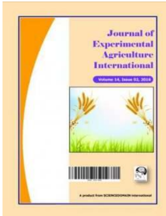

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Evaluation of Cowpea (*Vigna unguiculata* L.) Genotypes' Growth and Yield Performance and Resistance to the Cowpea Seed Beetle, *Callosobruchus maculatus* F.

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Authors' contributions

This work was carried out in collaboration between all authors. Author BA performed the work, wrote the protocol and the first draft of the manuscript. Authors EAO and SA supervised the work and worked on the final manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The search for resistant cowpea (*Vigna unguiculata* (L.)) Walp. genotypes against the cowpea seed beetle *Callosobruchus maculatus* F. (Coleoptera: Chrysomelidae) in order to minimize the use of synthetic insecticides due to problems associated with their use led to field and laboratory experiments to evaluate the growth and yield of 10 cowpea genotypes on the field and their responses to the insect in the laboratory. A randomized complete block design with three replications and a completely randomized design with four replications were used for the field and laboratory experiments, respectively. The study was undertaken at Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana from the period August 2016 to February 2017. The cowpea genotypes were Asontem, Sanzisanbili, Hans Adua, Zamzam, Nketewade, Videza, IT97K-819-132, Agyenkwa, Bengah and Bianga. The results of the field experiment showed that there were significant differences among the cowpea genotypes in all the growth and yield

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parameters evaluated. Asontem, Hans Adua, Zamzam, Videza and Bianga produced significantly (P < .05) taller plants at maturity. Nketewade produced the highest grain yield (466.1 kgha^{-1}) whiles Sanzisanbili, Hans Adua, Zamzam, Videza, Agyenkwa and Bengah recorded the lowest grain yields. The grain yield ranged from 75.4 to 466.1 kgha^{-1}. Results from the laboratory experiment showed that Videza supported the least number of F_1 progeny, prolonged the development period of *C. maculatus* and suffered the least storage weight loss. Videza was thus the only genotype that showed moderate resistance to *C. maculatus* whiles the other genotypes were either susceptible or highly susceptible.

Keywords: Cowpea genotypes; Callosobruchus maculatus; resistance; susceptibility index; storage.

1. INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) is one of the most important food legumes grown in the arid and semi-arid regions of the world including Africa, Asia, Southern Europe and Central and South America. Cowpea serves as important staple food for humans in poor arid regions and also as soil fertility enrichment [1]. It is mostly grown for its edible grains, although the leaves and green pods can also be consumed before the dried pods are harvested [2]. Cowpea seeds serve as a rich source of proteins, calories, minerals and vitamins and it is also low in anti-nutritional factors [3]. It is estimated that about 200 million people consume cowpea on daily basis [4].

A major constraint to sustainable production and post-harvest preservation of cowpea grains in the tropics is infestation by insect pests. Cowpea is attacked by insects on the field in all stages of the plant growth and also during storage. The storage bruchid, *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) is a cosmopolitan and destructive pest of stored pulses which causes severe economic losses to farmers and traders [5,6]. *Callosobruchus maculatus* begins infestation in the field, but heavy infestation is done in storage [7] and about 95% grain loss could be observed after months of storage depending on location, time and type of cultivar [8].

Various synthetic insecticides in the form of fumigants and grain protectants have been applied or used by farmers for the management of *C. maculatus*. Due to the high costs, unavailability of some of these chemicals in the local markets and the associated negative health and environmental risks posed, chemical control of the pest appears unsustainable.

To minimize the problems associated with the use of synthetic chemicals, the search for host plant resistance in cowpea has intensified. The development and use of resistant cultivars offer a simple and cost effective method of minimizing the bruchid infestation. It also requires little knowledge by farmers, free of extra cost to farmers and enhances the effectiveness of other pest management methods such as cultural and biological control [9]. It was against this background that this study which evaluated agronomic performance of 10 improved cowpea genotypes and their responses to *C. maculatus* infestation in the store, geared towards identification of resistant ones to warrant the use of no or minimal amount of insecticides for their preservation, was undertaken.

2. MATERIALS AND METHODS

2.1 Experimental Location and Source of Cowpea Genotypes

Field and laboratory experiments were conducted at the Department of Crop and Soil Sciences of Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. The field study was conducted at the plantation section of KNUST from the period August 2016 to November 2016 whiles the laboratory experiment spanned the period December 2016 to February 2017. The plantation section is located at 6.40^{o}N latitude and 1.37^{o} E longitude. The soil texture of the land is sandy loam. The temperature in the insect laboratory during the research was 30.1 ± 5℃ and relative humidity was 58.5 ± 12%. The field experiment was conducted with the aim of multiplying the cowpea genotypes and determining their growth and yield characteristics. The laboratory experiment was done to evaluate the responses of the cowpea genotypes to *C. maculatus* infestation. The cowpea genotypes - Asontem, Sanzisanbili, Hans Adua, Zamzam, Nketewade, Videza, IT97K-819-132 and Agyenkwa - were obtained from Crops Research Institute (CSIR-CRI) whiles Bengah and Bianga were obtained from Plant

Genetic Resources Research Institute (CSIR-PGRRI), both of the Council for Scientific and Industrial Research (CSIR) located at Fumesua and Bunso, Ghana, respectively.

2.2 Source and Rearing of Experimental Insects (*C. maculatus*)

Adults of *C. maculatus* obtained from a stock maintained in the insect laboratory of the Department of Crop and Soil Sciences, KNUST were reared on Vita 7, a susceptible cowpea variety in 1.0 litre Kilner jars. The jars were sterilized in an oven at a temperature of 60℃ for 4 h. Twenty pairs of *C. maculatus* were introduced into the Kilner jars containing 500 g of the cowpea grains. The Kilner jars were then covered with muslin cloth to allow gas exchange. Four of such cultures were set up. The adult *C. maculatus* introduced into the Kilner jars were sieved out after seven days of mating and egg laying. The set-up was left on laboratory benches at the insect laboratory and monitored. The offspring (F_1) that emerged from the cultures after 25 days were used as the parent generation to produce the F_2 generation used for the main work.

2.3 Experimental Design and Procedure

For the field experiment a randomized complete block design (RCBD) with three replications was used. There were three blocks and each block contained 10 plots. Each plot measured 5 m × 1.8 m. An alley of 1 m was left between plots in each block and 2 m alley was left between blocks.

A completely randomized design (CRD) with four replications was used for the laboratory experiment. Two separate laboratory set-ups were maintained in 0.5 liter plastic containers and glass Petri dishes (9 cm diameter). The harvested cowpea grains used for the laboratory experiment were put in a freezer for 14 days to sterilize them and thereafter kept in the laboratory for five days for conditioning before being used for the experiment. One hundred grains of each cowpea genotype were counted and put in separate glass Petri dishes that were sterilized in an oven at a temperature of 60℃ for 4 h. In order to monitor oviposition, adult mortality, F_1 progeny emergence and development period, five pairs of 0-1 day old *C. maculatus* were then introduced into separate Petri dish containing cowpea grains. The Petri dishes were then covered and placed on laboratory benches in the insect laboratory and monitored. There were four replications for each treatment (genotype). In order to monitor grain damage and grain weight loss, another set-up was maintained by weighing 200 g of each cowpea genotype using a dial spring scale (CAMRY, Yongkang, China) into plastic containers. Ten pairs of 0-1 day old *C. maculatus* were then introduced into separate plastic container containing the cowpea grains and the containers were then covered with muslin cloth. There were also four replications for each treatment (genotype). The set-up was maintained on the laboratory benches and monitored.

2.4 Data Collection

Growth performance and yield data collected from the field experiment were: number of days to 50% emergence, number of days to maturity, plant height at physiological maturity, number of pods per plant, 1000 seed weight and grain yield which was determined in kg/ha using the formula below:

$$\text{Grain yield (kg / ha)} = \frac{\text{Plot seed weight (kg)}}{\text{Plot area } (\text{m}^2)} \times 10,000 \text{ m}^2 \quad (1)$$

The data collected from the Petri dish and plastic container experiments were: number of eggs laid (oviposition), adult mortality, adult emergence (F_1 progeny), development period, susceptibility index using the formula described by Dobie [10] as:

$$\text{S} = \frac{\log_e F_1}{\text{D}} \times 100 \quad (2)$$

where F_1 = the total number of adults emerged and D = the mean development period which was estimated as the total number of days from middle of oviposition to 50% emergence. Grain weight loss and number of damaged seeds were also taken respectively after two months of storage using the formulas:

$$\text{Grain weight loss (\%)} = \frac{\text{(UNd) - (DNu)}}{\text{U(Nd + Nu)}} \times 100 \quad (3)$$

Where U = weight of undamaged grains, Nu = number of undamaged grains, D = weight of damaged grains and Nd = number of damaged grains using method of Proctor and Rowley [11] and

$$\text{Grain damage (\%)} = \frac{\text{Weight of grains with holes (g)}}{\text{Total weight of grains after storage (g)}} \times 100 \quad (4)$$

Cowpea grains with at least a hole was considered damaged.

For data on oviposition, eggs laid on the surfaces of the grains of each treatment were counted and recorded daily for a period of seven days. For adult mortality, the number of adults that died from each Petri dish was recorded daily for a period of seven days. For each day, any adult found to be inactive when probed with a camel's hair brush was considered dead and removed from the Petri dish. For F_1 progeny emergence, the number of adults that emerged from each Petri dish was recorded daily after emergence of the first offspring until no adult emerged again. This lasted for over 14 days.

2.5 Statistical Analysis

The data collected from both the field and laboratory were subjected to analysis of variance (ANOVA) using Genstat statistical package (Version 12). Numerical data and that in percentages were transformed by square root and arc sine, respectively to correct for heterogeneous variances and to stabilize variances. Treatment means found to be significant were separated using Tukey's honest significant difference (HSD) at 5% level of probability. A correlation analysis was done to ascertain the relationship between the parameters evaluated in the laboratory experiment.

3. RESULTS AND DISCUSSION

3.1 Growth and Yield Performance of Cowpea Genotypes

Table 1 presents the results for the growth and yield performance of the various cowpea genotypes. There were significant differences in the number of days to 50% emergence. IT97K-819-132, Zamzam, Bianga, Bengah and Agyenkwa genotypes took significantly more days for 50% of the plants to emerge compared to the other cowpea genotypes. The mean least number of days taken for 50% emergence was 4.7 days. The number of days to maturity ranged from 53.0 to 59.3 days from the sowing date. The least number of days to maturity was observed in Bengah and Bianga. Mean plant height ranged from 23.1 to 41.9 cm and there were significant differences in plant height among the various cowpea genotypes. Videza produced the tallest plants at physiological maturity which was significantly different from Sanzisanbili, Nketewade, Agyenkwa and Bengah. There were significant differences in the number of pods produced per plant for the cowpea genotypes. Hans Adua produced significantly more pods per plant (17.8 pods) than the other genotypes, with the exception of Nketewade and Bianga which recorded 14.9 and 14.4 pods, respectively. Zamzam produced significantly the heaviest 1000-seed. The least 1000-seed weight was produced by Sanzisanbili. Nketewade produced significantly the highest grain yield (466.1 kgha^{-1}) whiles Asontem and IT97K-819-132 produced the lowest yield (75.4 and 75.6 kgha^{-1} respectively).

Table 1. Effect of cowpea genotypes on growth and yield components in a study conducted in Kumasi, Ghana

Genotype	Number of days to 50% emergence	Number of days to maturity	Plant height at physiological maturity (cm)	Number of pods per plant	1000-seed weight (g)	Grain yield (kg/ha)
Asontem	5.0 cd	57.3 ab	39.8 ab	7.5 cd	103.5 e	75.4 d
Sanzisanbili	5.0 cd	58.0 ab	24.9 c	9.3 cd	72.7 f	173.6 c
Hans Adua	5.3 bcd	58.7 a	32.6 abc	17.9 a	165.5 b	296.2 c
Zamzam	6.3 abc	58.0 ab	32.3 abc	6.3 d	195.6 a	173.4 c
Nketewade	5.0 cd	58.0 ab	26.1 c	14.9 ab	152.8 cd	466.1 a
Videza	4.7 d	59.3 a	41.9 a	11.6 bc	145.8 d	211.7 c
IT97K-819-132	7.0 a	59.3 a	30.9 abc	8.20 cd	148.5 cd	75.6 d
Agyenkwa	7.0 a	59.3 a	29.5 bc	10.0 bcd	159.2 bc	179.7 c
Bengah	6.0 abcd	53.0 c	23.1 c	10.8 bcd	148.6 cd	209.7 c
Bianga	6.7 ab	54.7 bc	33.1 abc	14.4 ab	102.3 e	257.7 b
CV (%)	8.0	2.2	12.2	8.9	2.9	9.3

Means with the same letter(s) are not significantly different (P < .05, according to Tukey)

3.2 Responses of Cowpea Genotypes to *C. maculatus* Infestation

Significantly more eggs were laid on Sanzisanbili grains than all the genotypes with Nketewade and Videza attracting the least oviposition (Table 2). There were no significant differences among the cowpea genotypes with respect to adult mortality by the seventh day of storage. Percentage F_1 adult emergence generally ranged from 39.1 to 82.4%. Significantly more adults emerged from Sanzisanbili, Hans Adua, Zamzam, Nketewade and IT97K-819-132 than Videza and Bianga. Again, significant differences were observed in the development period of *C. maculatus* on the various cowpea genotypes. *C. maculatus* took significantly more days to emerge from grains of Videza than Sanzisanbili, Hans Adua, IT97K-819-132, Agyenkwa and Bianga. There were significant differences among the cowpea genotypes in grain damage after two months (60 days) of storage. The greatest grain damage (80.2%) was recorded in Bianga genotype but that was similar to Asontem (61.8%) and Sanzisanbili (58.3%). The least grain damage was produced in Agyenkwa (20.0%) and Videza (20.1%) but that was similar to Hans Adua, Zamzam and IT97K-819-132 which were not more than 29.8%. There were significant differences among the cowpea genotypes in grain weight losses after two months (60 days) of storage. The least grain weight loss after storage was produced in Videza (Table 2). The susceptibility index analysis shown in Fig. 1 indicates that Sanzisanbili, Hans Adua, Zamzam, IT97K-819-132 and Bengah are highly susceptible to the cowpea seed beetle whiles Asontem, Nketewade, Agyenkwa and Bianga are susceptible. Only Videza was classified as moderately resistant. None of the cowpea genotypes was resistant. The correlation analysis results from Table 3 shows that the mean number of eggs laid (oviposition) showed positive significant correlation with mean percentage adult emergence (r = 0.47), mean percentage storage losses (r = 0.36) and susceptibility index (r = 0.70). There was no significant correlation between mean percentage adult mortality and the rest of the performance parameters. Susceptibility index also showed strong negative significant correlation with mean development period (r = -0.56) but showed strong positive significant correlation with mean percentage adult emergence (r = 0.80) and mean percentage storage losses (r = 0.59). A negative significant correlation was also established between mean percentage storage losses and mean development period (r = -0.47) and also between mean development period and mean percentage adult emergence (r = -0.44).

The susceptibility index ranging from 0 - 11, was used to classify the cowpea genotypes. 0 – 3 means resistant, 4 – 7 means moderately resistant, 8 – 10 means susceptible and ≥ 10 means highly susceptible Dobie [10].

The various cowpea genotypes showed variations in the growth parameters evaluated, corroborating the assertions of [2] that cowpea exhibits a wide range of growth habits. The genotypes showed significant differences in the number of days to 50% emergence which is an indication that some of the seeds of the genotypes were of poor quality and viability. The variation in maturity in the cowpea genotypes

Table 2. Responses of cowpea genotypes to *Callosobruchus maculatus* in an experiment conducted in Ghana

Genotype	Number of eggs laid (Oviposition)	Adult emergence (%)	Adult mortality (%)	Development period (Days)	Grain weight loss (%)	Grain damage (%)
Asontem	59.8 ab	50.5 ab	12.5 a	33.3 abc	22.3 ab	61.8 ab
Sanzisanbili	98.5 a	70.7 a	40.0 a	31.5 bcd	22.8 ab	58.3 ab
Hans Adua	44.8 abc	73.6 a	25.0 a	29.5 bcd	16.2 bc	29.2 de
Zamzam	60.8 ab	68.0 a	10.0 a	33.3 abc	15.6 bc	24.2 de
Nketewade	38.0 bc	69.0 a	30.0 a	32.3 abc	17.1 abc	33.2 cd
Videza	29.5 bc	39.1 b	10.0 a	40.3 a	2.8 d	20.1 e
IT97K-819-132	74.5 ab	82.4 a	25.0 a	24.8 d	29.0 a	29.8 de
Agyenkwa	53.8 abc	53.2 ab	10.0 a	28.5 bcd	9.3 c	20.0 e
Bengah	93.5 ab	61.8 ab	25.0 a	35.3 ab	20.2 ab	47.5 bc
Bianga	19.5 c	40.5 b	27.5 a	27.8 cd	20.8 ab	80.2 a
CV (%)	11.8	5.8	19.9	2.7	8.8	5.2

Means with the same letter(s) are not significantly different (P < .05, according to Tukey)

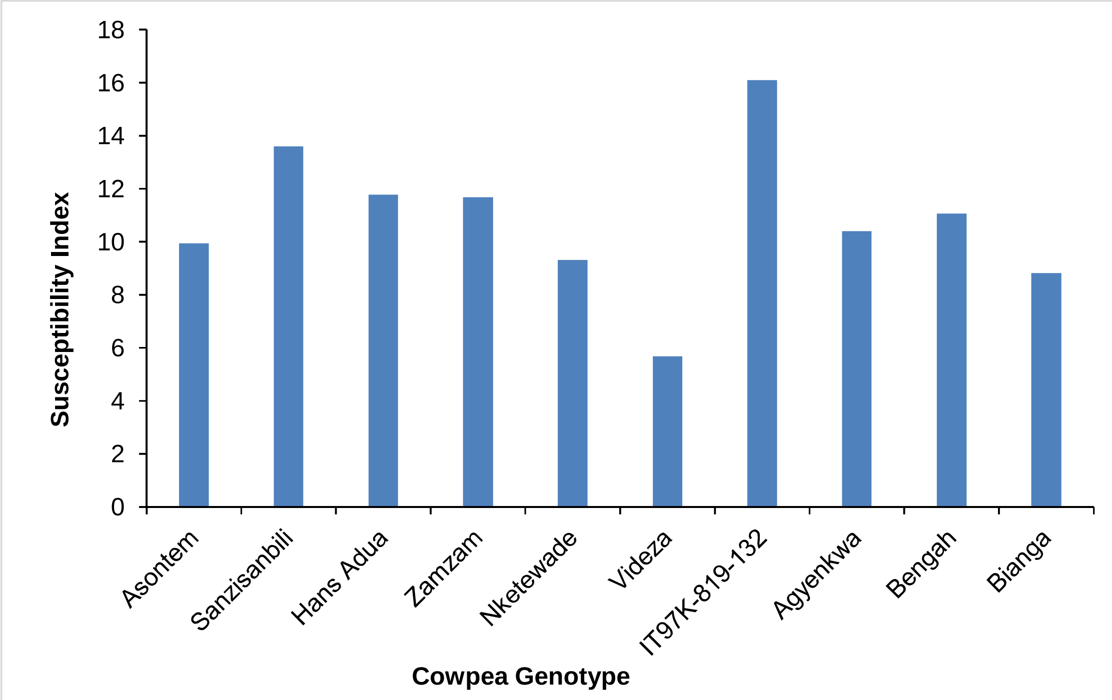

Fig. 1. Susceptibility indices of the various cowpea genotypes against *Callosobruchus maculatus*

Table 3. Correlation analysis of performance parameters

Variety	Adult emergence	Development period	Grain weight loss	S.I	Adult mortality	Oviposition	Grain damage
Adult emergence	_						
Dev. Period	-0.442*	_					
Grain w. loss	0.4056*	-0.4739*	_				
S. I.	0.8011*	-0.5646*	0.5927*	_			
Adult mortality	0.0605	0.0007	0.2547	0.1242	_		
Oviposition	0.4717*	-0.0461	0.3680*	0.7022*	0.0542	_	
Grain damage	-0.2185	-0.1213	0.5403*	0.0150	0.2978	0.0904	_

*Correlation coefficient (r) values with the sign * means significant at 5% level*
Dev. = Development; S. I. = Susceptibility Index; W = Weight

was expected. Ehlers and Hall [2] also observed variation in growth habits of various cowpea genotypes under similar conditions and noted that environmental factors such as rainfall, air and soil temperatures can have negative effect on the growth of some cowpea genotypes. The variation observed in plant height among the cowpea genotypes could be inherent genetic nature of the various cowpea genotypes. Generally, grain yield was low for all the genotypes, and it must be explained that there was erratic rainfall during the flowering and pod forming stages of the plants and this might have impacted negatively on grain yield. Mortimore et al. [12] indicated that, although cowpea is considered a drought-resistant crop, inadequate rainfall or lack of irrigation is a frequent cause of shortfall in production. IT97K-819-132 was among the genotypes that took longer number of days to mature but was among the lowest producing grain yield genotypes. Interestingly, Futuless and Bake [13] also observed that early maturing cowpea genotypes performed very well in terms of yield than relatively late maturing types.

The cowpea seed beetle is known to prefer cowpea as its main host [14]. The study showed that each of the cowpea genotypes suffered varying degrees of attack by *C. maculatus*. This agrees with the findings of Oke and Olajire [15] who observed that cowpea varieties exhibited varying levels of resistance and susceptibility to *C. maculatus* in their work. Significant differences were observed in the various parameters used for evaluating the response of the various cowpea genotypes to *C. maculatus* infestation except mortality. The short life span of *C. maculatus* might have influenced the results on mortality even though Derera et al. [16] suggested that poor handling of insects during infestation can cause increase in mortality and not necessarily a result of genotypic effect. Generally, it can be suggested that adult mortality could be a poor indicator to measure the resistance of cowpea genotypes against *C. maculatus.*

Sanzisanbili, Bengah, Asontem, Hans Adua, Zamzam, Agyenkwa, Bengah and IT97K-819-132 which have smooth textured seed coats attracted more oviposition - they supported the highest number of eggs laid by *C. maculatus.* These genotypes were also found to be either susceptible or highly susceptible. Amusa et al. [17] also reported that cowpea genotype that was preferred most for oviposition by *C. maculatus* was found to be the most susceptible genotype in a study to assess bruchid tolerance of some elite cowpea varieties. Boeke et al. [18] found that more eggs were laid on larger grains than smaller grains of cowpea genotypes. Zamzam produced the heaviest seed weight among the genotypes yet supported less number of eggs laid by *C. maculatus* compared to Sanzisanbili. These suggest that other factors in addition to the size of seeds can determine the preference of *C. maculatus* for oviposition.

Videza supported least number of adults that emerged and hence suffered the least grain damage and grain loss by *C. maculatus.* It has also been reported that cowpea genotypes with the least adult emergence recorded the lowest grain weight loss and grain damage after some period of storage [19,20]. Boeke et al. [18] found that larger seeds supported the emergence of more adults of *C. maculatus* since it provided more food for them. Videza is lighter in weight than Hans Adua, Zamzam, Nketewade, IT97K-819-132, Agyenkwa and Bengah, yet supported the lowest number of adult emergence. It appears that other factors apart from seed size influence progeny emergence in cowpea grains. Miura et al. [21] and Xavier-Filho et al. [22] noted that biochemicals such as trypsin inhibitors, tannins, lectins, lignin, alpha-amylase inhibitors, beta 1, 3-glucanases and chitinases were found in resistant cowpea varieties at varying concentrations. Morrison et al. [23] found twice more lignin in pigmented resistant cowpea seeds than those unpigmented susceptible varieties against *C. maculatus*. Friends [24] noted that the lignin in cowpea seeds helps in cementing and anchoring cellulose fibers in the seeds together to provide mechanical rigidity, chemical, physical and biological protection of cowpea seeds against attack of insects and other pathogens.

Videza significantly delayed the development period of *C. maculatus.* Amusa et al. [17] observed that development period of *C. maculatus* had a significant negative correlation with susceptibility index. Badii et al. [20] also reported that development period was inversely proportional to susceptibility index. It was therefore not surprising that Videza was among the genotypes that supported the least adult emergence of *C. maculatus* and the least susceptibility index.

Videza was also the best genotype in terms of storage weight loss and among the best in terms of seed damage. These results agree with that of Mogbo et al. [25] and Amusa et al. [17] who reported that cowpea genotypes with the lowest number of adult emergence sustained lower seed damage and storage weight loss compared to the other cowpea genotypes with higher adult emergence.

Comparing the performance of the cowpea genotypes to Dobie's scale, none of the genotypes was found to be resistant. However, Videza was found to show some level of resistance (moderate resistance) against *C. maculatus* whiles all the other cowpea varieties ranged from susceptible to highly susceptible. Amusa et al. [17] noted that seed damage, grain weight loss, development period and adult emergence have significant influence on the susceptibility of a cowpea genotype.

There was significant positive correlation among susceptibility index, adult emergence and storage weight loss. As the number of emergence increased, the storage weight loss increased and susceptibility also increased. Singh et al. [19] reported similar results and noted that the higher the number of adult emergence, the higher the grain damage sustained*.* Susceptibility index was found to show significant negative correlation with development period. Beck and Blumer [26] also reported that susceptible cowpea varieties recorded lower number of days for the development of *C. maculatus* than other genotypes they used in their work.

4. CONCLUSION

It can be concluded that Videza was the only cowpea genotype that showed moderate resistance against *C. maculatus*; all the other genotypes were either susceptible or highly susceptible. The most susceptible genotype was IT97K-819-132. Further work can be conducted to identify more resistant genotypes against *C. maculatus* to minimize use of synthetic chemicals to forestall problems associated with the use of these chemicals.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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