that showed characteristics symptoms in pathogenicity test. Pure culture obtained by single spore showed cent per cent similarity with the inoculated test pathogen, like olive-green to grey colored colony with irregular growth. Conidia were solitary, subcylindrical, fusiform to obclavate, straight to somewhat curved with length and width 27.5-55.1×4.13-10.62 µm, respectively having 1-4 septations, hence pathogenicity of *P. griseola* was proved and isolate was maintained to conduct further experiments.

Effect of leaf wetness duration on disease development

The effect of leaf wetness duration on disease development was studied under *in-vivo* conditions on common bean cultivar Jwala and the data regarding incubation period and number of lesions per leaf were recorded and presented in Table 3. Effect of different wetness durations revealed that increase in leaf wetness duration from 3 to 12 hours showed a corresponding decrease in incubation period from 12 to 10 days while lesion number increased from 8.22 to 35.22. However, further increase in leaf wetness did not exert any effect on incubation period whereas there was a progressive increase in lesion number per leaf with the increase in wetness duration up to 24 hours (48.50 lesions/leaf) after which lesion number per leaf declined with the increase in wetness duration. Mathew (1999) also reported that 24-hour leaf wetness duration increase the rate of disease development. Alvarez and

Table 3 Effect of different leaf wetness durations on disease development

Leaf wetness period (hours)	Incubation period (days)	Lesions/Leaf
` 3	12.33	8.22
6	12.00	23.25
9	10.33	29.25
12	10.00	35.22
18	9.00	39.22
24	9.00	48.50
48	9.00	43.25
C.D.(p=0.05)	-	2.16

Schwartz (1979) also revealed that infection might develop after a period of 24-48 hours of leaf wetness

Effect of intermittent leaf wetness durations on disease development

The study on the role of intermittent leaf wetness on disease development was done on Jwala plants. The data on the effect of intermittent leaf wetness (Table 4) revealed that the number of lesions increased with the interruption of leaf wetness with dry periods up to 3 cycles (40.25 lesions) and further extension of wet and dry periods resulted decrease in lesion number. Mathew (1999) also reported that 3 cycles of leaf wetness with dry periods increase the efficiency of disease.

The study investigating the impact of leaf wetness duration on angular leaf spot development in the common bean reveals a clear relationship between moisture availability and disease progression. Increasing leaf wetness duration from 3 to 12 hours correlates with a decrease in the incubation period and a significant rise in lesion number per leaf, highlighting the critical role of moisture in facilitating pathogen germination and infection. While further extension of wetness duration beyond 12 hours does not affect the incubation period, lesion formation continues to escalate, peaking at 48.50 lesions per leaf after 24 hours. Additionally, the examination of intermittent leaf wetness demonstrates a non-linear relationship between wet and dry cycles and lesion development, emphasizing the complex interplay between moisture management and disease severity in common bean cultivation. These findings highlight the importance of implementing targeted management strategies to mitigate the impact of angular leaf spot and safeguard crop productivity.

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Conflict of interest: The authors declare that there is no conflict of interest in this research paper.

Table 4 Effect of intermittent leaf wetness durations on disease development

Duration of wet and dry period (hours)	Wet/dry sequence (hours)								Lesions/leaf
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	*Mean
24	12	12	-	-	-	-	-	-	32.25
48	12	12	12	12	-	-	-	-	36.50
72	12	12	12	12	12	12	-	-	40.25
96	12	12	12	12	12	12	12	12	38.97
C.D.=(p=0.05)									0.68

*Average of five replications

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