

Evaluation of Cowpea (*Vigna unguiculata* (L.) Walp Genotypes for Response to Wilt Disease caused by *Fusarium oxysporum* f.sp. *tricheiphilum*

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Abstract

Wilt disease caused by *Fusarium oxysporum* f. sp. *tracheiphilum* (Fot), is a primary constraint to global cowpea production. It is a systemic disease which impairs water absorption and translocation processes leading to death of infected plants. This study aimed to evaluate 11 cowpea genotypes for sources of resistance to *Fusarium* wilt. The experimental pots were inoculated with 3 g of wheat seeds which served as inoculum carrier. Disease severity was evaluated on a six-point scale which ranged from mild to very severe wilting. Area under disease progress curve (AUDPC) was determined using severity values. Data were collected on growth, yield, and disease incidence. Statistical analysis was done using analysis of variance (ANOVA) and means separated with Duncan Multiple Range Test (DMRT) at $p < 0.05$. There was significant ($p < 0.05$) difference among the genotypes on the incidence and severity of *Fusarium* wilt disease. AUDPC values showed that 27.3, 45.5 and 18.2% of the genotypes were resistant, moderately resistant and susceptible, respectively. Three genotypes, IT07K-282-10, IT99K-573-11 and IT98D-610 were resistant to *Fusarium* wilt disease in this study, while genotypes IT07K-297-13 and Ife brown control were susceptible and highly susceptible, respectively. Two resistant genotypes, IT96D-610 and IT07K-230-2-9, with low severity values had the highest yield of 2.66 and 2.74 tones/ha⁻¹ and these will be useful to breeders for improvement of the low yielding types. These resistant genotypes identified in this study could serve as sources of genes in breeding for resistance to cowpea wilt disease.

Keywords: AUDPC, Cowpea, Fot, Genotype, Wilt disease

Introduction

Cowpea (*Vigna unguiculata* (L.) Walp) is an annual herbaceous legume from the genus *Vigna*. It thrives well in soils with low fertility and moisture content. It is a valuable crop for poor farmers and has the ability to produce significant yield in areas with low rainfall (USDA, 2021). It has the highest protein content among the cultivated legumes and is a good amino acid source in animal diet (Basaran *et al.*, 2011; Falade, 2016). Cowpea is an important food crop in sub-Saharan Africa with much of its produce for domestic consumption. The commodity plays a significant role in the Nigerian economy as it occupies a unique

position in the internal food chain, an income earner, and is capable of providing solution to the protein-carbohydrate imbalance in nutrition. (Taiwo *et al.*, 2014). Increase in the demand for this commodity has led to extensive cultivation of the crop in many parts of the country making Nigeria a main producer in the world. Four subspecies of cowpea are recognised, of which three are cultivated (Leticia *et al.*, 2017). A high level of morphological diversity is found within the species with large variations in size, shape, and structure of the plant (Ibro *et al.*, 2015; Rengadu *et al.*, 2020). The crop is mainly grown for its seeds, although the leaves and immature seed pods can also be consumed.

Wilt caused by *Fusarium oxysporum* f. sp. *tracheiphilum* (*Fot*) (E.F. Sm.) Snyder & Hans is a devastating disease that is responsible for about 60% yield reduction in cultivated cowpea (Pirayesh *et al.*, 2018; Metsena *et al.*, 2021). It is a systemic disease which attacks the vascular bundles of cowpea where the pathogen secretes extracellular polysaccharides that interfere with water absorption and translocation processes in the xylem and phloem vessels, respectively (Porttorf *et al.*, 2014; Gordon, 2017). This impairs plant growth and ultimately reduces yield among susceptible cowpea varieties. The initial symptoms are expressed in older leaves which show mild discolouration of leaves that later change to greenish yellow (Sabo *et al.*, 2014). Advanced stage of infection shows drooping of leaves, wilting and ultimate death of infected plants. Wilting often begins on one side, then the whole parts, and later the death of the plant (Wamalwa, 2018). The *Fot* pathogen is polyphagous with a wide host range and has been identified as the causative agent of wilt disease in many leguminous crops and is also widely distributed in several countries where cowpea is grown (Ratnaningsih *et al.*, 2020; Ana *et al.*, 2020). The pathogen is soil-borne which gains entry through the proximal regions of apical roots into the vascular bundles where it proliferates and impairs metabolic and physiological processes in affected plants thereby causing wilt disease (Dominguez-Perles *et al.*, 2015). *Fusarium* species including *Fot* have the unique ability to produce resting spores such as chlamydo spores which enhance their inherent ability to survive unfavourable environmental conditions for many years when either the main or alternative hosts are unavailable (Pujari *et al.*, 2015; Khare *et al.*, 2016) This strategy also enables *Fot* to survive between growing and off-seasons.

Cowpea wilt disease is conventionally controlled through the application of systemic fungicides such as benomyl in Nigeria. However, this method is constrained by reported cases of poisoning of humans and livestock. There is also the knotty issue of pathogens becoming resistant through mutation emanating from consistent and indiscriminate use of fungicides (Horn *et al.*, 2015; Pande *et al.*, 2017). Breeding

for resistance is eco-friendly and economically viable approach in the management of plant diseases. Resistant varieties could also provide sources of genes in the breeding programme against cowpea wilt disease. This study sought to evaluate cowpea genotypes for resistance to *Fusarium* wilt of cowpea.

Materials and methods

Study site

The experiment was set up at the screenhouse of the Department of Crop Protection and Environmental Biology, Ibadan, Nigeria. Ibadan, with the coordinates: 7° 22' 36".25N and 3°56' 23".23E is located in the forest transition agroecological zone characterised by an annual bimodal rainfall of 1497-1844 mm with mean minimum and maximum temperatures of 26°C and 32°C, respectively (Dania and Nurudeen, 2019).

Experimental design and treatments

The experimental layout was a Completely Randomized Design (CRD) which consisted of 11 genotypes including a susceptible check with three replications. There were three pots per genotype in each replicate and one plant per pot, which equals to three plants per genotype in a replicate. This translates to nine pots per genotype in three replicates, which equals to nine plants per genotype in three replicates. Therefore, there were 33 experimental pots (3 pots × 11 genotypes) per replicate with a total of 99 experimental unit (33 pots × 3 replicates). The pots were arranged at a spacing of 75 cm between replicates and 40 cm within replicate. The list of cowpea genotypes used in the study are presented in Table 1.

Plant material and inoculation technique

Eleven cowpea genotypes used in the experiment were sourced from Genetic Resource Centre of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, while a susceptible check, Ife brown, was obtained from the Institute of Agricultural Research and Training (IAR&T), Ibadan. A pathogenic isolate of *Fusarium oxysporum* f.sp. *tracheiphilum* used in the screenhouse experiment was collected from the Germplasm Health Unit of IITA. The

Table 1. List of cowpea genotypes used in the study

Genotype	Size	Colour	Texture	Growth habit
IT95K-207-22	M	W	Smooth	Semi-Erect
1T07K-292-10	M	W	Smooth	Erect
IT13K-1308-5	L	W	Smooth	Erect
IT99K-573-11	M	B	Smooth	Erect
IT07K-243-12	M	W	Smooth	Erect
IT07K-297-13	M	W	Rough	Semi-Erect
IT96D-610	M	W	Smooth	Erect
IT90K-76	M	W	Smooth	Erect
1T07K-243-1-10	L	B	Rough	Erect
IT0K-230-2-9	L	W	Smooth	Erect
Ife brown	M	B	Rough	Semi-Erect

M= Medium, L=Large, W=White, B=Black

inoculum was multiplied in the plant pathology laboratory at the University of Ibadan, Nigeria by sub-culturing from agar slants in McCartney bottles unto 10 freshly prepared potato dextrose agar (PDA) in glass Petri dishes. PDA contains dextrose as the major carbohydrate source and potato infusion that provide nutrient base for luxuriant growth of many fungi. Water agar consisting of agarose and agarpectin is 1.5% additive-free and is usually not recommended for growth of most fungi, but enhances sporulation. Therefore, the inoculum was further transferred to water agar plates containing less nutrients than PDA to facilitate sporulation of the fungus. The culture plates were incubated under 12 hours of alternating light and darkness for 14 days in an incubator with model number 1-5611-D USA. The sandy loam soil used in the experiment was sterilized using an electrical sterilizer model BR 12-00-220 Suzhou, China at 170°C for three hours. Sterilized wheat seeds were used as carrier of the pathogen for inoculation of soil at one week ahead of cowpea sowing (Dania and Ewongo, 2022). Sterilized soil was filled into 25-cm diameter 5 kg experimental pots laid out in a CRD with three replications. The pots were watered with sterile distilled water to field capacity before inoculation with *Fot*. Each pot was inoculated with 3 g of wheat seeds impregnated with *Fot mycelia* seven days before sowing to allow colonization of the soil. Healthy cowpea seeds of 10 genotypes and a susceptible Ife brown check were sown a week later. Data

were collected on growth parameters regularly for seven consecutive weeks. Plant height was measured from the soil level to the leaf tip of three randomly selected apical leaves, while number of branches per plant were counted. Fresh weight of three representative plant samples were randomly taken and weighed at harvest stage using a mettler analytical balance ME308TE India. Leaf area was calculated according to Osei-Yeboah *et al.* (1983):

$$\text{Leaf area (cm}^2\text{)} = L \times W$$

Where L = Leaf length (cm)

W = Leaf width (cm)

Grain yield (kg/ha⁻¹) was obtained by threshing and weighing total seeds produced by cowpea plants therein and calculated as:

$$\frac{a \times b \times 1000}{c}$$

Where a = Number of cowpea grains per treatment

b = Total number of treatments

c = Area of hectare (10,000 m²)

This was divided by 1000 to obtain grain yield (tons/ha).

Inoculated cowpea plants were evaluated for disease incidence and severity on weekly basis up till seven week after inoculation (WAI). Disease incidence was assessed by counting the number of plants showing typical wilt symptoms including drooping of leaves, browning of the vascular tissues and eventual death. The recorded values were expressed as a percentage

of the total number of plants per treatment:

$$\text{Disease incidence} = y/Y \times 100$$

Where:

y = Number of infected plants per treatment

Y = Total number of plants per treatment

Disease severity among cowpea varieties inoculated with *Fot* was determined on a six-point rating scale using the modified method of Lebeda and Buczkowski, (1986) (Table 2).

disease on growth of cowpea varieties inoculated with *Fusarium oxysporum* f.sp. *tricheiphilum*.

Disease incidence among cowpea plants artificially inoculated with *Fot* pathogen increased with the number of weeks after inoculation and peaked at seven weeks after inoculation (WAI). A similar trend was also observed for disease severity at 7WAI. Disease expression ranged from mild to very severe symptoms of wilt disease on inoculated cowpea

Table 2: Disease severity scale for *Fusarium* wilt disease among cowpea genotypes

Rating	Symptom description	Reaction
1	Whole plants with no visible symptoms	Immune (I)
2	Very mild wilting, 1-5% wilting of one or two leaves	Resistant (R)
3	Mild wilting, 6 – 10% plant tissue wilted, few leaves affected	Moderately resistant (MR)
4	Moderate wilting, 11 – 20% yellowing of lower leaves and necrosis	Moderately susceptible (MS)
5	Severe wilting, 21 – 50% plant tissue wilted, drooping of leaves	Susceptible (S)
6	Very severe wilting, >50% plant tissue wilted, drooping and death	Highly Susceptible (HS)

Area Under Disease Progress Curve (AUDPC) was calculated using mean severity values according to Campbell and Madden (1990):

$$AUDPC = \sum_{i=1}^{n-1} 0.5(x_{i+1} + x_i)(t_{i+1} - t_i)$$

Where t = time in days after *Fot* inoculation at ith observation, “

xi = disease severity at the ith observation

ti + 1-ti = interval between two consecutive observations.

n = total number of observations

AUDPC values were computed using Statistical analysis system (SAS, 2009) version 9.2.

Data Analysis

Data were analyzed using one-way Analysis of Variance (ANOVA) with Statistical analysis system (SAS, 2009) version 9.2. and significant means were separated with Duncan Multiple Range test (DMRT) at 5% level of probability.

Results

Effect of incidence and severity of wilt

plants showing drooping of foliage, while very severe symptoms were manifested as total collapse and eventual death of infected plants (Fig. 1). The resistance status of the treatments to wilt disease varied from resistant (R), moderately resistant (MR) moderately susceptible (MS) to highly susceptible (HS). Three genotypes, IT07K-282-10, IT99K-573-11 and IT98D-610 were resistant to *Fusarium* wilt disease in this study, while variety IT07K-297-13 and Ife brown control were susceptible and highly susceptible to *Fusarium* wilt disease, respectively. The remaining four genotypes were moderately susceptible to the disease. The area under disease progress curve (AUDPC) varied between 25.3 and 60.7% (Fig. 2). The susceptible check had the highest AUDPC value of 60.7% followed by genotype IT07K-297-13. Two of the genotypes IT99K-573-11 and IT96D-610 had the lowest AUDPC value of 10.01%. Disease expression in Ife brown variety was significantly ($p < 0.05$) higher than the other treatments. Plant height ranged from 31-08 to 47.04 cm. Ife brown check was the tallest with a height of 47.04 cm. which was significantly

($p < 0.05$) higher than the other genotypes, while genotype IT0K-230-2-9 was the shortest (Table 3). However, the number of branches and leaf area did not differ significantly ($p > 0.05$) among the treatments including the control.

Effect of *Fusarium* wilt disease on cowpea yield

Fresh weight of cowpea at harvest varied between 21.08 and 40.5 g/plant. Ife brown variety had the highest fresh weight of 40.5

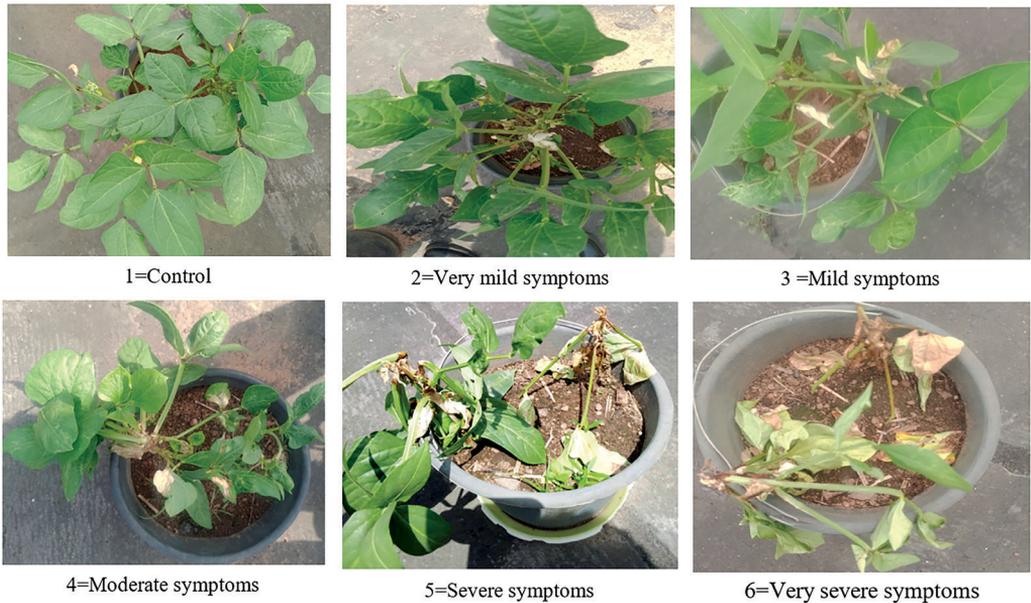


Figure 1: Symptom expression among cowpea genotypes inoculated with *Fusarium oxysporum* f.sp. *tricheiphilum*

Table 3: Effect of *Fusarium* wilt disease on cowpea growth and reaction at seven weeks after inoculation

Genotype	Plant height (cm)	No. of branches	Leaf area (cm ²)	Incidence (%)	Severity rating	Reaction
IT95K-207-22	32.73d	6.01a	20.77b	10.03e	3.01d	MR
1T07K-292-10	36.96c	4.08a	24.33b	8.21f	2.17d	R
IT13K-1308-5	43.44b	5.33a	21.16b	12.02e	3.10d	MR
IT99K-573-11	32.58d	5.13a	28.12a	9.14e	2.03d	R
IT07K-243-12	33.87d	4.77a	24.01b	15.01d	3.15d	MR
IT07K-297-13	36.01c	4.22a	28.22a	31.01b	5.01b	S
IT96D-610	32.07d	6.08a	31.19a	7.33f	2.13d	R
IT90K-76	31.49d	5.41a	19.92b	13.01e	3.19d	MR
1T07K-243-1-10	37.02c	5.62a	23.11b	26.11c	4.08c	MS
IT0K-230-2-9	31.08d	5.71a	25.05b	12.05e	3.08d	MR
Ife brown	47.04a	6.19a	32.73a	42.17a	6.04a	HS
CV (%)	21.75	11.67	22.30	18.71	24.38	
SE	2.61	0.18	0.57	3.60	1.17	

Means with the same superscript along the column are not significantly different using Duncan Multiple Range Test at $p < 0.05$. R = Resistant, MR = moderately resistant, MS = moderately susceptible HS = Highly susceptible, S = Susceptible. CV = Coefficient of variation, SE = Standard error of mean.

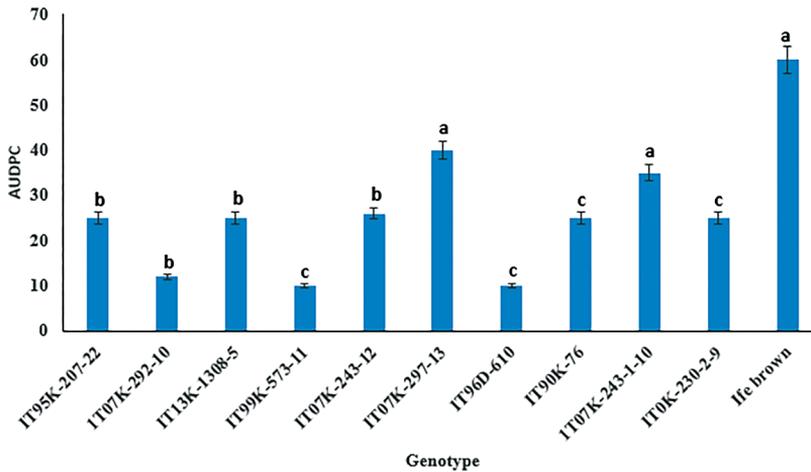


Figure 2: Percent area under disease progress curve (AUDPC) values for *Fusarium oxysporum* f.sp. *tricheiphilum* causing wilt disease among cowpea genotypes

g, while genotype IT07K-243-1-10 recorded the lowest weight (Table 4). Genotypes IT07K-243-1-10 and IT90K-76 produced the highest and lowest number of 5.01 and 3.17 peduncles per plant, respectively. There was no significant ($p>0.05$) difference among the treatments in the number of peduncles produced per plant. Similarly, the number of pods produced per peduncle did not differ

significantly ($p>0.05$) among the genotypes. However, the number of seeds per pods produced per peduncle differed significantly ($p<0.05$) among the genotypes. Three of the genotypes IT0K-230-2-9, IT96D-610 and IT99K-573-11 produced highest number of 14.88, 14.71 and 14.31 seeds per pod respectively and these were significantly higher than other treatments, while the susceptible check had 9.17 seeds per pod.

Table 4: Effect of *Fusarium* wilt disease on cowpea yield

Genotype	Fresh plant weight (g)	No. of Peduncles per plant	No of pods per Peduncle	No. of seeds per pod	Yield (tons/ha ⁻¹)
IT95K-207-22	24.67bc	3.89a	2.33a	10.05b	1.73b
IT07K-292-10	27.32b	3.60a	2.18a	13.11a	2.27a
IT13K-1308-5	20.88c	4.62a	3.31a	9.22b	1.60b
IT99K-573-11	28.11b	3.56a	1.77a	14.31a	2.33a
IT07K-243-12	30.77b	5.50a	2.44a	13.04a	2.10a
IT07K-297-13	24.03bc	3.33a	3.22a	10.45b	1.96b
IT96D-610	29.51b	3.17a	2.09a	14.71a	2.66a
IT90K-76	26.93bc	3.37a	1.82a	9.83b	1.72b
IT07K-243-1-10	21.08c	5.01a	3.08a	10.18b	1.88b
IT07K-230-2-9	25.19bc	3.70a	2.07a	14.88a	2.74a
Ife brown	40.05a	3.01a	1.32a	9.17b	1.04b
CV (%)	20.8	9.81	8.94	21.72	22.30
SE	0.32	0.23	0.41	0.58	0.62

Means with the same superscript letter along the column are not significantly different using Duncan Multiple Range test (DMRT) at 5% level of probability. CV=Coefficient of variation, SE=Standard error of mean.

The same genotypes with the highest no of seeds also produced corresponding higher yields of 2.33, 2.66 and 2.74 tons/ha⁻¹ respectively. The lowest yield of 1.04 kg/ha⁻¹ was recorded in the control treatment.

Discussion

Cowpea is an important legume crop that has the unique ability to fix atmospheric nitrogen in the soil thereby increasing its fertility. Regardless of this potential, however, it requires a fertile soil with essential nutrients for optimal performance (Hamid *et al.*, 2015). A fertile soil with requisite nutrients will inhibit deficiency diseases in crop plants which may be misconstrued as disease caused by biotic factors. Symptom expression in this study showed wilting of leaves and browning of the vascular tissues of severely infected plants that were inoculated with the pathogen. These distinct symptoms confirmed *Fot* as the causative agent of cowpea wilt disease. This finding agrees with the reports of Walmawa *et al.* (2018) and Ana *et al.* (2020) which indicated that cowpea wilt disease is caused by *Fot*. The disease usually begins with defoliation and wilting on a side of the plant which later spreads to the whole plant, eventually leading to its death. Rodrigues and Menzes (2005) reported that *Fusarium* wilt disease accounts for over 40% of the losses incurred in cowpea cultivation under field conditions. The initial symptoms observed among the susceptible genotypes and control were mild necrosis and drooping of infected leaves which later turned brown and wilted at advanced stage of infection. These, however, contradict the previous findings of Gordon (2017) who reported chlorosis and wilting as major symptoms of cowpea plants inoculated with *Fot*. Yellowing of leaves may not necessarily be a disease symptom in plants but may be attributed to nutrient deficiencies such as nitrogen, sulphur and iron. Although cowpea has the inherent ability to fix atmospheric nitrogen, chlorotic symptoms may appear if cultivated in soils deficient in the other two essential nutrients for its growth. This may be responsible for the disparity between the two experiments.

There was significant ($p < 0.05$) difference

among the inoculated genotypes in the incidence and severity of *Fusarium* wilt disease. Several authors have reported significant variation in the incidence of wilt disease among cowpea accessions (Wamalwa *et al.*, 2018; Pirayesh *et al.*, 2018; Ana *et al.*, 2020). The disparity in cowpea response to wilt disease may have been influenced by the individual genome of the F1 hybrids used in this study, arising from the genetic constitution of their parental lines. The disease incidence among treatments inoculated with *Fot* ranged between 7.3 to 42.1% at seven weeks after inoculation. The low level of infection rate among some of the genotypes could be attributed to their resistance status to disease infection, while those with high incidence may be due to their possession of recessive and susceptible genes to *Fusarium* wilt. Generally, early planting reduces the infectivity potential of plant pathogens and the occurrence of plant diseases as a result of unfavourable environmental conditions (Goher *et al.*, 2020). Higher incidences of cowpea wilt disease are often experienced at the peak of the rains, while low infection rate occurs at the onset of the planting season (Ana, 2020).

It was observed in this study that genotype IT07K-297-13 and the Ife brown check were susceptible (S) and highly susceptible (HS) to *Fusarium* wilt disease, respectively. Ana *et al.* (2020) had reported variability in susceptibility among cowpea genotypes to *Fusarium* seed rot disease. Timing of assessment is also very important in the evaluation of plant genotypes for resistance to disease as they are bound to have varying severity rating over a given period of time. Lower severity values are usually recorded at the onset which ultimately increases with duration of study (Gordon, 2017; Dania and Nurudeen, 2019). The significant ($p < 0.05$) difference recorded among the genotypes in response to wilt disease in this experiment could be attributed to the variation in their sources of resistance genes to *Fot*. Wilting and eventual death were recorded in susceptible genotypes at advanced stage of infection. The death of the infected cowpea plants may be due to the systemic nature of *Fot*. The pathogen attacks the xylem and phloem tissues of the host where it produces metabolites that impede

water absorption and translocation processes respectively. These impairs plant growth and ultimately reduce yield among susceptible cowpea varieties.

Two cowpea genotypes, IT96D-610 and IT07K-230-2-9, had the highest yield potential and these will be useful to breeders for improvement of the low yielding types. However, yield was significantly ($p < 0.05$) reduced in cowpea genotypes that were either susceptible or highly susceptible to *Fusarium* wilt disease. This may be attributed to the systemic interruption of the pathogen with the absorption of nutrients necessary for metabolism and translocation in the plant. Several authors have reported decreased productivity among susceptible cowpea cultivars (Gordon, 2017; Metsena *et al.*, 2021).

Conclusion

Fusarium wilt disease poses serious threat to cowpea production with a significant reduction in yield. The adoption of integrated management strategies that involve a combination of genetic resistance, cultural practices, and proper sanitation would minimize the impact of plant diseases on agricultural systems. The resistance status of the genotypes to wilt disease varied from resistant (R), moderately resistant (MR) to moderately susceptible (MS). Three varieties, IT07K-282-10, IT99K-573-11 and IT98D-610 were resistant to *Fusarium* wilt disease in this study, while variety IT07K-297-13 and Ife brown control were susceptible. The remaining four genotypes were moderately susceptible to the disease. The three resistant genotypes, IT07K-282-10, IT99K-573-11 and IT98D-610 identified in this study could serve as sources of genes in breeding for resistance to cowpea wilt disease.

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