



RESEARCH ARTICLE

Prevalence and severity of anthracnose diseases of green gram [*Vigna radiata* (L.)] caused by *Colletotrichum truncatum* (Schw.) in arid ecosystem of India

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Abstract

Production and productivity of green gram are mainly constrained by fungal diseases which led to a yield loss of 20-80 %. In the present study, geo-referenced surveys were conducted weekly during July-August of, 2023-24 and 2024-25 in three major green gram producing districts of Rajasthan namely, Bikaner, Jodhpur and Nagaur, to assess the prevalence and severity of green gram anthracnose disease (GAD) in the arid ecosystem of India. The disease index in the range of 25.02-31.85 % and 28.40-35.19 % were recorded in 2023-24 and 2024-25 respectively. Correlation studies between per cent disease index (PDI) and weather parameters, maximum temperature (T_{max}), minimum temperature (T_{min}), morning relative humidity (RHM), evening relative humidity (RHE) and rainfall (RF) revealed that RHM and RHE had a positive correlation with PDI across all three districts. The predisposition of the disease found in this study provided an overall study for disease management programme in the speedily expanding of green gram cultivation in India.

Keywords: anthracnose; arid ecosystem; green gram; prevalence; severity

Introduction

Green gram (*Vigna radiata* L.) is a widely cultivated *Kharif* and summer season legume grown across tropical, subtropical and arid agro ecologies in the world. It is a short duration and fast-growing pulse crop require less input and performs well under heat and drought conditions. It is also known as “Golden gram” because of its nutritional richness like protein (24.5 %), fat (1.3 %), minerals (3.5 %), carbohydrates (56.7 %) etc. The ability for increasing the soil fertility by the incorporation of nitrogen (30 kg/ha/annum) (1, 2). It also provides high quality of some essential amino acids like lysine (460 mg/g N) and tryptophane (60 mg/g N). It can provide some of the antioxidant in the form of ascorbic acid when sprouted and contains riboflavin (0.21 mg/100 g) (3). In India, the crop is one of the most important crops for smallholder farmers for household consumption.

In India, it is largely cultivated in the states of Rajasthan, Madhya Pradesh, Maharashtra, Uttar Pradesh, Orissa andhra

Pradesh and Tamil Nadu. It is primarily cultivated during the *Kharif* season, covering an area of 15.93 million hectares and yielding a production of 3.74 million tonnes. Rajasthan is a leading state in terms of both area (2.4 million hectares) and production (1.22 million tonnes) of green gram (4).

Both biotic factors, such as diseases and insect pests and abiotic stresses are major constraints to green gram production and productivity worldwide. It is affected by all phytopathogens like nearly 60 fungal, 3 bacterial and 5 viral diseases (5) and out of which the fungal diseases viz. cercospora leaf spot, anthracnose, web blight, powdery mildew and dry root rot are the most prevalent (6). Among the fungal diseases, anthracnose caused by *Colletotrichum truncatum* (Schw.) is one of the economically important diseases and it occurs in all the growing track of the crop (7). The disease results in both qualitative and quantitative losses (8). The grain yield loss is high due to attack of anthracnose disease causing pathogen leads to almost 18.20 to 86.50 % pre and post-harvest yield losses (9).

Some diseases are distributed world-wide and are endemic to all green gram cultivating regions, although others are limited to certain geographical areas. As a result, the importance of a particular disease could vary from one geographical area to another. Therefore, information on disease occurrence from farm survey data obtained from evenly distributed fields across geographical locations or ecological zones are prerequisite to effective disease management (10). Such surveys can reveal the effectiveness of existing disease management practices and the desirability and conditions required for introduction of new methods (11). Therefore, the main aim of the present study is to determine the prevalence and severity of GAD in farmers' fields across arid ecosystem of India and to assess the relationship between disease prevalence and the climatic variables in surveyed locations.

Materials and Methods

Survey area

Anthraco disease survey was conducted on weekly basis during July-August, 2023-24 and 2024-25 in three major green gram producing districts of Rajasthan i.e., Bikaner, Jodhpur and Nagaur districts in arid ecosystem of India (Fig. 1). The survey routes were determined using the road maps of Western Rajasthan, India included highways, secondary roads and feeder roads. As much as possible, the routes were chosen to intersect the main green gram growing areas and to ensure that sufficient green gram fields were available for sampling. The coordinates of each field visited, were recorded using a GPS. In each district, three tehsils were selected. From each tehsil, five villages were chosen and from each village, five farmers' fields were randomly selected and a total of 225 fields assessed.

Sampling and data collection

To assess the GAD, 25 plants were randomly selected along two diagonals across the field at each survey site. The PDI of anthracnose disease of green gram was calculated by the using the formula given earlier and by referring the scale (1-9) given in Table 1 (12, 13).

$$\text{PDI} = \frac{\text{Sum of numerical disease rating}}{\text{Total no. of samples} \times \text{maximum disease grade}} \times 100$$

Isolation and identification of associated pathogens in the laboratory

Isolation of the putative pathogen was done from the leaves showing the typical diseased symptoms i.e., sunken lesions with black margins which were collected from each site. Diseased leaf tissues were excised into small pieces and then surface sterilized for 2 min in 1 % sodium hypochlorite solution, rinsed in 3 changes of sterile distilled water and dried on sterilized filter paper before inoculating on solidified PDA (potato dextrose agar) media. The inoculated plates were incubated at $27 \pm 2^\circ\text{C}$ for 5 - 7 days and further pure culture was done by hyphal tip method. Confirmation of *Colletotrichum truncatum*, the causal pathogen of GAD was carried out based on morphological and cultural characteristics on PDA and microscopic observation following the fungi identification key like ellipsoidal conidiophore and falcate shape conidia (14). The samples were observed under phase contrast microscope (Magnus MX21iLEDFS11) at 100X magnification for presence of *C. truncatum* based on the type of mycelium, fruiting structure, shape and size of conidia (14).

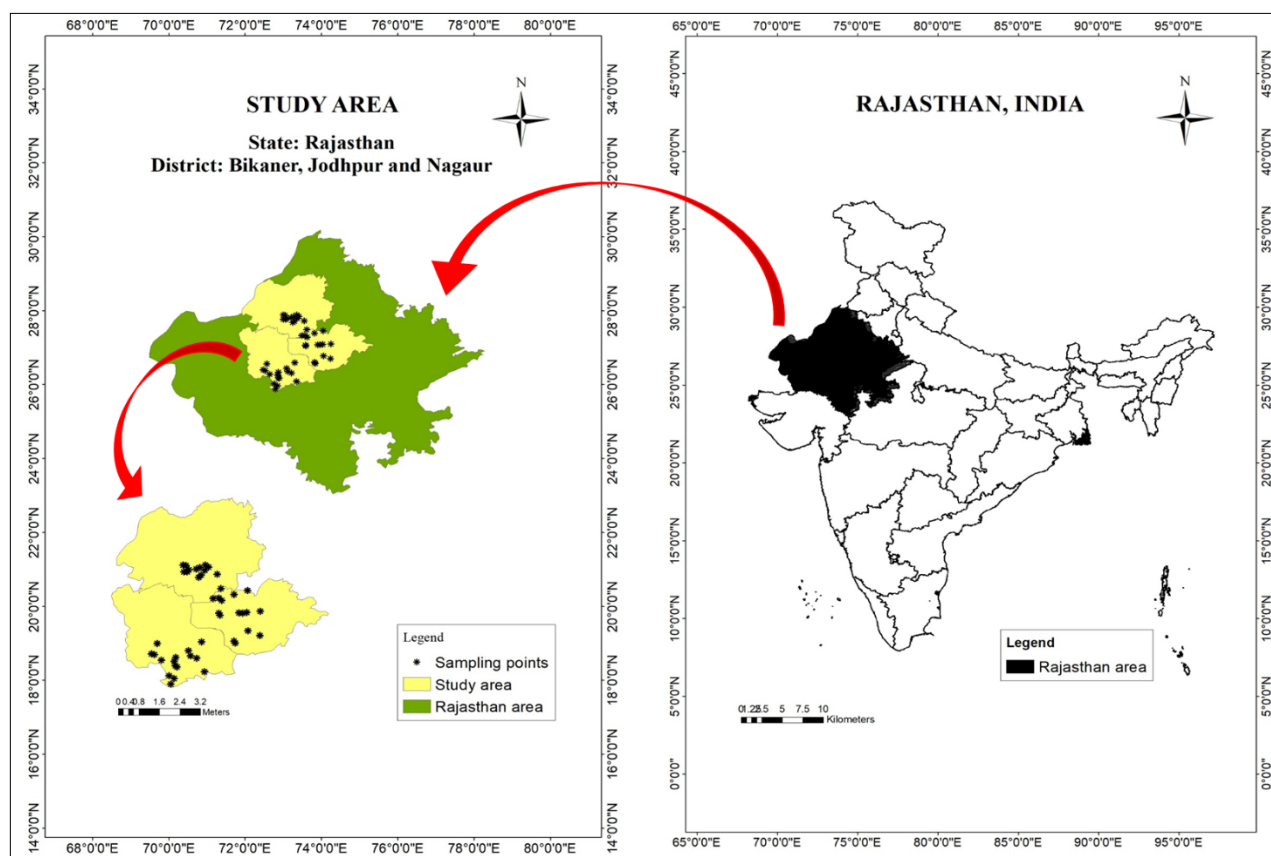


Fig. 1. Map of India showing the locations in arid ecosystem where GAD surveys were conducted in 2023-24 and 2024-25.

Table 1. PDI against anthracnose disease with PDI range and severity ratings

Severity ratings	Symptoms and lesion type on the leaves	PDI range
1	0 to <1 % leaf area covered with hypersensitive reaction with mild yellow flecks	0 to 20 %
2	1-5 % leaf area covered with hypersensitive lesions without acervuli	21 to 35 %
3	5.1-10 % leaf area covered with hypersensitive lesions without acervuli	36 to 45 %
4	10.1-20 % leaf area covered with hypersensitive and restricted necrotic lesions with acervuli	
5	20.1-30 % leaf area covered with hypersensitive and restricted necrotic lesions with acervuli	46 to 70 %
6	30.1-40 % leaf area covered with coalescing necrotic lesions with acervuli	
7	40.1-50 % leaf area covered with coalescing necrotic lesions with acervuli	More than 70 %
8	50.1-75 % leaf area covered with coalescing necrotic lesions with acervuli	
9	75.1-100 % leaf area covered with coalescing necrotic lesions with acervuli	

Data analysis

Prevalence of GAD within each village was summarized as the percentage of surveyed fields in the village with disease symptoms. The number of farmers' fields in each severity category was expressed as a percentage of total infected fields to obtain severity frequency distributions. The Pearson correlation analysis was carried out between weekly PDI and weather parameters such as T_{max} , T_{min} , RHM, RHE and RF using SPSS software.

The meteorological data of the whole survey period of two *Kharif* seasons were obtained from ARS, SKRAU, Bikaner; ICAR-CAZRI, Jodhpur and District Collector Office, Nagaur and Krishi Vigyan Kendra, Athiyasan, Nagaur, Rajasthan, India.

Results and Discussion

In 2023-24, it was observed that PDI was in the range of 25.02 %-31.85 % across all the three surveyed districts. Among all the surveyed villages of Bikaner district, maximum PDI (44.67 %) was recorded in the 30th standard meteorological week (SMW) of July in Biramsar village of Nokha tehsil which was followed by Kawani (43.98 %) of Bikaner tehsil and minimum PDI of 7.18 % was observed in 27th SMW of July in Inda Ka Bala village of Kolayat tehsil. The data on PDI of anthracnose disease of green gram in Jodhpur district revealed that both maximum and minimum disease index of 46.50 % and 2.92 % were recorded in Agolai village of Balesar tehsil in 30th and 27th SMW of August and July respectively. In Nagaur district, the maximum disease index of 52.81 % was observed in Bagar village of Merta tehsil in 31st SMW of August and minimum (4.84 %) was observed in Surpaliya village of Jayal tehsil in 27th SMW of July. It was also observed that the mean PDI of Nagaur district was 31.85 % which was comparatively higher than that of other two districts (Table 2).

Similarly, in 2024-25, the PDI was in the range of 28.40-35.19 % across all the three surveyed districts. In Bikaner district, the maximum disease index was also observed in

Biramsar village of Nokha tehsil with disease index of 51.64 % and minimum was observed in Inda Ka Bala village of Kolayat tehsil with disease index of 8.26 % in 31st and 27th SMW of August and July respectively. Likewise, in all the surveyed villages of Jodhpur district, the maximum disease index (47.39 %) was observed in Doli village of Luni tehsil in 31st SMW of August and minimum (8.35 %) was observed in Agolai village of Balesar tehsil in 27th SMW of July. In Nagaur district, maximum and minimum PDI was observed in Igyar village (57.78 %) and Surpaliya village (11.08 %) of Jayal tehsil in 32nd and 27th SMW of August and July, respectively. It was also noticed that the comparatively higher mean PDI was observed in Nagaur district which was 35.19 % (Table 2).

Correlation and multiple linear regression analysis between PDI of anthracnose disease of green gram and weather parameters

Effect of weather parameters on the development and spread of anthracnose disease of green gram

Understanding how weather conditions influence the disease is essential to weather condition is necessary to organize Agro Advisory Services (AAS) for the farmers to take up management practices in advance. The data highlighted in Table 3 showed that in Bikaner district, the anthracnose symptoms were first observed on 27th SMW, when the crop was at 11-17 and 15-21 DAS in 2023 and 2024 respectively. The severity increased slowly and reached as high as 40.78 % and 44.89 % during 30th and 31st SMW of 2023 and 2024 respectively. In Jodhpur district, the appearances of first symptoms were observed when the crop was also at 11-17 and 15-21 DAS in 2023 and 2024 respectively. The disease severity increased slowly and reached maximum with disease index of 37.90 and 43.11 % during 30th and 31st SMW of 2023 and 2024 respectively (Table 4). Similarly, in Nagaur district, the data presented in Table 5 indicated that the anthracnose symptoms were first observed on 27th SMW, when the crop was at 11-17 and 15-21 DAS in 2023 and 2024 respectively. The disease severity increased slowly as the pathogen got favourable condition for its multiplication and the disease reached its maximum with disease index of 45.94 and

Table 2. District wise comparison of PDI of anthracnose of green gram during survey in *Kharif* 2023-24 & 2024-25

Districts	PDI in three districts in the standard meteorological week (SMW) of July-August								Mean
	27	28	29	30	31	32	33	34	
2023									
Bikaner	9.28	20.98	33.28	40.78	36.68	30.08	25.48	22.28	27.36
Jodhpur	6.60	17.70	23.70	37.90	36.56	31.95	26.32	19.42	25.02
Nagaur	9.14	23.54	27.84	41.94	45.94	41.14	29.64	35.64	31.85
2024									
Bikaner	10.36	22.64	28.36	32.01	44.89	38.45	33.56	27.56	29.73
Jodhpur	12.03	16.45	23.24	32.08	43.11	39.16	34.12	27.01	28.40
Nagaur	15.38	26.12	30.49	35.00	43.05	51.08	42.36	38.06	35.19

Table 3. Effect of weather parameters in relation to anthracnose disease of green gram at Bikaner district during *Kharif* 2023-24 & 2024-25

SMW*	Stage of crop (days)**	Weekly PDI	2023					Total RF (mm)	Stage of crop (days)**	Weekly PDI	2024					Total RF (mm)
			Temperature (°C)		Relative humidity (%)		Max.				Min.	Temperature (°C)		Relative humidity (%)		
			Max.	Min.	Mor.	Eve.						Max.	Min.	Mor.	Eve.	
27	11-17	9.28	38.92	25.90	74.85	46.14	26.4	15-21	10.36	39.40	25.81	74.57	50.43	52		
28	18-24	20.98	37.91	25.70	80.57	49.71	127.6	22-28	22.64	39.77	26.04	92.86	94.71	44.4		
29	25-31	33.28	37.72	25.87	80.28	55.85	35.8	29-35	28.36	41.14	28.44	96.71	96.57	0		
30	32-38	40.78	34.94	25.21	88.71	67.71	47.6	36-42	32.01	39.03	27.14	98.43	98.14	38		
31	39-45	36.68	35.92	25.45	82.85	54.57	0.2	43-49	44.89	34.97	24.59	94.29	91.29	69		
32	46-52	30.08	35.15	25.27	77.14	49.71	0	50-56	38.45	33.56	25.13	96.57	88.00	65		
33	53-59	25.48	36.41	25.27	75.57	47.14	0	57-63	33.56	33.61	24.93	94.29	87.43	44.6		
34	60-66	22.28	36.61	25.77	77.42	46.28	0	64-70	27.56	35.67	25.66	97.57	95.57	0		

*SMW= Standard meteorological week **Days after sowing

Table 4. Effect of weather parameters in relation to anthracnose disease of green gram at Jodhpur district during *Kharif* 2023-24 & 2024-25

2023										2024					
SMW*	Stage of crop (days)**	Weekly PDI	Temperature (°C)		Relative humidity (%)		Total RF (mm)	Stage of crop (days)**	Weekly PDI	Temperature (°C)		Relative humidity (%)		Total RF (mm)	
			Max.	Min.	Mor.	Eve.				Max.	Min.	Mor.	Eve.		
27	11-17	6.6	37.44	28.00	78.43	52.29	2.20	15-21	12.03	37.64	28.59	76.86	48.43	14.8	
28	18-24	17.7	35.26	27.01	84.00	58.14	16.20	22-28	16.45	38.26	28.61	75.14	43.71	14.9	
29	25-31	23.7	36.79	27.60	81.86	50.71	69.10	29-35	23.24	39.13	28.73	82.43	49.00	3	
30	32-38	37.9	34.56	27.33	86.14	62.14	23.10	36-42	32.08	36.17	28.53	85.71	69.29	25.6	
31	39-45	36.56	33.01	26.41	85.86	59.70	0.00	43-49	43.11	33.93	26.84	87.71	73.29	111.9	
32	46-52	31.95	33.34	25.90	81.29	57.98	0.00	50-56	39.16	30.54	25.79	90.14	70.57	60.7	
33	53-59	26.32	33.74	25.73	81.14	56.89	0.00	57-63	34.12	30.41	25.71	91.57	77.43	124.3	
34	60-66	19.42	34.33	26.19	80.57	54.00	10.30	64-70	27.01	33.17	25.81	87.29	67.57	99.8	

*SMW= Standard meteorological week **Days after sowing

Table 5. Effect of weather parameters in relation to anthracnose disease of green gram at Nagaur district during *Kharif* 2023-24 & 2024-25

2023										2024					
SMW*	Stage of crop (days)**	Weekly PDI	Temperature (°C)		Relative humidity (%)		Total RF (mm)	Stage of crop (days)**	Weekly PDI	Temperature (°C)		Relative humidity (%)		Total RF (mm)	
			Max.	Min.	Mor.	Eve.				Max.	Min.	Mor.	Eve.		
27	11-17	9.14	34.29	26.14	84.00	62.29	65.14	15-21	15.38	31.86	27.57	86.00	74.29	31.71	
28	18-24	23.54	34.00	26.86	87.57	65.71	150.86	22-28	26.12	34.00	28.29	84.60	66.14	53.29	
29	25-31	27.84	33.43	27.00	87.92	68.00	118.14	29-35	30.49	34.14	30.71	85.43	66.57	18.29	
30	32-38	41.94	32.71	27.14	89.29	71.43	159.00	36-42	35.00	32.43	28.43	90.12	78.14	68.43	
31	39-45	45.94	31.86	25.57	88.57	72.14	36.86	43-49	43.05	30.86	27.29	91.14	79.86	209.00	
32	46-52	41.14	32.43	26.42	86.86	69.71	0.00	50-56	51.08	26.57	26.00	92.57	94.14	67.71	
33	53-59	29.64	33.29	26.21	85.65	61.14	1.86	57-63	42.36	30.71	26.71	91.86	80.00	169.29	
34	60-66	35.64	32.86	24.31	87.00	63.29	34.71	64-70	38.06	30.43	27.14	91.14	80.14	40.00	

*SMW= Standard meteorological week **Days after sowing

51.08 % during 31st and 32nd SMW of 2023 and 2024 respectively. The data indicated that 27th and 28th standard weeks were highly favourable for the first appearance of the disease in all three surveyed districts during both the years. The weather factors play an important role in predisposing of disease. It gives information to design supervisory control measures of disease to get good yield.

Correlation and multiple linear regression analysis between PDI of anthracnose disease of green gram in relation to weather parameters

The influence of meteorological variables such as T_{max} , T_{min} , RHM, RHE and RF were statistically correlated with the PDI of anthracnose disease of green gram of three surveyed districts in both 2023-24 and 2024-25 as shown in Table 6.

It is evident that the correlation analysis showed positive significant correlation of PDI with RHM (0.7769 &

0.7287) and RHE (0.8030 & 0.6366) while, negatively correlated with T_{max} (-0.7734 & -0.6281) for Bikaner district in 2023-24 and 2024-25, respectively. The correlation between PDI of Jodhpur district and weather parameters revealed the positive significant of PDI with RHM (0.7622 & 0.8745) and RHE (0.7292 & 0.8782) while, negatively correlated with $T_{(Max)}$ (-0.7417 & -0.7235) in 2023-24 and 2024-25 respectively. Similarly, correlation analysis showed positive significant of PDI with RHM (0.7581 & 0.8610) and RHE (0.7165 & 0.7573) while, negatively correlated with T_{max} (-0.9547 & -0.7152) for Nagaur district during 2023-24 and 2024-25, respectively.

The multiple linear regression of PDI in relation to weather parameters of three surveyed districts (Table 7) indicated that the regression coefficients for T_{max} , T_{min} , RHM, RHE and RF were found to be -4.096, 5.664, 0.411, 0.585 and -0.022, respectively in 2023 and -4.784, 8.158, -1.684, 1.121 and

Table 6. Correlation between PDI of anthracnose of green gram in relation to weather parameters of three surveyed districts in 2023-24 & 2024-25

S. No.	Weather parameters	Correlation coefficient (r)					
		Bikaner		Jodhpur		Nagaur	
		2023	2024	2023	2024	2023	2024
1.	Maximum temperature (°C)	-0.7734*	-0.6281*	-0.7417*	-0.7235*	-0.9547*	-0.7152*
2.	Minimum temperature (°C)	-0.6017	-0.3216	-0.4318	-0.6473	-0.1528	-0.5022
3.	Relative humidity morning (%)	0.7768*	0.7287*	0.7622*	0.8745*	0.7581*	0.8610*
4.	Relative humidity evening (%)	0.8030*	0.6366*	0.7292*	0.8782*	0.7165*	0.7573*
5.	Total rainfall (mm)	-0.1418	0.2862	-0.0059	0.6816	-0.1781	0.5264

**significant at 1 % probability, * significant at 5 % probability

Table 7. Multiple linear regression coefficients and equations for anthracnose disease of green gram in relation to weather parameters of three surveyed districts in 2023-24 & 2024-25

Districts	Weather parameters and equations							
	Constant (a)	X ₁	X ₂	X ₃	X ₄	X ₅	R	R ²
2023								
Bikaner	-29.649	-4.096	5.664	0.411	0.585	-0.022	0.917	0.841
	-29.649 - 4.096 X ₁ + 5.664 X ₂ + 0.411 X ₃ + 0.585 X ₄ - 0.022 X ₅							
Jodhpur	277.316	-15.350	16.412	-2.905	1.350	0.382	0.977	0.955
	277.316 -15.350 X ₁ + 16.412 X ₂ - 2.905 X ₃ + 1.350 X ₄ + 0.382 X ₅							
Nagaur	268.782	-14.519	2.007	2.925	-0.950	-0.003	0.991	0.983
	268.782 - 14.519 X ₁ + 2.007 X ₂ + 2.925 X ₃ - 0.950 X ₄ - 0.003 X ₅							
2024								
Bikaner	45.197	-4.784	8.158	-1.684	1.121	0.225	0.934	0.872
	45.197 - 4.784 X ₁ + 8.158 X ₂ - 1.684 X ₃ + 1.121 X ₄ + 0.225 X ₅							
Jodhpur	-154.676	-0.043	2.299	1.175	0.340	0.020	0.899	0.809
	-154.676 - 0.043 X ₁ + 2.299 X ₂ + 1.175 X ₃ + 0.340 X ₄ + 0.020 X ₅							
Nagaur	-116.092	-5.866	5.045	3.025	-1.001	0.039	0.924	0.853
	-116.092 - 5.866 X ₁ + 5.045 X ₂ + 3.025 X ₃ - 1.001 X ₄ + 0.039 X ₅							

X₁ = T_{max} (°C), X₂ = T_{min} (°C), X₃ = RHM (%), X₄ = RHE (%), X₅ = RF (mm), R² = Coefficient of determination

0.225, respectively during 2024 in Bikaner district. In Jodhpur district it was found that the regression coefficients for T_{max}, T_{min}, RHM, RHE and RF were found to be -15.350, 16.412, -2.905, 1.350 and 0.382, respectively in 2023 and -0.043, 2.299, 1.175, 0.340 and 0.020, respectively during 2024. Similarly, the multiple linear regression analysis of PDI in relation to weather parameters of Nagaur district revealed the respective regression coefficients for, T_{max}, T_{min}, RHM, RHE and RF were found to be -14.519, 2.007, 2.925, -0.950 and -0.003 in 2023 and -5.866, 5.045, 3.025, -1.001 and 0.039 in 2024. The multiple linear regression equations were fitted to the data and the equation arrived for the weather parameters was mentioned in Table 7.

The present investigation on PDI of anthracnose disease of green gram was conducted in three districts of Rajasthan, India (Bikaner, Jodhpur and Nagaur) during *Kharif* 2023-24 and 2024-25. The study revealed that the disease was evident in all the surveyed villages of all three districts for two consecutive years. The observed PDI were in the range of 25.02 %-31.85 % in 2023-24 and 28.40 %-35.19 % in 2024-25 among all the surveyed districts. It was also observed that the mean PDI of Nagaur district was 31.85 % and 35.19 % during 2023-24 and 2024-25 respectively which was comparatively higher than other two districts. The probable reason for higher PDI in 2024-25 than 2023-24 among all the surveyed districts maybe due to prevailing environmental conditions of warm and humid weather. A roving survey during *Kharif* 2013-14 in major green gram regions of Northern Karnataka revealed widespread anthracnose incidence, with highest severity in Bidar, followed by Gulbarga and Bijapur (9). The present study was also supported by the work conducted in farmers' markets of 6 districts in Tamil Nadu during August-September 2012, where the maximum mean PDI of *C. lindemuthianum* ranged from 20.04 % to 21.52 % (15).

Influence of weather parameters on PDI of anthracnose disease of green gram

The investigated results between correlation of PDI of anthracnose of green gram (GAD) and five weather factors (T_{max}, T_{min}, RHM, RHE and RF) showed that the PDI in all the three districts i.e., Bikaner, Jodhpur and Nagaur was positively correlated with RHM and RHE, negatively correlated with T_{max}. The recorded average morning relative humidity of all the three

surveyed districts for 2 years was ranged between 83.06-88.96 % and average RHE of all the three surveyed districts for two years ranged between 58.44 %-75.86 %. These findings indicate that morning and RHE are key environmental factors influencing disease development. A previous experiment also demonstrated a statistically significant relationship between PDI and RHM/RHE in Karnataka, India (16). The present study was also supported by this work, which reported that rainfall, T_{min}, rainy days and RHM significantly influenced disease development, while T_{max}, RHE and sunshine hours had no significant effect under the Tarai zone of Uttarakhand (1).

Conclusion

From the present study, it can be concluded that GAD is likely to become a major problem as green gram production is rapidly increasing in arid and semi-arid regions of India. Disease resistant cultivars remain the most feasible approach to control of green gram diseases, but there is an urgent need to intensify the introduction of new improved GAD resistant cultivars to these regions where local landraces are more susceptible to disease were predominant in the surveyed fields. The observed distribution patterns of GAD disease in the surveys indicated a clear association between disease severity and specific weather parameters. Correlation analysis revealed that among T_{max}, T_{min}, RHM, RHE and RF, both RHM and RHE showed a positive relationship with disease severity across all surveyed locations and green gram-producing regions.

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Authors' contributions

KP designed the methodology and conducted the experiment as a survey. KP wrote the original manuscript. ALY provided the main concepts. ALY supervised the work. AK analysed and interpreted the data. PD planned the experiment. ARS helped with data analysis. ST

participated in the design of the study. JS provided concepts. RJ and JS wrote part of the original manuscript. ALY, AK, PD, ARS reviewed and gave final approval of the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical issues: None

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