PREVALENCE OF GREEN GRAM FUNGAL DISEASES IN THE EASTERN PART OF KENYA

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ABSTRACT

Green gram (*Vigna radiata* L.), also known as Mung bean, is an annual legume crop that is grown all over the world. Green gram yield in the arid and semi-arid lands of Kenya is still low due to various factors including plant diseases and poor soils. The objective of this study was to evaluate prevalence of green gram fungal diseases and the effects of soil factors on their occurrence in Tharaka-Nithi and Kitui Counties in Kenya. The study was conducted in Kitui Central and Kyangwithya wards in Kitui County, and Mukothima and Nkondi wards in Tharaka-Nithi County. Household surveys were conducted using a semi-structured questionnaire. Farm surveys were carried out using transects to evaluate disease incidence and severity. Symptomatic green gram samples were collected for isolation of pathogens. Isolates were identified based on morphological features and cultural characteristics. Soil samples were collected from farms and analyzed for physicochemical properties. Eight fungal diseases with diverse morphological and cultural characteristics were identified. The diseases were Downy mildew, Powdery mildew, Cercospora leaf spot, Macrophomina rot, Anthracnose, Late blight, Corynespora spot and Angular spot. Disease severity and incidences ranged from 6.13 to 32.2% and 16.67 to 67.79%, respectively. Powdery mildew, caused by *Erysiphe polygoni* DC, was the most prevalent disease with an incidence and severity mean of 59.09% and 30.92%, respectively. This could be attributed to susceptibility of the local cultivar, soil conditions and crop management practices. Soil physicochemical properties also varied among the wards and had a significant correlation with incidence and severity of diseases. These results could be useful in mapping green gram diseases and developing sustainable management options.

Key words: Green grams; incidence; morphological characterization; severity; soil factors

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INTRODUCTION

Green gram (*Vigna radiata L.*), also known as Mung bean, is an annual legume crop that belongs to Fabaceae family. The crop is cultivated in many countries of Europe, Asia, Africa and America (Sequeros *et al.,* 2021). Green gram is majorly produced in South Asia, where India is the largest producer among the countries that grow green gram (Nair and Schreinemachers, 2020). The crop provides significant amounts of nutrients in human diets such as potassium (K), sodium (Na), vitamins, iron (Fe), magnesium (Mg) and calcium (Ca), and is an excellent source of protein (Anwar *et al*., 2007). Green gram is known to fix atmospheric nitrogen (N) that enriches soils hence increases productivity and resilience of cropping systems (Keatinge *et al*., 2011). Unlike other crops, the yields are likely to increase substantially in the future as a result of climate change during both the long and short rainy seasons (Demissie *et al*., 2019). This ability enables green gram to be a preferential crop for climate-smart agriculture (CSA).

Kenya is the seventh largest global producer of green grams and the second leading producer in East Africa (Karimi *et al.,* 2019). The crop is a central income generating value chain in the arid and semi-arid areas (ASALs) of Kenya, ranking second after indigenous chicken (AgriFI CSAP, 2021). Up to 90% of green gram farming is carried out in the ASALs of Kitui, Makueni, Mbeere, Machakos, Tharaka-Nithi, and Meru Counties that depend on rain-fed agriculture (Kilimo, 2017; Karimi *et al.,* 2019; Sequeros *et al.,* 2021). More than 260,000 ha of land are under green gram cultivation mostly by small-scale farmers (Kilimo, 2017; Nzuma, 2020).

Although green gram has great economic significance for the Kenyan ASALs, its yields are still low. The potential yield of green gram should be 300-1500 kg/ha (Karanja *et al.,* 2006). However, the actual yield has been reported to be 30-416 kg/ha (Kimiti *et al.,* 2009), and the productivity continues to decline despite increase in production area (Karimi *et al.,* 2019). The production is yet to meet the local market demand as Kenya still has a deficit of

3,987 tonnes (Kilimo, 2017; Nzuma, 2020). The low production of green grams may be attributed to various abiotic and biotic constraints, especially fungal diseases and poor soils (Gharde *et al.,* 2018). Most common fungal diseases reported include cercospora leaf spot (Yundaeng *et al*., 2020), powdery mildew (Pandey *et al.*, 2018), anthracnose (Marak *et al.,* 2020), dry root rot and leaf blight (Sundaramoorthy *et al.,* 2013). The severity of yield loss may depend on the time of infection and crop stage at which the diseases attack occurs. Disease severity and incidence may be influenced by the cultivar type, planting period and soil conditions (Teferie *et al.,* 2020).

Limiting soil factors affect the survival of pathogen's propagules and limit or affect disease development (Agrios, 2005). These factors have effects on the number of propagules produced by the pathogen to initiate the infection process (Das *et al.,* 2016). Soil physico-chemical properties and nutrients influence fungal disease infection (Dordas, 2008). For instance, soil pH has a significant impact on the occurrence of soil-borne fungal diseases and is critical to the activity of soil amendment (Bailey and Lazarovits, 2003). It also influences the solubility, mobility, and bioavailability of trace elements. Depending on the fungal pathogen, the amount of micronutrients may influence pathogenicity (Marschner 1995; Huber *et al.* 2012). Calcium (Ca) deficiency for instance is associated with a weak middle lamella and susceptibility of the crop to diseases (Singh *et al.*, 2007). However, Ca treatment significantly reduces fungal spore germination and mycelial growth (Dordas, 2008, Huber *et al.* 2012). Sufficient Ca in the soil reduces incidences of fungal diseases such as damping-off, botrytis and anthracnose in different crops (Campbell and Arthur, 1990).

Adequate K in the soil minimize the incidence of fungal diseases such as the late blight caused by *Phytophthora infestans* (Mont.) de Bary, dry rot caused by *Fusarium* Link, early blight associated with *Alternaria solani* Sorauer, and powdery scab caused by *Spongospora subterranean* (Wallr.) Lagerh (Mitchell and Walters, 2004; Liljeroth *et al.,* 2016). Reduced N availability in many crops including green grams may enhance susceptibility to fungal diseases (Mitchell and Walters, 2004). The amount and quality of soil organic matter can also alter plant nutritional status, which can affect fungal disease incidence by enhancing plant resistance, improving plant development and influencing the pathogen's habitat (Stone *et al*., 2004). Knowledge of these factors and how they influence the development of a particular fungal disease in a given ecosystem are necessary towards the formulation of management strategies (Das *et al*., 2016).

The objective of this study was therefore to evaluate green gram fungal diseases and the effects of soil factors on their occurrence in Tharaka-Nithi and Kitui Counties in Kenya.

MATERIALS AND METHODS

Study area

This study was carried out in Tharaka-Nithi (0.2965° S, 37.7238° E) and Kitui (1.6833° S, 38.3166° E) Counties located in the ASALs of Kenya (Figure 1). The 2 Counties were purposively selected due to their great contribution to green gram production in Kenya. Tharaka-Nithi County receives an average annual rainfall of about 700 mm. Kitui County experiences high temperatures throughout the year, which ranges from 14 ^{o}C to 34^{o} C. The county is elevated at an altitude that ranges between 400-1800 meters above sea level and experiences an average annual rainfall of about 750 mm. Most farmers in these two Counties depend on the short rainy season for agricultural productivity.

Sampling and data collection

Four wards were purposively selected, 2 in Kitui (Kitui-Central and Kyangwithya wards) and 2 in Tharaka-Nithi County (Mukothima and Nkondi wards). In each of the wards, 25 farms were purposively selected giving a total sample size of 100. Out of the 100 farms, 6 farms were randomly selected from each ward for the actual farm survey on green gram diseases giving a total of 24 farms. Household survey was carried out between April to June, 2021 when the crop was about 1-2½ months old. Green gram farms were surveyed for actual disease occurrence by walking along transects in 2 diagonals (X-Fashion) according to Panduranga *et al.* (2012). Five plots measuring 0.5 m × 0.5 m were established along transect at equidistance of 5 m. In every plot, plants were assessed for the occurrence of diseases on foliar and stem. Where symptoms of wilting of root rot were observed, the whole plant was uprooted and assessed further. Disease severity was evaluated for every plot established and scored using different suitable scales. Symptomatic green gram samples were collected for pathogen isolation in the laboratory.

Diseases severity and incidence

Incidence of disease was done differently depending on the type of disease. This was according to Ramakrishnan and Savithramma (2014), Kumar *et al.* (2020), Ahmad *et al.* (2020), Sandeep *et al.* (2014) and Moore (2019). Disease incidence was calculated using the formula:

$$DI = \frac{ND}{N} x100$$

where *DI* = disease incidence, *ND*= number of disease plants and *N* = total number of plants assessed.

Disease severity was scored using different scale systems. Powdery mildew and downy mildew were scored on a scale of 0-9 according to Mallaiah *et al.* (2016). Cercospora leaf spot was scored using a scale of 1-5 (Ngegba *et al.,* 2017). Charcoal rot (Macrophomina rot) and Corynespora spot were scored on a scale of 0-5 (Fang *et al.,* 2011). Scoring for anthracnose disease was on a scale of 1-6 while Angular leaf spot was scored on a scale of 0-9 (Chaudhari and Gohel, 2016).

Morphological characterization of fungal pathogens of green gram diseases

The collected samples were cleaned to remove debris, then symptoms were observed and described. The samples were then stored at 4 °C awaiting pathogen isolation. About 40 g of potato dextrose agar (PDA) media was prepared and dispensed in Petri dishes. Sections of the diseased plant samples (about 3mm in size) were cut using a sterilized blade. The sections were cleaned in distilled water containing 3.5% Sodium Hypochlorite (NaOCl) then rinsed with distilled water (dH_2O). They were surface sterilized in 70% ethanol for 2 minutes, passed in dH_2O once again, and then dried using sterile blotting paper. The dried diseased section was aseptically placed on the surface of dried culture media on petri dishes and incubated at room temperature (approximately 20 °C).

Pure isolates of green gram fungal pathogens were obtained by culturing individual colonies in fresh media. The isolates were identified based on morphological features as observed under the microscope and cultural characteristics as observed during fungal growth. Morphological characteristics observed included conidia width and length, number of septa on the conidia and spore colour. Cultural characteristics comprised of type of margin (entire, regular or irregular) and rate of mycelia growth.

Evaluation of soil factors

Crop residues were removed from the soil surface prior to soil collection. Soil samples were then collected using sterile soil auger using zigzag soil sampling method. The auger was cleaned after every collection to ensure no mixing of soil nutrients from one point to the other. The soils collected for an individual farm were mixed to form a homogeneous mixture. From the mixture, about 0.5 kg of soil was packed in zip-lock polyethylene bags, labelled accordingly and transported to Chuka University soil laboratory for analysis. In the laboratory the samples of soil were dried at room temperature and ground to powder. They were analyzed using a soil scanner (Model SC0677D)**.** The scanner's probes were first cleaned and calibrated according to the manufacturer's instructions. Five scans were done per sample and printed out. The soils were analyzed for soil pH, N, P, K, cation exchange capacity and carbon (C) content.

Data analysis

Data were subjected to analysis of variance (ANOVA) using Statistical Analysis system (SAS) statistical software (version 9.4; SAS Institute). Pairwise comparison of means was conducted using the Least Significance Difference (LSD) at $P \leq 0.05$. Data from questionnaires were coded and analyzed using Statistical Package for Social Sciences (SPSS) version 21. Conidia sizes of individual isolates were compared using General Linear Model (GLM) and significant means separated using Least Significant Difference (LSD) at $P \leq 0.05$. Pearson correlation coefficient (*r*) was computed to determine the association between disease parameters and soil factors.

RESULTS

Incidence and severity of green gram diseases

Based on farmers' response, there was a significant correlation (r = 0.621***) between disease occurrence and climate conditions across the wards. The highest disease occurrence of 54.08% was observed during high humidity conditions, followed by wet conditions (33.67%). The least occurrence was observed during hot and dry conditions (2.04%). As per the wards, the highest occurrence of diseases (27.55%) was observed in Mukothima ward in Tharaka-Nithi County while the lowest proportion of 23.47% was observed at both

Kyangwithya in Kitui County. Several diseases and symptoms were mentioned by the farmers. These were anthracnose, powdery mildew, blight, dumping off, spots, rots and wilting. Majority of the farmers reported mild severity of anthracnose (32%), late blight (12%), leaf spot (12) and wilting (12%). For powdery mildew, majority of the farmers (48%) indicated severe presence of the disease, while about 23% reported mild disease severity.

Disease incidence

A total of eight diseases were identified. These were downy mildew, powdery mildew, cercospora leaf spot, macrophomina rot, Anthracnose, Late blight, corynespora spot and angular spot. Their causal agents are shown in Table I.

Nithi County (Table II). The most common disease was powdery mildew (mean of 59.09%) followed by Downy mildew (56.94%). The highest incidence of powdery mildew (65.85%) was reported in Mukothima while that of Downy mildew was reported in Nkondi (67.79%).

Disease severity.

Similar to disease incidence, powdery mildew showed the highest disease severity with a mean of 30.92%, followed by downy mildew (28.54%). The highest incidences of the both diseases were reported in Mukothima (32.2%) and Nkondi (31.87%), respectively (Table 3). There was a high variation in disease severity among the wards. Mukothima ward had the highest severity of powdery mildew (32.2%).

TABLE I -FUNGAL GREEN GRAM DISEASES AND THEIR CAUSAL AGENTS IN THARAKA-NITHI AND KITUI COUNTIES.

No.	Green gram diseases	Causative agent
	Downy mildew	*Hyaloperonospora parasitica* (Pers.) Constant.
	Powdery mildew	*Erysiphe polygoni* DC.
	Cercospora leaf spot	*Cercospora canesens* Fresen.
	Macrophomina rot	*Macrophomina phaseolina* (Tassi) Goid.
	Late blight	*Colletotrichum* sp.
	Anthracnose	*Colletotrichum* sp.
	Corynespora spot	*Corynespora* sp.
	Angular leaf spot	*Phaeoisariopsis griseola* (Sacc.) Ferraris.

There was a significant ($P<0.05$) difference due to incidence for Downy mildew, Macrophomina root rot, Cercospora spots and anthracnose. However, the highest proportion of incidence of Downy mildew was 67.79%, Cercospora spots, 54.67%, Anthracnose, 58.68%, late blight, 58.9% and Angular spot, 56% in Nkondi ward in Tharaka-Nkondi had the highest severity of downy mildew (31.87 %) and late blight (17.87%). Kyangwithya had the highest severity of Cercospora spot (18.53%), Corynespora spot (19.06%) and Angular spot (21.25% while Kitui-Central had the highest severity Anthracnose (22.67%) (Table III).

TABLE II - INCIDENCE OF GREEN GRAM FUNGAL DISEASES (%) IN THARAKA-NITHI AND KITUI COUNTIES.

Ward	PD	DmD	Cerc	Mac	Anthr	LBT	Coryn	Ang
Mukothima	65.85	45.62 [b]	32.67 [b]	16.67 [c]	40.0 [b]	42.0 [b]	32.0 [b]	45.33
Kyangwithya	59.35	59.38 [a]	52.67 [a]	33.33 [b]	56.667 [a]	46.67 [b]	52.67 [a]	53.33
Nkondi	56.47	67.79 [a]	54.67 [a]	46.0 [a]	58.67 [a]	58.9 [a]	46.03 [a]	56.0
Kitui Central	55.26	57.24 [a]	54.0 [a]	46.0 [a]	51.33 [a]	39.33 [b]	50.0 [a]	48.66
LSD ($\alpha = 0.05$)	1.234	1.197	1.259	1.301	1.215	1.271	1.178	1.287
Mean (%)	59.09	56.94	48.50	35.50	51.67	46.5	47.0	50.83
Cv (%)	10.077	8.699	11.554	11.965	9.471	12.294	11.832	12.599

[a]Means followed with the same latter are not statistically significant at $\alpha=0.05$. Where PD= Powdery mildew, DmD= Downy mildew, Cerc= Cercospora spot, Mac= Macrophomina rot, Anthr = Anthracnose, LBT= late blight, Coryn= Corynespora spot and Ang= Angular spot

TABLE III- SEVERITY OF GREEN GRAM FUNGAL DISEASES IN THARAKA-NITHI AND KITUI COUNTIES.

Ward	PmD	DmD	Cerc	Mac	Anthra	LBT	Coryn	Ang
Mukothima	32.20	25.233 [b]	9.33 [b]	6.13 [b]	16.0 [b]	12.93 [b]	11.6 [b]	16.8 [b]
Kyangwithya	31.33	29.733 [ab]	18.53 [a]	11.73 [a]	20.8 [ab]	14.53 [b]	19.06 [a]	21.25 [a]
Nkondi	31.47	31.867 [a]	17.33 [a]	16.0 [a]	18.53 [ab]	17.87 [a]	15.6 [b]	18.13 [ab]
Kitui Central	26.67	27.333 [ab]	18.40 [a]	16.0 [a]	22.67 [a]	11.73 [b]	18.0 [a]	17.16 [ab]
LSD (α = 0.05)	1.301	1.244	1.313	1.440	1.323	1.374	1.339	1.322
Means (%)	30.92	28.542	15.9	12.467	19.50	14.267	16.067	18.567
Cv (%)	15.486	13.049	19.647	24.800	18.839	24.484	22.475	19.088

[a]Means followed with the same latter are not statistically significant at α=0.05. Where PD= Powdery mildew, DmD= Downy mildew, Cerc= Cercospora spot, Mac= Macrophomina rot, Anthr = Anthracnose, LBT= late blight, Coryn= Corynespora spot and Ang= Angular spot

Morphological characterization of pathogens

Green gram leaves infected with *Macrophomina phaseolina* appeared to dry out while turning brown and had a charcoal rot and black specks of sclerotia. The vascular system had dark charcoal-like spores. The color of the spores ranged from smoke gray to dark gray, though, in some cases, the initial growth was whitish before turning grayish to blackish. The texture of colonies was either fine or coarse in texture. The mycelium of the isolates had a hyaline to brownish appearance, branched and septate. The lengths and sizes of the sclerotia of the isolates were significantly ($P < 0.05$) different. Conidia length of the isolates ranged from 55.23 to 89.83 µm. The width of the sclerotia did not differ significantly ($p > 0.05$) and ranged from 46.62 µm to 35.7 µm.

Cercospora leaf spot disease symptoms were observed on green gram leaves, stems, pods and petioles. Margins of the lesions were brownish with a whitish center. Growth of the *Cercospora* isolates on PDA was ranged from slow-growing, medium growing to rapid growing colonies. Spore colour was gray or olivaceous green while the mycelia were hyaline and irregular.

The diameter and length of mycelia growth of the *Cercospora canesens* isolates were significantly ($P < 0.05$) different after 2 weeks of growth and ranged from 5.40 to 3.51 cm and 43.96 to 92.75 µm, respectively. However, the width of conidia was not significantly different ($P > 0.05$) and ranged from 3.55 to 3.94 µm. Conidia were septate with septa ranging from 5-8 µm.

Anthracnose disease symptoms caused by *Colletotrichum* species were observed on pods, leaves and the stem. Their characteristic sunkened and circular spots identified the symptoms, with dark centers and red-orange margins. The diameter of mycelia growth of *Colletotrichum* isolates was significantly different after 1 week of growth. The length ranged from 6.16-6.75 cm. The length and sizes of the conidia were significantly ($P < 0.05$) different and ranged from 8.10-11.4 µm. The conidia width and the overall size differed significantly ($P < 0.05$) and ranged from 2.89-4.02 µm and 166.19-456.09 µm, respectively.

The symptoms of the *Corynespora* leaf spot had lesions that had concentric rings with circular to irregular margins. Initially, the necrotic lesions progressed from light brown to dark brown and were surrounded by halo with a dark border. *Corynespora* isolates had greyish colour at the center with whitish margin, brown pigmentation with hyaline and septate *Conidia.* . The isolates differed in their cultural characteristics. Whereas some had rapid growth, others had medium and slow growth. The isolates had profuse spore texture, and the mycelia were septate. The diameter of mycelia growth of the *Corynespora* isolates significantly ($P < 0.05$) differed after 1 week of growth and ranged from 4.27-5.86 cm. The length, sizes and width of the *Corynespora* conidia were significantly ($P < 0.05$) different. *Conidia* length of the isolates ranged from 24.26-150.84 µm while the width ranged from 11.05-19.14 µm.

Soil factors and green gram diseases

Correlation among soil factors showed that soil organic carbon had a significant negative correlation (r = -0.487*) with pH and a positive significant correlation (r = 0.974***) with total N and K (r = 0.724***). However, there was no significant ($P > 0.05$) difference

in soil pH, P and CEC among the 4 wards. The values ranged from 5.4- 5.9 and 0.66-0.78 mg/kg and 123-141 cmol/kg, respectively. There was a significant ($P < 0.05$) difference in soil organic C, N and K among the 4 wards and the values ranged from 16.2-24.02 cmol/kg, 1.23-1.95% and 5.33-10.28 cmol/kg, respectively.

In this study there was no association between soil factors and disease incidence (Table IV). Late blight had a negative significant correlation with pH (r = -0.389; P = 0.0048). However, pH had a positive correlation with disease incidence of Downy mildew, Cercospora spot, Angular spot and Corynespora spot (Table III). There was no association between K with incidence of Downy mildew Powdery mildew and Cercospora spot (Table III). N associated with the incidence of Powdery mildew and Cercospora but a negative correlation with incidence of Downy mildew. Soil organic content on the other hand had a positive correlation with incidence of all diseases except Corynespora spot (Table IV). There was a positive correlation between K and incidence of *Cercospora* leaf spot and *Macrophomina* rot (Table V).

TABLE IV-ASSOCIATION OF SOIL FACTORS WITH INCIDENCE OF GREEN GRAM FUNGAL DISEASES

Soil factor	PmD	DmD	Cerc	Mac	Anthra	LBT	Ang	Coryn
pH	-0.066 (0.643)	0.108 (0.450)	0.087 (0.543)	-0.103 (0.471)	-0.091 (0.521)	-0.389 (0.004)	0.067 (0.639)	0.030 (0.832)
Organic Content	0.147 (0.302)	-0.070 (0.621)	-0.150 (0.292)	-0.049 (0.732)	-0.052 (0.716)	0.166 (0.242)	0.050 (0.726)	-0.101 (0.477)
Total Nitrogen	0.047 (0.740)	-0.102 (0.472)	-0.069 (0.626)	0.011 (0.938)	-0.034 (0.809)	0.228 (0.107)	0.001 (0.997)	-0.102 (0.474)
Total Phosphate	0.163 (0.252)	-0.039 (0.784)	-0.062 (0.665)	-0.240 (0.088)	-0.073 (0.610)	-0.183 (0.198)	0.069 (0.630)	0.029 (0.837)
Potassium	-0.066 (0.642)	-0.194 (0.171)	-0.029 (0.836)	0.021 (0.884)	-0.126 (0.378)	0.093 (0.513)	-0.033 (0.814)	-0.161 (0.256)
Cation exchange	0.023 (0.870)	0.174 (0.221)	0.104 (0.465)	0.041 (0.771)	0.219 (0.122)	0.051 (0.717)	-0.061 (0.670)	0.076 (0.591)

PD= Powdery mildew, DmD= Downy mildew, Cerc= Cercospora spot, Mac= Macrophomina rot, Anthr = Anthracnose, LBT= late blight, Coryn= Corynespora spot and Ang= Angular spot. Values in parenthesis are p values at alpha = 0.05.

TABLE V-ASSOCIATION OF SOIL FACTORS WITH SEVERITY OF GREEN GRAM FUNGAL DISEASES

Soil factor	PmD	DmD	Cerc	Mac	Anthra	LBT	Ang	Coryn
pH	0.048 (0.734)	-0.100 (0.483)	-0.006 (0.962)	-0.032 (0.822)	0.058 (0.684)	-0.331 (0.017)	0.192 (0.176)	0.060 (0.671)
Organic Content	0.197 (0.165)	0.011 (0.934)	-0.122 (0.393)	-0.057 (0.690)	0.016 (0.909)	0.142 (0.319)	0.073 (0.608)	(-0.121) (0.395)
Total Nitrogen	0.079 (0.581)	-0.009 (0.947)	-0.044 (0.758)	0.008 (0.952)	-0.019 (0.893)	0.156 (0.273)	0.125 0.380)	-0.163 (0.252)
Total Phosphate	0.116 (0.415)	-0.114 (0.422)	-0.080 (0.575)	-0.202 (0.154)	0.077 (0.591)	-0.148 (0.297)	-0.004 (0.975)	(0.043) (0.764)
Potassium	-0.045 (0.752)	-0.173 (0.224)	0.036 (0.798)	0.016 (0.907)	-0.081 (0.567)	0.029 (0.836)	0.080 (0.576)	-0.133 (0.350)
Cation exchange	0.164 (0.248)	0.077 (0.587)	0.149 (0.296)	0.022 (0.876)	0.268 (0.056)	-0.002 (0.987)	0.084 (0.557)	-0.056 (0.694)

PD= Powdery mildew, DmD= Downy mildew, Cerc= Cercospora spot, Mac= Macrophomina rot, Anthr = Anthracnose, LBT= late blight, Coryn= Corynespora spot and Ang= Angular spot. Values in parenthesis are p values at alpha = 0.05.

DISCUSSION

Incidence and severity of green gram fungal diseases

There was a significant correlation between disease occurrence and climate conditions. These results are similar to those reported by Jeong and Sung (2010) and Rajashree *et al.* (2020). The two studies reported a positive significant relationship between weather factors (rainfall, relative humidity-RH and temperature) and incidence, distribution, and severity of green gram diseases. Climatic conditions influence the occurrence, prevalence and severity of plant diseases as well as disease management with regard to timing, preference and efficacy of control measures (Juroszek and Von Tiedemann, 2011). The highest disease occurrence was observed during high humidity conditions. This affirms the fact that pathogen spore germination and infection of the host plant often require close to 100% RH (Juroszek and Von Tiedemann, 2011). In addition, changes in weather may modulate host susceptibility or resistance responses to pathogens (Van Maanen and Xu, 2003). In a systematic study estimating green gram disease severity and incidence in Berlin, Campbell and Neher (1994) showed that the severity of Anthracnose, leaf spots, wet rot, Powdery mildew, rust and wilting in green gram corresponded to weather factors. Projected climate change will thus affect the interaction between crops and pathogens as well as management options (Zayan, 2019).

In this study, a total of 8 diseases were identified. Powdery mildew was the most common and has long been known as an important disease of plants in all parts of the world. Sandeep *et al.* (2014) reported powdery mildew incidence range of 15%-63% and severity range of 16%-68%; while Channaveeresh and Shripad (2017) reported severity range of 20.23%-59.73%. This variation may be attributed to the susceptibility of the local cultivar and varying climatic conditions such as temperature, rainfall and drought (Ramakrishnan and Savithramma, 2014). Severity of this disease could be reduced by establishing and planting resistant genotypes (Mandhare and Suryawanshi, 2008).

Cercospora leaf spot is a critical overwhelming fungal disease of green gram that causes a great loss of yield (Kumar *et al.,* 2020). During favorable conditions, the spots increase in size and at the time of flowering and pod formation they lead to severed defoliation. *Cercospora* leaf spot severity in this study varied among the study areas. This range is within that reported by Bhat (2019) of 5.9%-28.4% with a similar variation in all study areas. However, Gunasri *et al*. (2018) reported a very high severity range of 37.77%- 63.89%. The disease can be managed by the application of fungicides (Khunti *et al.,* 2002) (Uddin *et al.,* 2013), but the most appropriate recommendation is the use of resistant green gram varieties (Kumar *et al.,* 2020). The severity range of *Macrophomina* rot in this study ranged from 6.13% to 16%. The symptoms are more severe under dry and warm growing conditions with soil being the primary source of inoculum (Farr and Rossman, 2014). Expression of the disease also depends on the age of the crop and varieties. Very little attention has been paid on the epidemiology and management of this disease, which has become a problem in hindering the production of green gram particularly in the sandy and sandy loam soils (Mehta, 2004). However, *Macrophomina* rot and blight can be managed by the use of chemicals, botanicals and microorganisms such as *Trichoderma* (Ushamalini *et al*., 1997).

Anthracnose disease severity ranged from 16% to 22.67% with an incidence range of 40.0%-58.67%. The severity range is within that reported by Laxman (2006) of 18.2% to 86.57% and by Kulkarni and Benagi (2013) of 19.71% to 60.21%. Variation in weather factors such as rainfall, humidity and the existence of different strains of a pathogen may have contributed to the variation observed (Pastor Corrales and Tu, 1989). Sunil and Raja (2019) reported that the presence of ambient temperature (26 – 30 ^{o}C) and RH range 85-96%) could favour the development, spread and severity of *Anthracnose*. Significance of environmental factors on variation of *Anthracnose* disease intensity has also been reported by Aggarwal *et al*. (2017). Further, possibility of existence of different pathogen races has also been cited for variability of *anthracnose* severity (Kulkarni and Benagi, 2013). To maintain an anthracnose-free environment, management practices that reduce free moisture would restrict the spread of pathogen propagules (Freeman, 2008). Severity of *Corynespora* leaf spot in this study ranged from 11.6% to 19.6%. Sandeep *et al*. (2014) reported disease severity and incidence range of 22.22%-53.33% and 19 to 59%, repectively. The variability may be attributed to differences in prevalent soil factors.

Characterization of fungal pathogens of green grams diseases

Macrophomina phaseolina may cause seedling blight, charcoal rot, root rot, leaf blight and damping off of over 500 species of plants (Farr and Rossman, 2014). In this study, leaves of green gram infected by *M. phaseolina* had charcoal rot and black specks of *sclerotia*. These are characteristic of post-emergence symptoms of *sclerotia* that allow the fungus to survive for prolonged periods of time in the soil (Baird *et al*., 2003). The sizes of the *sclerotia* observed in this study (46.62-35.7 µm) are within the range detected by Wagan *et al.* (2018), Mehta (2004) and Tandel *et al.* (2018) of 40-218 mm. Abundant aerial mycelium is produced in the culture plate with *sclerotia* imbedded within the hyphae or engrossed in the agar or on the agar surface with smooth precincts (Kaur *et al*., 2012). Spores of the *M. phaseolina* isolates in this study were whitish, turning greyish to dark in color, while the mycelia were branched, septate and appeared hyaline to brownish. The observed color of the mycelia is concurrent with that of Tandel *et al.* (2018). According to Almomani *et al.* (2013), single species of *M. phaseolina* isolates may display heterogeneity regarding to the color of the mycelium, distribution of the *microsclerotia*, *pycnidia*, among other traits. Tok (2019) and Pandey *et al.* (2020) have attributed the heterogeneity of a single phytopathogen to variations of weather and host species. Genetically, *M. phaseolina* may at some point have genetically diverse strains with varied virulence capacity (Mehta, 2004).

Cercospora canesens is the principal pathogen causing leaf spotting and defoliation in several legumes including green gram (Joshi *et al*., 2006). Morphological traits observed for *C. canesens* isolates in this study (small-sized and large-sized lesions) are similar to those observed by Bhat (2011), Rewal and Bedi (1997) and Arya *et al*., (1997). Occurrence of different lesion types indicates the occurrence of more than one strain (Joshi *et al.,* 2006). *Conidia* sizes were within those observed by Bhat (2019) and Dombroski (2005). According to Chand *et al.* (2013), *C. canescens* grow slowly and sporulation occurs between 10-12 days but with low spores. *Cercospora* spp. produce a perylenequinone toxin called cercosporin which is non-selective affecting bacteria, plants, fungi and animals unless these produce protective antioxidants (Daub and Ehrenshaft, 2000) *Colletotrichum* spp. are broad-ranged pathogens, meaning that many species can infect a single host and a single species can infect diverse hosts. In this study, *Colletotrichum* was observed in various green gram plant parts. *Colletotrichum* isolates of green gram significantly differs in their conidial length and breadth (Marak *et al.,* 2019). This variability may be due to response to varied climatic conditions (Chaudhari *et al.*, 2017; da Silva *et al.,* 2021). *Colletotrichum* may be considered as a group species and teleomorph exists within the species, which may contribute to genetic diversity and heterogeneity (Johnston and Jones, 1997). However, *Colletotrichum* isolates may not cluster based on their geographical origin of sampling (Naveen *et al.*, 2020). Resistant varieties could be used for management of this pathogen although Sunil *et al*. (2009) screened 30 green gram genotypes against *Colletotrichum truncatum* and none of the genotypes were found to be immune except a few that showed a resistant reaction. Morphological traits observed for the *Corynespora* pathogen isolates in this study were comparable to those reported by Sushmita *et al.* (2021). The pathogenic causes target spot especially on leaves but also on stems, roots and flowers of a wide range of host plants. The severity of the disease is greatly influenced by the susceptibility of the host genotype.

Association between soil factors and green gram disease

Potassium had a negative correlation with incidence and severity of powdery mildew, downy mildew and Corynespora spot. Potassium in plant enhance resistance to plant fungal pathogens by stimulating quick and vigorous response against pathogen (Kowalska and Drożdżyński, 2018; Mohammadi *et al*., 2019;). According to Perrenoud (1990), application of K fertilizers significantly reduces the incidence of fungal diseases by 70%, bacteria diseases by 69% and virus diseases by 41%. Increasing the level of K may subsequently decrease the occurrence of fungal diseases (Marschner, 1995; Mitchell and Walters, 2004; Liljeroth *et al.*, 2016).

Nitrate (NO_3) had a positive correlation with incidence and severity of powdery mildew, *Macrophomina*, late blight and angular spot. This indicates that increasing nitrogen content in the soil may likely increase the incidence and severity of the green gram diseases. According to Huang *et al.* (2017), severity of stripe rust

during grain filling escalated with increase in nitrogen. The role of nitrogen in escalating crop diseases such as downy mildew, powdery mildew, leaf rust, stem rot and rice blast diseases has been reported by Sun *et al.* (2020). Nitrogen is capable of making crop vulnerable to diseases since it promotes plant growth and increases succulent tissues and plant compounds that favour pathogen growth (Dordas, 2008). On the contrary, high N supply to plants has been observed to lower severity and incidence of some diseases (Blachinski *et al.,* 1996)

There were both negative and positive correlations between soil pH and incidence of green gram fungal diseases. Contrasting soil pH effects on plant pathogens suggest functional redundancy in carbon mineralization (Rousk and Baath, 2009). However, soil pH has no effect on the relative abundance of fungi and has only a slight influence on fungal diversity (Rousk *et al*., 2010). Generally, in lower pH conditions, the growth of fungi is increased. Decreased pH has a direct influence on microbial diversity and the soil ecosystem, leading to an imbalance in the soil micro-ecosystem and an abundance of soil-borne diseases in arable soil (Li *et al*., 2017).

CONCLUSION AND RECOMMENDATION

Powdery mildew, caused by *Erysiphe polygoni* DC, was the most prevalent disease. Incidence and severity of green gram diseases varied according to geographical areas and climate. Their distribution is majorly influenced by soil factors. The causative pathogens of these diseases differ in their morphological and cultural characterization. In order to successfully manage green gram fungal diseases, it would be important to identify all of them, map geographic distribution and determine their severity status. This can be achieved through comprehensive characterization of the diseases using an integrated approach of cultural, morphological and molecular methods. Green gram farmers should be advised on proper management strategies of soils to mitigate against fungal diseases infestation.

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