

## Comparative Morphological and Symptomatic Analysis of *Alternaria* leaf spot Disease in Green Gram (*Vigna radiata*) Across Six Districts in Rajasthan

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### ABSTRACT:

This study investigates the morphological and symptomatic characteristics of *Alternaria alternata* causing leaf spot disease in green gram (*Vigna radiata* L.) across six districts in Rajasthan, namely—Jodhpur, Nagaur, Ganganagar, Ajmer, Bikaner, and Jaipur, during the Kharif seasons of 2022 and 2023. The research aims to provide a comprehensive analysis of the variation in disease symptoms, fungal morphology, and growth patterns in different geographical locations. Samples of infected green gram leaves were collected from selected fields and subjected to surface sterilization and isolation on Potato Dextrose Agar (PDA) for fungal cultures. The macroscopic and microscopic features of *A. alternata* isolates, including colony color, texture, conidial shape, and septation, were examined. Symptomatic observations focused on the development and severity of leaf spot symptoms in relation to environmental factors. The results revealed that Ganganagar and Bikaner districts exhibited the highest disease severity, with dense dark brown lesions and rapid fungal growth on PDA. Microscopic analysis showed that isolates from Jaipur had longer, more septate conidia compared to those from other regions, indicating morphological diversity. The findings highlight the variability in disease expression across districts, with significant differences in colony morphology and disease severity. This comparative study offers valuable insights into the regional dynamics of *A. alternata* in green gram cultivation, providing essential data for more targeted disease management strategies in Rajasthan.

**Keywords:** *Alternaria alternata*, fungal disease, Rajasthan, leaf spot, green gram, disease management

### 1. INTRODUCTION

*Alternaria alternata* is a significant fungal pathogen that causes leaf spot disease in various crops, including green gram (*Vigna radiata*). Green gram is a crucial leguminous crop grown extensively in India, especially in arid and semi-arid regions like Rajasthan, which faces unique environmental challenges that influence disease dynamics (Singh et al., 2020). *Alternaria alternata* infection results in reduced photosynthetic activity, leading to yield losses and impacting the overall quality of the crop. The disease is characterized by distinctive symptoms such as dark brown lesions with concentric rings on the leaves, often leading to premature leaf drop and reduced plant vigor.

Rajasthan, with its diverse agro-climatic conditions, is home to significant green gram cultivation, and the disease's impact may vary across regions. Despite the widespread occurrence of *Alternaria alternata* in the state, limited studies have focused on understanding the morphological variability and symptom expression of the pathogen across different districts. Environmental factors such as temperature, humidity, and soil conditions can significantly influence the prevalence and severity of the disease, leading to regional differences in fungal growth patterns and disease manifestation (Kumar et al., 2019).

This research aims to perform a comparative analysis of the morphological and symptomatic characteristics of *A. alternata* from green gram leaves collected across various districts of Rajasthan. By examining fungal colony features, conidial morphology, and disease symptoms in relation to local environmental conditions, the study seeks to identify

regional variations in disease severity and pathogen morphology. Understanding these factors will provide valuable insights for developing region-specific strategies for managing *Alternaria* leaf spot disease and mitigating its impact on green gram production in Rajasthan.

## 2. MATERIALS AND METHODS

Infected green gram (*Vigna radiata*) leaves showing characteristic *Alternaria* leaf spot symptoms. Collected from six districts of Rajasthan: Jodhpur, Nagaur, Ganganagar, Ajmer, Bikaner, and Jaipur during the Kharif seasons of 2022 and 2023.

### Sample Collection and prevalence of the disease

Infected green gram (*Vigna radiata* L.) leaves exhibiting typical symptoms of *Alternaria* leaf spot disease were collected from green gram fields across six districts of Rajasthan Jodhpur, Nagaur, Ganganagar, Ajmer, Bikaner, and Jaipur. During the Kharif seasons of 2022 and 2023. Samples were placed in brown paper bags and further put in sterile poly bags and brought to the laboratory for further analysis. The disease symptoms of *Alternaria* leaf spot were recorded in the fields as well as in the sample collected as mentioned above.

### Isolation and identification of the Pathogen

The Leaf samples were surface sterilized using 1% sodium hypochlorite aqueous solution for 30 sec., followed by three rinses with sterile distilled water to eliminate surface contaminants. Sterilized segments (3–5 mm) were cut from the lesion margins and placed on sterilized Petri dishes containing Potato Dextrose Agar (PDA) medium. Plates were incubated at  $25 \pm 2^\circ\text{C}$  for 5 days in a BOD incubator. Fungal colonies emerging from the infected leaf tissues were carefully sub-cultured onto fresh PDA plates to obtain pure cultures. These isolates were maintained at  $4^\circ\text{C}$  on PDA slants for further morphological and microscopic studies.

Colony characteristics such as color, texture, margin, and growth pattern were recorded. The diameter of the colonies (in mm) was measured at regular intervals (24, 48, 72, and 96 hours) to determine radial growth rates under controlled laboratory conditions. Microscopic features were studied using slides prepared with Lactophenol Cotton Blue staining. Structures including conidia, septation, beak length, and conidiophores were examined under a compound microscope at 400x–1000x magnification. Observations were compared with standard taxonomic keys and existing literature to confirm identification (Ellis, 1971).

### Disease Symptoms

In the present study in green gram, the symptoms of *Alternaria* leaf spot disease infection begin as small, circular, necrotic spots on the leaves. As the infection progresses, these spots coalesce and form larger lesions with concentric rings, which are characteristic of the disease. The lesions can cause premature leaf drop, reducing the plant's photosynthetic capacity and ultimately leading to yield losses (Sharma et al., 2013).

### Comparative Morphological Studies

Morphological characteristics of *A. alternata* isolates from green gram were compared with previously reported isolates from other crops such as mustard, tomato, and sunflower. Parameters such as colony color, sporulation, and spore dimensions were evaluated to assess intra-species variability.

## 3. RESULTS AND DISCUSSION:-

### Sample Collection and Disease Prevalence

During the Kharif seasons of 2022 and 2023, green gram (*Vigna radiata* L.) leaves showing typical *Alternaria* leaf spot symptoms were collected from six districts of Rajasthan: Jodhpur, Nagaur, Ganganagar, Ajmer, Bikaner, and Jaipur. Infected leaves exhibited necrotic brown spots with concentric rings and chlorotic halos. Disease prevalence was highest in Jaipur and Jodhpur, moderate in Nagaur and Bikaner, and lowest in Ganganagar and Ajmer.

### Isolation and identification of the Pathogen

Surface-sterilized leaf segments were cultured on PDA and incubated at  $25 \pm 2^\circ\text{C}$ . Fungal colonies developed within 48

hours, becoming dark and velvety with irregular margins. Pure cultures were maintained on PDA slants. Radial growth averaged 65–75 mm after 96 hours. Microscopic analysis using Lactophenol Cotton Blue revealed conidia characteristic of *Alternaria alternata*—obclavate, brown, with transverse and longitudinal septa, and short beaks—confirming identity as per Ellis (1971).

### Pathogen Biology of *Alternaria alternata*

*Alternaria alternata* is a necrotrophic fungus known to infect a wide range of plant species, including pulses, oilseeds, and vegetables (Patil et al., 2017). The fungus reproduces asexually through conidia, which are spread by wind, rain, and contaminated soil. The pathogen produces toxins that kill host cells, which facilitates its entry and proliferation in plant tissues (Thomma, 2003). *A. alternata* can survive in crop debris and other plant residues, making it a persistent threat in regions like Rajasthan where crop rotation practices may not be strictly followed (Neergaard, 1977).

### Fig 1(A-F) Morphological variations in conidia of *Alternaria alternata* isolates under microscopic observation

**A. JOD Aa-1** – Conidial morphology of *A. alternata* isolate collected from **Jodhpur**, showing multi-septate, obclavate conidia with light pigmentation.

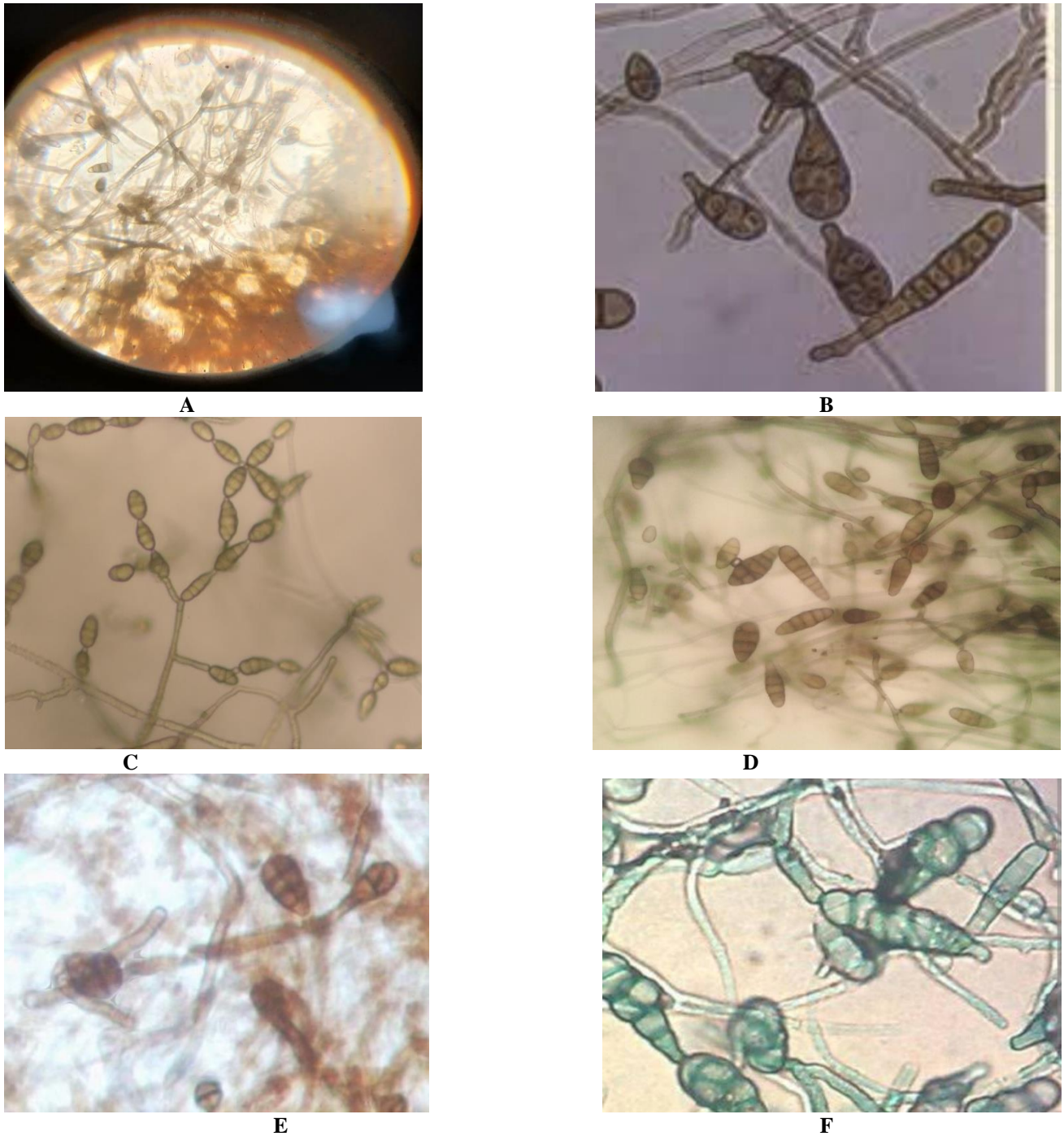
**B. NAG Aa-1** – Isolate from **Nagaur** depicting conidia with transverse and longitudinal septa and variable pigmentation.

**C. GAN Aa-1** – Conidial structure of isolate from **Ganganagar**, displaying moderately curved, beaked conidia under high magnification.

**D. AJM Aa-1** – *A. alternata* conidia from **Ajmer** with pronounced septation and dark pigmentation, typical of aged conidia.

**E. BIK Aa-1** – Morphological traits of isolate from **Bikaner**, characterized by short-beaked, broad conidia with variable septation.

**F. JAI Aa-1** – Conidia of *A. alternata* isolate from **Jaipur**, showing distinct morphological traits such as elongated shape and moderate pigmentation.



**Fig 1(A-F) Conidial morphology of six isolates of *Alternaria alternate* from various places showing variability in conidia and conidiophores.**

**Table 1: Conidial Traits of *Alternaria alternata* Isolates of collected from various location**

Isolate Code	Location	Conidial Shape	Color / Pigmentation	Septation	Arrangement	Notable Adaptation
JOD Aa-1	Jodhpur	Elongated, slightly curved	Dark brown	Multiple transverse + longitudinal	Chains or singly	Adapted to arid desert; UV & desiccation resistance
NAG Aa-1	Nagaur	Oval to obclavate	Moderately brown	Clear transverse septa	Mostly singly	Shorter spores; semi-arid climate adaptation
GAN Aa-1	Shri Ganganagar	Broad, thick-walled	Light brown	Oblique septa	Clusters or singly	Likely due to canal irrigation, milder stress
AJM Aa-1	Ajmer	Long and narrow	Light to moderate brown	Well-defined transverse septa	Chains	Optimal dissemination; central Rajasthan traits
BIK Aa-1	Bikaner	Short and broad	Highly brown	oblique septa	Scattered or singly	Arid adaptation; compact for survival
JAI Aa-1	Jaipur	Elongated, slightly curved	Dark brown	Densely septate	Chains or grouped	Transition zone adaptation; high sporulation

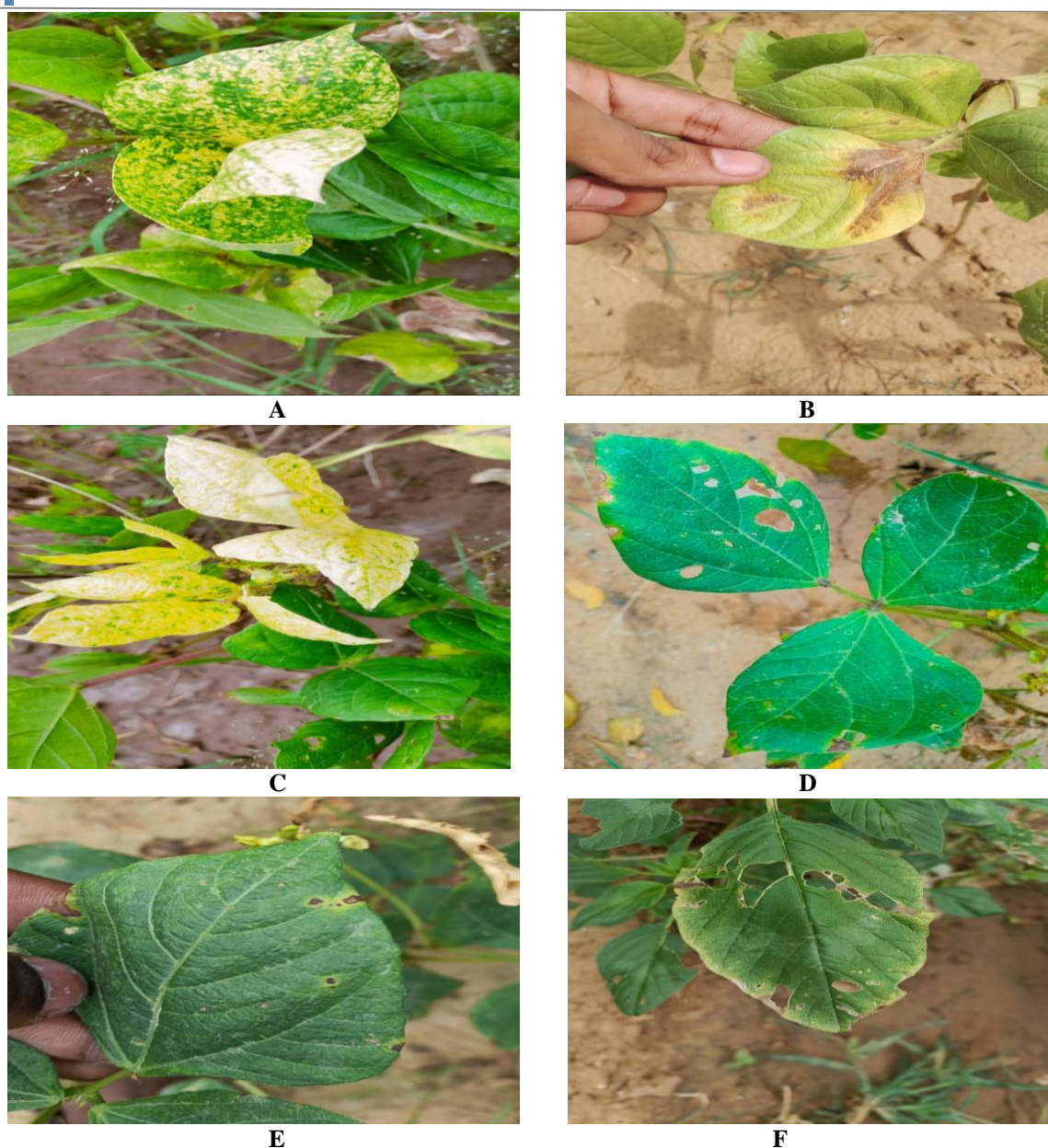
### Disease Symptoms

Field observations revealed that infected green gram plants exhibited characteristic symptoms of *Alternaria* leaf spot. Initial symptoms appeared as small, circular, necrotic spots on the leaves, which gradually enlarged and coalesced to form larger lesions with prominent concentric rings. In severe cases, affected leaves showed chlorosis and premature defoliation. These typical symptoms were consistently observed across all surveyed districts, with the most pronounced manifestations recorded in Jaipur and Jodhpur. The extent of foliar damage indicated a significant potential for reduced photosynthetic activity and yield loss, particularly in heavily infected areas.

**Fig. 2 (A–F): Field observations and *Alternaria* leaf spot disease symptoms in Green gram in different places of Rajasthan.**

- A. Salori, Jodhpur – Yellow to brown necrotic spots observed on leaves.
- B. Balwanta, Ajmer – (Leaf spots suggestive of *Alternaria* infection; similar symptoms expected).
- C. Vijayapura, Jaipur – (Lesions on foliage; likely *Alternaria*-induced chlorotic zones).
- D. Chugawas, Bikaner – (Foliar spotting and marginal yellowing due to disease progression).
- E. Baswani, Nagaur – (Multiple concentric leaf spots indicative of *Alternaria alternata*).
- F. Ganganagar, Haripura – (Leaf with necrotic patches and possible defoliation signs).





**Fig. 2 (A–F):** Disease symptoms of *Alternaria* leaf spot in the infected plants of green gram (*Vigna radiata* L.) collected from places of Rajasthan:

#### Disease Symptoms in Green Gram across Rajasthan: A Comparative Overview

Field assessments across six agro-climatic zones—Jodhpur, Nagaur, Ganganagar, Ajmer, Bikaner, and Jaipur—revealed diverse foliar symptoms associated with *Alternaria alternata* infection in green gram.

**Fig. 2 (A–F):** Early and advanced disease symptoms of *Alternaria* leaf spot in green gram (*Vigna radiata* L.) observed on infected plant leaves collected from various locations in Rajasthan:

(A) **Salori, Jodhpur:** Early symptoms included bright yellow, mosaic-like chlorosis, indicating initial chlorophyll degradation due to pathogen activity. At maturity, lesions expanded and merged, leading to large necrotic areas and reduced photosynthetic capacity.

(B) **Balwanta, Ajmer:** Initial necrotic spots with well-defined margins were visible. As disease progressed, lesions coalesced under moderate humidity conditions, suggesting favorable microclimate for *Alternaria*

*alternata* growth (Sharma et al., 2013).

**(C) Vijayapura, Jaipur:** Early-stage infection showed small, circular brown spots. With time, these developed into larger, irregular lesions, often causing partial wilting under semi-humid transitional conditions.

**(D) Chugawas, Bikaner:** Rapid appearance of leaf spots in early stages, followed by extensive leaf desiccation and curling. Later symptoms indicated accelerated tissue collapse, possibly due to high-temperature-induced stress.

**(E) Baswani, Nagaur:** Initially, yellowing around small necrotic lesions with concentric rings. At later stages, the lesions expanded, forming typical *Alternaria* patterns with a distinct dark center and chlorotic halo.

**(F) Haripura, Sri Ganganagar:** Early symptoms appeared as browning along leaf veins. As infection advanced, tissue collapse intensified, resulting in premature drying and complete foliar necrosis.

Epidemiology and Favorable Conditions

*Alternaria alternata* thrives in environments with high humidity (over 80%) and moderate temperatures (25–30°C) (Meena et al., 2017).Rainy seasons, irrigation practices that keep leaves wet, and dense plant canopies provide ideal conditions for the development of *Alternaria alternata*. In Rajasthan, the changing climate with unpredictable rainfall patterns and higher humidity during key crop growth periods—has led to more frequent and severe outbreaks of *A. alternata* in recent years (Gupta et al., 2011; UMass Extension, 2023).

Comparative Morphological Studies

The morphological characteristics of *Alternaria alternata* isolates obtained from green gram were compared with reported isolates from other crops including mustard, tomato, and sunflower. Green gram isolates exhibited grayish-white to dark olive colonies with rapid sporulation and dense mycelial growth. Conidia were obclavate to elliptical with 3–8 transverse and 0–3 longitudinal septa, measuring on average 18–30 µm in length and 7–12 µm in width, consistent with standard descriptions. Compared to isolates from mustard and tomato, green gram isolates showed slightly larger conidial dimensions and more frequent beak formation. These observations highlight moderate intra-species variability, suggesting possible host-specific adaptation or environmental influence on morphological traits.

Table 2: Epidemiology and Favorable Conditions for *Alternaria alternata* in Green Gram

Region	Climatic Zone	Favorable Conditions for Infection	Key Epidemiological Factors
Jodhpur	Hot Arid	Dry winds, temp 30–38°C, low RH (<40%), sandy soils.	High temperature and low humidity enhance conidial sporulation.
Nagaur	Semi-Arid	Moderate humidity, 25–35°C, post-irrigation or monsoon showers.	Intermittent moisture boosts pathogen growth post-dormancy.
Ganganagar	Canal-Irrigated Plains	Higher RH (60–70%), moderate temp 25–30°C, dense canopy.	Moist soil and irrigation favor long spore survival.
Ajmer	Transitional Semi-Arid	Diurnal temp variation, light rain, humidity 45–60%.	Fluctuating weather triggers sporulation and symptom flare-ups.
Bikaner	Extreme Arid	Intense sunlight, low RH, minimal rainfall, high UV tolerance.	Pathogen survives as saprophyte in plant debris under stress.
Jaipur	Semi-Humid	Moist canopy, RH 60–70%, occasional showers, cloudy days.	High vegetative growth increases leaf wetness periods.

*Alternaria alternata*, a necrotrophic fungus, causes leaf spot in green gram (*Vigna radiata* L.) and thrives under specific environmental conditions. The pathogen persists in infected plant debris and seeds, serving as primary inoculum, while conidia dispersed by wind and rain splash contribute to secondary spread.

Optimal conditions for disease development include moderate temperatures (25–30°C), high relative humidity (>80%), and prolonged leaf wetness caused by dew, rainfall, or irrigation. These factors significantly influence conidial production, spore germination, and lesion expansion. The disease typically becomes severe during the flowering to pod-setting stages, especially under physiological stress and dense canopy cover (Sharma et al., 2013).

**Table 3: Comparative Analysis of *A. alternata* in Other Crops in Rajasthan**

Crop	Disease	Symptoms	Economic Impact
Green Gram	Leaf spot	Necrotic lesions with concentric rings, defoliation	15–30% yield loss
Chickpea	Blight	Brown irregular spots on leaves and stems	Up to 25% yield loss
Mustard	Leaf blight	Black spots on leaves and pods	20–40% yield loss
Tomato	Early blight	Leaf spots, yellow halos, fruit rot	20–50% loss if uncontrolled
Cotton	Leaf spot	Defoliation, boll drop	10–35% yield loss

Source: Sharma, P., Saharan, M. S., & Singh, R. (2013); Agrios, G. N. (2005)

This comparison highlights the polyphagous nature of *Alternaria alternata* and its ability to cause significant economic losses across diverse crops in Rajasthan (Sharma et al., 2013; Agrios, 2005).

### Management Strategies

Management of *A. alternata* in green gram requires a combination of cultural, chemical, and biological control measures.

- **Cultural Practices:** Crop rotation, optimum plant spacing, and removal of infected plant debris help reduce the survival and spread of *A. alternata* in the field environment (Sharma et al., 2013).
- **Chemical Control:** Fungicides such as mancozeb, chlorothalonil, and azoxystrobin have demonstrated effectiveness in controlling *A. alternata*. However, frequent application and over-reliance may contribute to the development of fungicide-resistant strains (Gupta & Tripathi, 2011).
- **Biological Control:** Antagonistic microorganisms such as *Trichoderma harzianum* and *Pseudomonas fluorescens* have shown promise in suppressing the pathogen through competition and antibiosis, offering an eco-friendly alternative (Rathod et al., 2020).
- **Host Resistance:** Breeding for resistance remains a long-term strategy. Efforts are ongoing to develop green gram varieties with resistance to *Alternaria* leaf spot; however, identification and incorporation of resistant genes into commercial lines remain limited and require further research (Kumar et al., 2019).

### Future Prospects: Sustainable Disease Management

The future of fungal disease management in green gram hinges on the development and implementation of integrated disease management (IDM) strategies. With the rising threat of climate change, it is anticipated that fungal pathogens like *A. alternata* will become more aggressive and widespread, particularly in semi-arid areas like Rajasthan where climate variability is pronounced (Meena et al., 2017; Maheshwari & Krishna, 2013).

Several critical components must be included in future disease management programs:

1. **Climate-Resilient Varieties:** Breeding efforts must prioritize resistance to both biotic and abiotic stresses. Molecular tools such as QTL mapping and marker-assisted selection (MAS) can accelerate the development of resistant green gram cultivars (Soni & Choudhary, 2020).
2. **Early Detection and Forecasting:** AI-based models and remote sensing tools are being explored to predict disease outbreaks by integrating weather, soil, and crop data. Early warning systems can enable timely intervention, reducing the need for curative fungicide applications (Devamani et al., 2017).
3. **Eco-Friendly Control Measures:** The use of plant-based formulations, microbial consortia, and organic amendments (e.g., neem extract, jeevamrut) offers environmentally safe alternatives for disease suppression (Bisht & Choudhary, 2021; Reddy & Padmodaya, 1996). Large-scale trials and extension services are essential to promote these methods.
4. **Farmer Education and Policy Support:** Awareness programs focusing on IDM and good agricultural practices (GAPs) are vital. Government-backed incentives for adopting sustainable inputs and IPM strategies could further enhance disease management efficacy.



5. **Pathogen Surveillance and Resistance Monitoring:** Establishing a statewide network for monitoring pathogen variability and fungicide resistance will help fine-tune region-specific disease control measures (Yadav et al., 2020).

### Conclusion

The present investigation confirms *Alternaria alternata* as the major causative agent of *Alternaria* leaf spot in green gram (*Vigna radiata* L.) across six key districts of Rajasthan—Jaipur, Jodhpur, Nagaur, Bikaner, Ganganagar, and Ajmer—during the Kharif seasons of 2022 and 2023. The highest disease prevalence was observed in Jaipur and Jodhpur, indicating regional vulnerability due to possible environmental or varietal factors. Morphological and microscopic identification revealed typical features of *A. alternata*, including obclavate brown conidia with transverse and longitudinal septa, validating its identity based on standard taxonomic keys.

Colony growth on PDA media reached up to 75 mm within 96 hours, showing rapid radial expansion under controlled conditions. Comparative morphological analysis with isolates from other crops like mustard and tomato revealed noticeable intra-species variability, suggesting adaptive potential of the pathogen.

Given the considerable impact on green gram foliage, which may lead to reduced photosynthesis and yield loss, it is imperative to implement targeted disease management strategies. These should include the use of region-specific fungicides, cultivation of resistant green gram varieties, and awareness campaigns for early disease detection.

Future research should focus on screening green gram genotypes for resistance, refining cultural practices, and integrating biological control agents to formulate a comprehensive Integrated Disease Management (IDM) approach. Strengthening farmer-level interventions and climate-based disease forecasting models will be key to mitigating the threat of *Alternaria alternata* and securing sustainable green gram production in Rajasthan.

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