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Yield Loss Assessment in Cowpea Genotypes due to Stem and Root Rot Caused by *Macrophomina phaseolina*

D. Gireesha[1,2], H. Virupaksha Prabhu[1], G.R. Vishwas Gowda[1], J. Vamshi[3], S.K. Deshpande[4], P.V. Patil[1]

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ABSTRACT

Background: Cowpea crop suffer from various stresses, both biological and environmental, that reduce their quality and yield, leading to significant economic losses. Stem and root rot caused by *Macrophomina phaseolina* is a major root disease responsible for yield losses of 50-55 per cent.

Methods: A study was conducted by the Department of Plant Pathology, University of Agricultural Sciences (UAS), Dharwad, Karnataka, India, during the *rabi* season of 2022-23 to assess the impact of *Macrophomina phaseolina* infection on cowpea yield in split plot design. Ten released cowpea genotypes (C 152, DC 15, DC 16, DCS 47-1, KBC 11, KBC 9, KM 5, PKB 6, PKB 4, IT 38956-1) were grown in plots that were both inoculated and uninoculated with *M. phaseolina*.

Result: The disease caused an estimated grain yield loss of 19.64-43.74 per cent in all the genotypes. The moderately resistant genotype KBC 9 had the lowest grain yield loss (19.64%), while the farmer preferred genotype C 152 was susceptible to the root rot disease and had high yield loss of up to 43.74 per cent. among the genotypes, KBC 9 showed the highest reduction in stem and root rot incidence (78.22%), followed by IT 38956-1 (77.68%) while C 152 had the lowest root rot reduction (70.34%) when compared to pathogen inoculated plots. The principal component analysis (PCA) of different yield parameters of cowpea genotypes shows that, first four components (PC-1 to PC-4) with Eigen values of 4.16, 1.47, 1.29 and 1.14, collectively explained 89.82 per cent of the total variance. PC-1 (46.32% variability) was primarily influenced by the number of pods per plant, root length, root nodules per plant and plant height. Seed yield was significantly ($p \leq 0.05$) and strongly correlated to number of root nodules per plant (r=0.50) and root length (r=0.49).

Key words: Cowpea, *Macrophomina phaseolina,* Principal component analysis, Stem and root rot.

INTRODUCTION

Cowpea (*Vigna unguiculata* L.), a member of the Fabaceae family, is an ancient food source cultivated for centuries in tropical and subtropical regions. It's a highly versatile legume, thriving in various cropping systems. Farmers in India often grow cowpea as a cover crop, mixed crop, catch crop or green manure crop (Alexandre *et al.,* 2016). Globally, cowpea cultivation spans an estimated 23.4 million hectares; with a production of 18.29 million tonnes and an average yield of 637 kg/ha (FAO, 2020). In India, cowpea covers roughly 4 million hectares, producing 2.7 million tonnes with an average yield of 567 kg/ha. Unfortunately, cowpea is susceptible to various fungal, bacterial and nematode diseases. Among these, stem and root rot caused by *Macrophomina phaseolina* is considered the most destructive. This disease can cause significant yield losses, ranging from 5 to 39 per cent (Mohanapriya *et al.,* 2017; Gireesha *et al.,* 2023). Cowpea is susceptible to a variety of diseases caused by viruses, bacteria and fungi. In India, where the majority of cowpea and other pulse crops are grown under rainfed conditions, the tropical environment favours disease incidence. Among the fungal diseases, charcoal rot, caused by *Macrophomina phaseolina* (Tassi.) Goid, is a major cause of yield loss. The concurrent occurrence of heat and moisture stress exacerbates the development of charcoal rot, often making cowpea cultivation uneconomical (Singh *et al.,* 2012). *M. phaseolina* infects plants by clogging the vascular bundle, thereby reducing the plants' ability to uptake water and nutrients, resulting in wilting and death, leading to severe yield losses (Amrate *et al.,* 2024).

[1]Department of Plant Pathology, University of Agricultural Sciences, Dharwad-580 005, Karnataka, India.
[2]Department of Plant Pathology, School of Agriculture, SR University, Warangal-506 371, Telangana, India.
[3]Department of Plant Pathology, Malla Reddy University, Kompally, Hyderabad-500 100, Telangana, India.
[4]Department of Genetics and Plant Breeding, University of Agricultural Sciences, Dharwad-580 005, Karnataka, India.

Corresponding Author: D. Gireesha, Department of Plant Pathology, University of Agricultural Sciences, Dharwad-580 005, Karnataka, India; Department of Plant Pathology, School of Agriculture, SR University, Warangal-506 371, Telangana, India. Email: girishag829@gmail.com

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M. phaseolina is a soilborne plant pathogen with a very wide host range. It attacks a variety of oilseeds, legumes and vegetable crops, as well as a wide range of unrelated plants. The fungus forms microsclerotia in senescing shoot tissues, which survive well in soil and serve as primary source of inoculum (Rahman *et al.,* 2021). The soilborne nature of the fungus makes it difficult to manage the disease. Successful control of *M. phaseolina* is challenging due to its persistence as sclerotia in the soil and plant debris (Rangaswami and Mahadevan, 2008). There is no clear estimation report on yield loss of cowpea due to *M. phaseolina* and also, lack of knowledge about pathogenesis of this disease. Using resistant line is the best way to control disease and reduce yield loss. Therefore, the present study was undertaken to estimate the actual yield loss caused by stem and root rot disease to the cultivated genotypes of cowpea.

MATERIALS AND METHODS

Preparation of giant culture of *Macrophomina phaseolina*

The pathogen, *M. phaseolina* was isolated from the stem and root rot infected cowpea plants collected from cowpea experimental plots of Department of Plant Pathology at Main Agricultural Research Station (MARS), University of Agricultural Sciences (UAS), Dharwad, Karnataka by tissue segment method on potato dextrose agar (PDA) medium. Sorghum seeds were used as substrate for giant culture preparation. The substrate was prepared by mixing 200 g of crushed sorghum seeds and 50 ml distilled water in 500 ml conical flask and sterilized at 15 psi for one hour for two consecutive days. Flasks was subsequently inoculated with 4-5 discs of seven days old culture of *M. phaseolina* and incubated at 28±1°C for 20 days (Choudhary *et al.,* 2011). During incubation, the culture was mixed thoroughly to get uniform growth.

Loss assessment in cultivated cowpea genotypes due to stem and root rot

Field experiment was conducted at Main Agricultural Research Station (MARS), University of Agricultural Sciences, Dharwad during *rabi* 2022-23 to assess the losses in ten cultivated genotypes of cowpea due to stem and root rot caused by *M. phaseolina.* The trial was conducted in split plot design with protected and unprotected conditions. Before sowing, all the seed furrows were uniformly applied with mass multiplied inoculum of *M. phaseolina* on sterilized sorghum grains medium to the unprotected plots to induce disease incidence. In protected plots, seed treatment with carboxin 37.5% + thiram 37.5% WP @ 2 g/kg of seeds and drenching with carbendazim 50% WP @ 3 g/l was followed at regular intervals to protect the genotypes.

The experiment was laid out using a split-plot design with three replications. The main plots consisted of two treatments: M1 (Protected with fungicide treatment) and M2 (Unprotected without fungicide treatment). The subplots included ten cowpea genotypes: C 152, DC 15, DC 16, DCS 47-1, KBC 11, KBC 9, KM 5, PKB 6, PKB 4 and IT 38956-1. The main plot size measured 21.6 m × 4.0 m, while each sub-plot had a size of 1.35 m × 4.0 m, consisting of three rows, each four meters in length. The spacing between plants was 45 cm × 20 cm.

Observations recorded

Per cent disease incidence (PDI) was recorded separately in both fungicide treated and untreated blocks at 30, 60 and 75 days after sowing (DAS) by using the formula given by Wheeler (1969).

Per cent disease incidence (PDI) =

$$\frac{\text{Number of plants infected}}{\text{Total number of plants observed}} \times 100$$

The per cent loss in grain yield was calculated by using the following formula (Robert and James, 1980).

$$\text{Per cent yield loss} = \frac{Yp - Yt}{Yp} \times 100$$

Where,

Yp = Grain yield (q/ha) from protected block.

Yt = Grain yield (q/ha) from unprotected block.

Plant height (cm), number of pods per plant, pod length (cm), number of seeds per pod, grain yield (q/ha), 100 seed weight (g), root length (cm) and number root nodules per plant of five randomly selected plants were measured in both protected and unprotected blocks.

Statistical analysis

Experimental data were statistically analyzed using split plot design (Panse and Sukhatme, 1985) and Principal Component analysis (PCA) was done by using OriginLab software. Per cent data was converted into arc sin values and square root transformed values. Fischer's method of analysis of variance was used for analysis and interpretation of the data (Gomez and Gomez, 1984). Other statistical analysis *viz.*, OP STAT online statistical analysis program developed by Hissar Agricultural University, IBM SPSS and MS-excel were used to analyze the data.

RESULTS AND DISCUSSION

Significant differences in per cent disease incidence (PDI) were observed due to fungicide treatment. Irrespective of genotypes, the protected block exhibited a markedly lower PDI (9.68%) compared to unprotected block (38.64%). Among the various genotypes, genotype C 152 had the highest PDI (31.85%), followed by PKB 6 (29.20%) and IT 38956-1 (28.96 %). In the protected block, C 152 displayed the highest incidence of 14.57 per cent and it is on par with PKB 6 (12.75%). Conversely, KBC 9 had the lowest PDI (4.17%). Similarly, in the unprotected block, C 152 had the highest PDI (49.12%), while KBC 9 exhibited the lowest (19.15%). Genotype KBC 9 showed the most substantial reduction in disease incidence (78.22%), with IT 38956-1 also displaying a significant reduction (77.68%), while C

Table 1: Effect of fungicide drenching on disease incidence, grain yield and 100 grain weight in cowpea genotypes during *rabi* 2022-23.

Genotypes	Per cent disease incidence (PDI)			Per cent reduction in disease over weight unprotected	Grain yield(q/ha)			Per cent loss in grain yield	100 seed weight(g)			Per cent loss in 100 grain
	Protected	Unprotected	Mean		Protected	Unprotected	Mean		Protected	Unprotected	Mean	
C 152	14.57 (22.52)	49.12 (44.80)	31.85 (33.37)	70.34	11.34	6.38	8.86	43.74	10.89	7.85	9.37	27.96
DC 15	8.93 (17.34)	38.16 (37.86)	23.55 (27.68)	76.60	13.10	8.37	10.74	36.06	11.56	8.89	10.22	23.15
DC 16	8.22 (16.60)	36.42 (36.81)	22.32 (26.80)	77.43	12.35	8.29	10.32	32.90	11.82	9.36	10.59	20.81
DCS47-1	10.32 (18.71)	42.57 (40.54)	26.45 (29.64)	75.76	11.51	7.43	9.47	35.49	12.89	9.25	11.07	28.26
KBC 11	6.04 (14.16)	24.18 (29.16)	15.11 (21.66)	75.02	12.70	9.66	11.18	23.93	14.79	13.15	13.97	11.07
KBC 9	4.17 (11.72)	19.15 (25.69)	11.66 (18.70)	78.22	13.03	10.47	11.75	19.64	11.07	9.53	10.30	13.97
KM 5	9.83 (18.23)	43.43 (41.07)	26.63 (29.66)	77.37	13.87	8.48	11.18	38.87	10.65	8.17	9.41	23.27
PKB 6	12.75 (20.94)	45.65 (42.47)	29.20 (31.62)	72.07	11.10	6.66	8.88	40.01	13.77	11.25	12.51	18.30
PKB 4	11.42 (19.74)	40.37 (39.19)	25.90 (29.50)	71.71	10.69	6.88	8.78	35.68	13.11	10.47	11.79	20.17
IT 38956-1	10.57 (18.95)	47.35 (43.59)	28.96 (31.13)	77.68	12.01	7.44	9.72	38.11	12.32	9.72	11.02	21.13
Mean	9.68 (17.89)	38.64 (38.12)			12.17	8.00			12.29	9.76		
	S.Em. ±	**C.D. @ 5%**			**S.Em. ±**	**C.D. @ 5%**			**S.Em. ±**	**C.D. @ 5%**		
Fungicide treatment (F)	0.30	1.99			0.13	0.84			0.16	1.09		
Genotypes (G)	0.60	1.73			0.21	0.61			0.19	0.57		
G at the same level of F	0.96	2.84			0.40	1.06			0.53	1.10		

* Arc sine values.

Table 2: Effect of fungicide drenching on yield parameters in cowpea genotypes due to stem and root rot during *rabi* 2022-23.

Genotypes	No. of pods per plant			Per cent reduction in no. of pods per plant over unprotected	Pod length (cm)			Per cent reduction in pod length over unprotected	No. of seeds per pod			Per cent reduction in no. of seeds per pod over unprotected
	Protected	Unprotected	Mean		Protected	Unprotected	Mean		Protected	Unprotected	Mean	
C 152	12.10	7.35	9.72	39.27	14.64	10.24	12.44	30.03	14.35	10.17	12.26	29.14
DC 15	13.62	10.13	11.88	25.57	12.33	9.85	11.09	20.15	10.90	8.39	9.65	23.00
DC 16	14.85	10.62	12.74	28.48	13.24	10.36	11.80	21.78	11.57	9.30	10.43	19.59
DCS47-1	13.78	9.42	11.60	31.64	14.63	11.35	12.99	22.46	10.09	8.14	9.11	19.35
KBC 11	15.93	13.39	14.66	15.93	15.12	13.57	14.35	10.27	15.31	13.52	14.41	11.69
KBC 9	13.78	11.57	12.67	16.03	17.75	14.95	16.35	15.76	14.96	13.61	14.28	9.06
KM 5	10.21	7.34	8.77	28.12	16.08	11.74	13.91	27.03	16.03	11.77	13.90	26.59
PKB 6	14.51	10.67	12.59	26.49	17.01	11.46	14.24	32.60	16.62	12.04	14.33	27.55
PKB 4	13.65	10.53	12.09	22.88	17.30	12.07	14.68	30.22	15.51	11.10	13.31	28.45
IT 38956-1	11.77	8.18	9.97	30.55	11.13	7.51	9.32	32.56	16.44	11.53	13.98	29.89
	13.42	9.92			14.92	11.31			14.18	10.96		
	S.Em. ±	**C.D. @ 5%**			**S.Em. ±**	**C.D. @ 5%**			**S.Em. ±**	**C.D. @ 5%**		
Fungicide	0.07	0.47			0.22	1.44			0.21	1.40		
treatment (F)	0.27	0.79			0.33	0.96			0.32	0.93		
Genotypes (G)												
G at the same	0.39	1.17			0.69	1.70			0.67	1.65		

Table 3: Effect of fungicide drenching on yield parameters in cowpea genotypes due to stem and root rot during *rabi* 2022-23.

Genotypes	Plant height (cm)			Per cent reduction in plant height over unprotected	Root length (cm)			Per cent reduction in root length over unprotected	No. of root nodules per plant			Per cent reduction in no. of root nodules per plant over unprotected
	Protected	Unprotected	Mean		Protected	Unprotected	Mean		Protected	Unprotected	Mean	
C 152	50.26	40.79	45.53	18.85	21.46	14.48	17.97	32.53	66.10	42.67	54.39	35.45
DC 15	54.78	44.31	49.54	19.12	23.93	18.55	21.24	22.49	68.22	48.08	58.15	29.53
DC 16	54.31	41.51	47.91	23.57	20.36	15.83	18.10	22.23	62.24	50.86	56.55	18.29
DCS47-1	47.11	38.49	42.80	18.31	22.08	16.52	19.30	25.22	60.21	42.74	51.47	29.01
KBC 11	49.50	43.88	46.69	11.34	25.09	20.69	22.89	17.56	75.66	56.72	66.19	25.03
KBC 9	57.76	51.24	54.50	11.28	23.24	19.04	21.14	18.09	72.00	52.63	62.32	26.91
KM 5	43.36	34.84	39.10	19.66	17.80	12.75	15.28	28.37	57.49	40.50	49.00	29.55
PKB 6	57.31	47.05	52.18	17.91	18.45	13.11	15.78	28.92	64.41	44.39	54.40	31.08
PKB 4	53.51	37.28	45.40	30.33	20.68	14.15	17.41	31.60	59.49	38.79	49.14	34.79
IT 38956-1	45.02	35.87	40.44	20.32	15.98	12.83	14.41	19.71	70.04	53.77	61.91	23.23
	51.29	41.53			20.91	15.79			65.59	47.11		
	S.Em.±	**C.D. @ 5%**			**S.Em.±**	**C.D. @ 5%**			**S.Em±**	**C.D. @ 5%**		
Fungicide treatment (F)	0.46	3.06			0.26	1.70			0.46	3.00		
Genotypes (G)	0.89	2.56			0.37	1.08			0.90	2.60		
G at the same level of F	1.48	4.24			0.82	1.94			1.45	4.27		

152 had the lowest reduction (70.34%) in disease incidence. In general, grain yields from unprotected blocks were lower than the protected blocks with yield loss estimates higher among the susceptible genotypes. It was observed that the moderately resistant genotype, KBC 9 recorded highest grain yields of 10.47 q ha^{-1} and 13.03 q ha^{-1} from both unprotected and protected blocks. However, the genotypes C 152 (6.38 q ha^{-1}) and PKB 6 (6.66 q ha^{-1}), respectively in the unprotected blocks had the least grain yields. The genotype C 152 had the highest yield loss of 43.74 per cent while KBC 9 had the least yield loss of about 19.64 per cent (Table 1).

Among the ten cowpea genotypes DCS 47-1 recorded the maximum loss in 100 seed weight (28.26%), followed by KBC 9 (13.97%), while KBC 11 had the minimum loss (11.07%). C 152 had the highest decrease in the number of pods per plant (39.27%), followed by DCS 47-1 (27.91%), while KBC 11 had the least reduction in pods per plant (15.93%). Highest per cent decrease in pod length was recorded in the genotype PKB 6 (32.60%) followed by IT 38956-1 (32.56%) and it was lowest in KBC 11 (10.27%). Highest per cent decrease in number of seeds per pod was recorded in the genotype IT 38956-1 (29.89%) and it was lowest in KBC 9 (9.06%) (Table 2). Highest per cent decrease in plant height was recorded in the genotype PKB 4 (30.33%) followed by DC 16 (23.57%) and it was lowest in KBC 9 (11.28%). Highest per cent decrease in root length was recorded in the genotype C 152 (32.53%) followed by PKB 4 (31.60%) and it was lowest in KBC 11 (17.56%). Highest per cent decrease in root nodules was recorded in the genotype C 152 (35.45%) and it was lowest in KBC 11 (25.03%) followed by KBC 9 (26.91%) (Table 3).

According to Nair *et al.* (2012), dry root rot results in 10 - 44 per cent yield loss in the production of mungbean in India and 33-44 per cent yield loss owing to *Rhizoctonia* root rot. In addition to decreasing crop yields, pathogen damage also decreases their ability to fix nitrogen in the soil (Khaledi *et al.,* 2015). The results are in accordance with Mohanlal (2006), who studied the impact of root rot, caused by *M. phaseolina*, on groundnut varieties. The highest yield loss, totaling 435 kg/ha, occurred in the rainfed conditions of Keshod tehsil in Junagadh district. This loss was associated with a plant mortality rate of 29.3 per cent and was most pronounced in the groundnut variety GG 2. In contrast, the lowest yield loss, at 19 kg/ha, was recorded in the same area, specifically with variety GG 10, where the plant mortality or root rot incidence was only 1.0 per cent. A study by Vamsikrishna *et al.* (2021) examined yield losses in three major pigeonpea genotypes *viz.,* Maruthi, TS3R and GRG-811 due to stem canker caused by *M. phaseolina*.

Table 4: Principal component analysis of different yield parameters in cowpea genotypes.

Component	Initial eigen values			Extraction sums of squared loadings		
	Total	Percentage of variance	Cumulative (%)	Total	Percentage of variance	Cumulative (%)
1	4.16	46.32	46.32	4.16	46.32	46.32
2	1.47	16.40	62.73	1.47	16.40	62.73
3	1.29	14.42	77.15	1.29	14.42	77.15
4	1.14	12.66	89.82	1.14	12.66	89.82
5	.609	6.77	96.59			
6	.206	2.29	98.88			
7	.069	.763	99.64			
8	.023	.261	99.90			
9	.008	.091	100.00			

Table 5: Correlation matrix for different yield parameters in cowpea genotypes.

	Disease incidence (%)	Yield (q/ha)	Test weight (g)	No. of pods /plant	Pod length (cm)	No. of seeds /pod	Plant height (cm)	Root length (cm)	No. of root nodules/ plant
Disease incidence (%)	1.000								
Yield (q/ha)	-.793*	1.000							
Test weight (g)	-.297	-.083	1.000						
No. of pods/plant	-.670*	.207	.766*	1.000					
Pod length (cm)	-.528	.243	.239	.386	1.000				
No. of seeds/pod	-.188	.137	.333	.026	.422	1.000			
Plant height (cm)	-.511	.166	.203	.646	.460	.066	1.000		
Root length (cm)	-.745	.498	.287	.690*	.341	-.245	.501	1.000	
No. of root nodules/plant	-.617	.506	.398	.518	-.103	.271	.363	.513	1.000

* Significant at 1%.



Fig 1: Scree plot showing eigen value against principal component.

The findings revealed that the Maruthi genotype, classified as Grade 5 (highly susceptible), had a yield of 514 kg per hectare, which represents a significant 40.61 per cent decrease compared to healthy plants. In contrast, the TS3R genotype, also Grade 5 (highly susceptible), yielded 912 kg per hectare, showing a 28.71 per cent reduction compared to healthy plants.

Principal component analysis

Ten cowpea varieties underwent principal component analysis (PCA) based on multiple traits, including PDI, plant height, pods per plant, pod length, seeds per pod, grain yield, 100 grain weight, root length and root nodules per plant. The first four principal components (PC-1 to PC-4) with Eigen values of 4.16, 1.47, 1.29 and 1.14, collectively explained 89.82 per cent of the total variance. PC-1 (46.32% variability) was primarily influenced by the number of pods per plant, root length, root nodules per plant and plant height. PC-2 (16.40% variability) was characterized by the number of seeds per pod and test weight. PC-3 (14.42% variability) was dominated by the number of seeds per pod and seed yield, while PC-4 (12.66% variability) emphasized the importance of the number of root nodules per plant for yield enhancement (Table 4). Traits like pod number, root length, root nodules, plant height and seed yield contributed significantly to diversity, aligning with Kaiser's criteria (Kaiser, 1960 and Zhu *et al.,* 2024). The fifth principal component (PC-5) had an Eigen value less than 1.0 and contributed less to divergence (6.77% variability) (Singh *et al.,* 2017). The Correlation studies between different yield parameters of cowpea genotypes shows that, seed yield was significantly ($p \leq 0.05$) and strongly correlated to number of root nodules per plant (r=0.50) and root length (r=0.49) (Yadav *et al.,* 2024). Significantly ($p \leq 0.05$) negative (weak) correlation was observed between seed yield and per cent disease incidence -0.79 (Table 5). The above results are in accordance with Jakhar and Kumar (2018) and Mahalingam *et al.* (2020). Scree plot (Fig 1) graphs the Eigen value against the components described above. Flat line from the fourth component onwards suggests that each successive component is accounting for smaller and smaller amounts of the total variance.

CONCLUSION

The disease caused an estimated grain yield loss of 19.64 - 43.74 per cent in the ten genotypes namely C 152, DC 15, DC 16, KM 5, DCS 47-1, KBC 9, KBC 11, PKB 6, PKB 4 and IT 38956-1. The moderately resistant genotype KBC 9 had the lowest grain yield loss (19.64%), while the farmer preferred genotype C 152 was susceptible to the root rot disease and had high yield loss of up to 43.74 per cent. among 10 different cowpea genotypes, KBC 9 showed the highest reduction in disease incidence (78.22%), followed by IT 38956-1 (77.68%) while C 152 had the lowest disease reduction (70.34%). Crop losses varied significantly between genotypes and the losses were directly proportional to disease incidence. The higher per cent disease incidence observed in the genotype in turn reflected on the magnitude of loss. It may be concluded that losses due to root rot disease can be effectively managed by development of improved genotypes.

Conflict of interest

The authors declare that they have no conflict of interest.

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