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Physical and biochemical attributes of seeds for screening cowpea genotypes for resistance to cowpea weevil {C*allosobruchus maculatus* (f.)}

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Abstract

Cowpea weevil {*Callosobruchus maculatus* (F.)} is an important field-to-store pest responsible for post-harvest loss of cowpea {*Vigna unguiculata* (L.) Walp.} seeds. One of the most economical and environment-friendly ways of preventing losses from the beetle is through the use of resistant cowpea genotypes. Physical and biochemical attributes of seeds play a key role in conferring resistance to the pest. Therefore, seeds of twenty-three cowpea genotypes were assessed for resistance to weevil infestation, nutritional and anti-nutritional factors. Relationship between the resistance parameters and the biochemical components of the seeds were also examined with the use of correlation analyses. Results showed that variability existed among the cowpea genotypes for seed physical resistance parameters such as initial seed weight, residual seed weight, seed weight loss, number of damaged seeds and weight of undamaged seeds. Association of reproductive efficiency with carbohydrate content, crude fibre and moisture content in the cowpea was positive and significant (r = 0.43*, 0.45* and 0.73**) while its relationship with protein content was negative and significant (r=-0.46*). The ash content correlated negatively and significantly (r=-0.46*) with the median developmental period. Cowpea genotype IT08K-125-107 exhibited the highest degree of tolerance to *C. maculatus* infestation, having suffered neither weight loss nor damaged seed and also had very low reproductive efficiency of the *C. maculatus*. The study concluded that while biochemical components played very little role in conferring resistance to *C. maculatus* in cowpea, increased carbohydrate content, crude fibre as well as reduced ash and protein content in the cowpea seeds decreased the reproductive efficiency and intensity of the *C. maculatus* infestation.

Keywords: Weevil; Nutrient; Anti-nutrient; Susceptibility; Resistance; Seed; Post-harvest loss

Introduction

Globally, grain legumes have been classified as the second most important family of crop species after cereals for the provision of food, feed and generation of income (Kebede and Bekeko, 2020). Cowpea, *Vigna unguiculata* (L.) Walp, a grain legume of African origin and a major source of plant protein and carbohydrate is of great social and economic importance in the developing countries of the world. The livelihoods of millions of people in tropical developing nations depend heavily on cowpea (Simion, 2018) and due to their low glycemic index, high quantities of protein, and high fiber content, cowpeas may have beneficial health consequences (Aguilera *et al*., 2013). It is a food that balances cereals and tuber staple crops due to its richness in the essential amino acids (lysine and tryptophan) which are lacking in most cereal crops. It is also an ideal crop for resource-limited farmers because of its ability to fix atmospheric nitrogen and improve the fertility status of the soil (Bisikwa *et al*., 2014, Ddamulira *et al*., 2015), with comparably high yields under harsh conditions where other legumes used as food cannot thrive (Shiringani and Shimelis, 2011). The average yields of cowpea in sub-Sahara Africa are very low because of the use of unimproved cultivars, substandard management practices and insufficient inputs (Kyei-Boahen *et al*., 2017). A host of biotic stress factors such as bacteria (*Xanthomonas campestris* pv.*vignicola*), virus (Cowpea Aphid-Borne Mosaic Virus, CABMV), parasitic plants (*Striga gesneroides* and *Alectra vogelii*) and insect pests are responsible for low yield recorded in the production of cowpea. Cowpea is attacked by complex insect pests throughout their stages of development, up to the store and an infestation particularly by the cowpea beetle {*Callosobruchus maculatus* (F.)}, a field-to-store pest which causes economic damage (Allotey *et al*., 2012).

Callosobruchus maculatus causes losses to both the quantity and quality of stored seeds with its infestation starting on the field and becoming more prevalent under storage. If unchecked, storage populations of *C. maculatus* can grow exponentially causing significant losses in seed weight, germination potential, viability and marketability (Adebayo and Eyo, 2014). This usually causes a reduction in the usefulness of seeds thereby, impairing them for use as food or for agronomic purposes (Ali *et al.,* 2004). This poses a major threat to farmers, traders and consumers because of its economic effect and contamination with mycotoxins which can compromise the nutritional status of infested cowpea (Atanda *et al*., 2012).

Several control measures for the management of bruchids such as physical, chemical, and biological methods have been expensive, not sustainable, and pose environmental or health hazards to the consumers (Adebowale and Adedire, 2006; Cissokho *et al*., 2015). The development and deployment of cowpea genotypes with a level of resistance to bruchids are one of the most economical, inexpensive, sustainable and environmentally friendly options to tackle the menace that is responsible for postharvest losses of cowpea. Reports have shown that the presence of phytochemicals such as anti-nutritional factors may confer a level of resistance to insect attacks and the presence of these factors commonly found in legumes is a major factor limiting the wider food use of these essential tropical plants (Liener, 1980). For instance, phytic and oxalic acid reduce mineral bioavailability that leads to various mineral deficiency diseases e.g., anaemia (Guthrie and Picciano, 1995) while anti-nutrients have been shown to possess pharmacological values. Tannins for example, possess anti-cancer and cytotoxic properties (Das and Mahato, 1983; Schopke and Hiller, 1990; Koratkar and Rao, 1997; Wakabayashi *et al*., 1997). Elevated levels of trypsin inhibitors have been reported as being responsible for resistance to *C. maculatus* in some cowpea genotypes (Gatehouse *et al*., 1979). Lattanzio *et al*., (2005) also reported a positive association of tannin in the seed coat with development time and mortality rate. To design an effective breeding strategy for developing resistant genotypes, it is importantto understand the mechanism that governs resistance (Tripathi *et al.,* 2015) and the role that metabolites play in conferring resistance in cowpea genotypes that are resistant to bruchid attack. Ajeigbe *et al*., (2008), Sharma and Thakur (2014) and Meisho *et al.,* (2018) reported that anti-nutritional factors may play an essential role in conferring resistance to bruchids in cowpea. The aim of this study was, therefore, to evaluate the physical and biochemical composition of seeds from twenty-three cowpea genotypes and investigate their influence in conferring resistance to *C. maculatus*.

Materials and Methods

Location of the experiment

The experiment was carried out in the Department of Agronomy, Faculty of Agriculture, University of Ilorin, Ilorin, Kwara State located in the Southern Guinea Savanna of Nigeria (8.49°North, 4.59° East). The study was conducted under a prevailing temperature of 24 ± 2℃, relative humidity of 65 ± 5% and 12-hour photophase.

Sources of experimental materials

The materials used for the study comprised twenty-three (23) cowpea genotypes collected from the International Institute of Tropical Agriculture (IITA), the Institute of Agricultural Research and Training (IAR&T) and the local market at Apata. The three locations are in Ibadan, Oyo State, southwestern Nigeria (Table 1).

Insect culture

The parental stock was obtained from an existing culture at the Department of Crop Protection, Faculty of Agriculture, University of Ilorin. Fifty unsexed *C. maculatus* adults were picked with the aid of a pooter (aspirator) and used to infest susceptible 500 g cowpea seeds in a 1 L Kilner jar which was covered with a muslin cloth to allow for aeration and prevent insect escape. The beetles were allowed to mate and lay eggs and then removed 5 days after infestation. Teneral adults (1-2 days old) that emerged from the culture were used for the study.

Sterilization of cowpea genotypes

Prior to the commencement of experiments, 100 clean seeds of each of the twenty-three cowpea genotypes were picked, weighed and stored in cold storage at -20℃ for about 14 days to kill any infesting beetle. After this period, the sterilized seeds were allowed to equilibrize with the environmental conditions of the laboratory for three days.

Table 1: Cowpea genotypes used in the study and their sources

S/N	Genotypes	Source
1	SAMPEA-15	IAR
2	SAMPEA-14	IAR
3	SAMPEA-11	IAR
4	IT98K-573-2-1	IITA
5	IT13K-993-6	IITA
6	IT13K-1427-3	IITA
7	IT13K-1424-12	IITA
8	IT13K8-1329-8	IITA
9	IT13K-1000-3	IITA
10	IT10K-973-1	IITA
11	IT10K-836-4	IITA
12	IT10K-836-3	IITA
13	IT10K-815-5-B	IITA
14	IT10K-815-5-A	IITA
15	ITI0K-292-10	IITA
16	IT07K-298-15	IITA
17	IT107K-125-107	IITA
18	IT07K-318-33	IITA
19	IT07K-298-9	IITA
20	IT07K-284-1-2	IITA
21	IT07K-210-1-1	IITA
22	Ife-Brown	IAR&T
23	Oloyin	Apata market

IAR: Institute of Agricultural Research, Samaru, Zaria; IAR&T: Institute of Agricultural Research and Training, Moor Plantation, Ibadan IITA: International Institute of Tropical Agriculture, Moniya, Ibadan.

Development of the insect on the different Cowpea Accessions

Ten sterilized seeds of each cowpea genotype were infested with four 1-2 days old adult *C. maculatus* in a sex ratio 1:1 inside 150 mm plastic containers. Lids made with nets were used to cover the containers for proper ventilation and to prevent bruchid escape. The experimental set-up, including the uninfested control, was arranged in a completely randomized design with four replications. The introduced adults were removed from the container after 10 days of oviposition to allow for the progeny emergence and the number

of eggs per container was counted from the 5^{th} to the 20^{th} day with the use of a hand-held lens.
The emergence of the first filial generation (F_1) progeny of cowpea weevil was checked on a daily basis from the 21^{st} day after infestation up to when there will be no more emergence for five consecutive days. All adult present per day were counted, eliminated and killed instantly by freezing at -5°C for 10 minutes. The process was repeated till there was no emergence of bruchids.

Determination of biochemical components
The samples of different cowpea genotypes were homogenized and analyzed for different biochemical components i.e., nutritional (carbohydrate, crude protein, crude fibre, crude fat, ash and moisture content) and antinutritional factors (flavonoids, phenolic compound, trypsin inhibitors, oxalate and phytates). The proximate analysis was conducted using standard methods of the Association of Official and Analytical Chemists (AOAC) (2003). The carbohydrate was calculated using the difference of the crude fat, crude fibre, moisture and ash content from 100 g. Phenolics were determined using the Folin-Ciocalteu reagent as described by Singleton and Rossi (1965). The calculations were carried out from a standardized base of a calibration curve on tannic acid (0-0.1 mg/mi) and the result was expressed in mg per g dry weight (mg TAE/g DW). Total flavonoids were determined using spectrophotometricericic protocols described by Tiwari *et al.,* (2013) while the phytate was extracted and precipitated in accordance with the method described by Wheeler and Ferrel (1971), the oxalate was however determined using the method described by Day and Underwood (1986).

Data collection
Data were collected on the following parameters:
NEPC: Number of eggs per container, PEW: Population of emerged weevil, ISW: Initial seed weight, MDP: Median developmental period NDS: Number of damaged seeds, NUDS: Number of undamaged seeds, RSW: Residual seed weight, SWL: WDS: Weight of damaged seeds, WUDS: Weight of undamaged seeds.

A number of parameters were determined as follows:
SWL: Seed weight loss, PWL: Percentage weight loss, PT: Percentage tolerance, RE: reproductive efficiency and SI: Susceptibility index
Seed Weight loss = Initial seed weight – Residual seed weight

$$\text{Percent weight loss} = \frac{\text{Initial weight of seed- Residual weight of seed}}{\text{Initial weight of seed}} \times 100$$

$$\text{Reproductive efficiency RE} = \frac{\text{Number of}F_1\text{ Adult}}{\text{Number of eggs}} \times 100$$

(Babarinde and Ewete 2008)

$$\text{Index of susceptibility} = \frac{\text{Log}_e\text{(total number of offspring)}}{\text{Median development time}} \times 100$$

(Dobie 1977)

The index rating was used in categorizing the cowpea genotypes as resistant or susceptible, where 1–5 = resistant; 6– 10 = moderately resistant; 11–15 = susceptible; and >16 = highly susceptible (Chakraborty, Mondal, and Senapati 2015)

Data Analysis

Data collected were subjected to analysis of variance (ANOVA) and where the F-value was significant, mean values were separated using Least Significant Difference (LSD) at $P \leq 0.05$. The relationship between the variables were investigated with PROC CORR statement on SAS v. 9.3 (SAS, 2003).

Results and Discussion

Results

The results of the mean square from the one-way analysis of variance of the cowpea weevil susceptibility parameters (Table 2) revealed that there were significant differences ($\rho \leq 0.05$ and 0.01) among the cowpea genotypes for only number of eggs per container (NEPC), initial seed weight (ISW), residual seed weight (RSW), weight of undamaged seeds (WUDS) and seed weight loss (SWL). The population of emerged weevils was not statistically different among the cowpea genotypes, although, more weevils emerged from variety IT107K-298-15 (55.75) while IT07K-125-107 recorded the least number of emerged weevils. There were significant differences among the cowpea genotypes with respect to the ISW and RSW, the values ranged from 1.29 g(IT07K-210-1-1) and 0.88 g (IT13K-1329-8) to 1.9 g(IT07K-292-10) and 1.74 g (IT13K-1424-12) with mean values of 1.64 g and 1.45 g respectively. In a similar manner, significant differences existed among the genotypes for WUDS with varieties IT07K-284-1-2, IT07K-125-107 recording the highest weight for undamaged seeds. All the twenty-three cowpea genotypes recorded loss in weight except IT07K-125-107. The percentage seed weight loss was lowest in IT08K-125-107 (0%) and highest in IT13K-1328-8 (33.83%) while the highest percentage of damaged seed was recorded in IT10K-973-1 (62.5%) with lowest damaged seeds obtained in IT08K-125-107. The percentage tolerance was within the range of 22.5% (IT107K-298-15) to 100% (IT08K-125-107) while the susceptibility index ranged from 0 (IT08K-125-107) to 15.46 in IT107K-298-15.

Nutritional and biochemical composition of the twenty-three cowpea genotypes

The nutritional (Carbohydrate, crude fibre, crude fat, crude protein, ash and moisture) composition of twenty-three cowpea genotypes are presented in Table 3. The values of the moisture content and total carbohydrate content among the genotypes were comparable while significant differences were observed among the genotypes for ash, crude protein, crude fat and crude fibre. Variety IT107K-125-107 had the highest ash content, SAMPEA-11 contained more crude protein, IT10K-836-4 had the highest crude fat and ITI0K-292-10 contained the highest crude fibre content.

The quantitative estimates of the other non-nutritional biochemical (Trypsin inhibitor, phenolic compound, oxalate, phytate and flavonoid) components presented in Table 4 revealed significant differences among the cowpea genotypes. IT07K-318-33 contained the highest phenolic compound, IT13K-1000-3 had the highest oxalate content, IT107K-125-107, IT10K-815-5-A and OLOYIN recorded the highest phytate composition, OLOYIN contained more flavonoids than the other genotype and SAMPEA-11 contained the highest trypsin inhibitor content.

Correlation of susceptibility parameters with biochemical composition of twenty-three cowpea genotypes

The nature of association between the *Callosobruchus maculatus* susceptibility parameters and the biochemical composition revealed that the percentage weight loss had a negative but significant correlation (r=0.76**) with the residual seed weight. The susceptibility index (SI) was significantly and negatively correlated (r=-0.79**) with the percentage tolerance. The phenolic compound had positive and significant correlation (r=0.54** and 0.61) with the flavonoid and oxalate composition of the cowpea genotypes respectively. The trypsin inhibitor had a significant and negative correlation (r=-0.43) with the oxalate compound while the moisture content. The carbohydrate content, crude fibre and moisture content in the cowpea were positivelyand significantly correlated with the reproductive efficiency with r values of 0.43*, 0.45* and 0.73** respectively while the crude protein was negatively and significantly correlated (*r* = - 0.46*) with the reproductive efficiency. The ash content was negatively and significantly correlated (*r* = - 0.46*) with the median developmental period

Table 2: Mean square from one-way analysis of variance (ANOVA) for *Callosobruchus spp* resistance screening parameters

Source of Variation	DF	PEW	NEPC	RSW	SWL	PWL	PT	WDS	WUDS
Replicate	3	27089**	0.06	0.96**	0.00	132.12	20963.2**	1.5**	4.34**
Genotype	22	1155.64*	0.62**	0.55**	0.26*	78.39	1660.03	0.19	0.68*
Error	66	1595.92	0.0	0.10	0.01	87.45	1437.79	0.13	0.34

*, **: Significant F- Test at 0.05 and 0.01 levels of probability respectively.
DF: Degree of freedom, PEW: Population of emerged weevil, NEPC: Number of emerged weevil per container, ISW: Initial seed weight, RSW: Residual seed weight, SWL: Seed weight loss, PWL: Percentage weight loss, PT: Percentage tolerance, WDS: Weight of damaged seeds, WUDS: Weight of undamaged seeds.

Table 3: *Callosobruchus maculatus* developmental parameters and seed weight loss analysis of twenty-three cowpea genotypes**.**

S/N	**Genotype**	**NEPC**	**PEW**	**MDP**	**ISW (g)**	**RSW (g)**	**WDS (g)**	**WUDS (g)**
1	IFE-BROWN	36.00	2.50	29.25	1.50	1.48	0.13	1.34
2	IT07K-210-1-1	86.30	30.75	27.00	1.29	1.24	0.21	1.02
3	IT07K-284-1-2	15.00	0.75	28.75	1.68	1.66	0.04	1.71
4	IT07K-292-10	134.50	27.25	28.00	1.90	1.46	0.04	1.42
5	IT07K-298-9	7.50	0.25	26.75	1.56	1.25	0.44	0.81
6	IT07K-318-33	227.30	29.5	28.5	1.66	1.44	0.24	1.19
7	IT08K-125-107	0.00	0.00	0.00	1.69	1.69	0.15	1.61
8	IT107K-298-15	371.50	55.75	26.00	1.68	1.39	0.57	0.81
9	IT10K-815-5-A	117.50	18.75	26.75	1.86	1.57	0.39	1.17
10	IT10K-815-5-B	256.50	34.25	27.00	1.67	1.66	0.05	1.69
11	IT10K-836-3	111.00	17.50	29.75	1.72	1.32	0.81	0.49
12	IT10K-836-4	102.30	20.50	27.00	1.70	1.63	0.34	1.29
13	IT10K-973-1	0.00	0.50	30.25	1.78	1.46	0.47	0.93
14	IT13K-1000-3	73.80	22.25	27.50	1.64	1.37	0.43	0.94
15	IT13K-1329-8	120.00	48.50	26.25	1.33	0.88	0.66	0.25

16	IT13K-1424-12	174.80	13.50	28.25	1.87	1.74	0.47	1.34
17	IT13K-1427-3	206.80	45.25	26.00	1.72	1.38	0.40	1.03
18	IT13K-993-6	340.50	26.50	28.00	1.71	1.59	0.00	1.71
19	IT98K-573-2-1	110.00	37.25	27.75	1.78	1.71	0.37	1.33
20	OLOYIN	26.30	7.75	26.25	1.54	1.51	0.06	1.49
21	SAMPEA-11	141.50	14.00	27.00	1.71	1.41	0.19	1.2
22	SAMPEA-14	7.50	0.25	30.00	1.32	1.08	0.24	0.85
23	SAMPEA-15	310.30	50.75	26.25	1.5	1.47	0.5	0.96
	Mean	129.43	20.86	26.45	1.64	1.46	0.31	1.16
	LSD	4.18**	1.10**	2.75**	0.08**	0.05**	0.06**	0.21**
	CV(%)	2.3	3.6	7.4	3.0	2.1	15.5	11.3

PEW: Population of emerged weevil, NEPC: Number of emerged weevil per container, MDP: Median Development Period, ISW: Initial seed weight, RSW: Residual seed weight, PT: Percentage tolerance, WDS: Weight of damaged seeds, WUDS: Weight of undamaged seeds.

Table 4: Classification of twenty-three cowpea Genotypes based on the index of susceptibility

S/N	Genotype	SWL	PT	RE	SI	Classification
1	IFE-BROWN	0.02	75	6.94	3.13	Resistant
2	IT07K-210-1-1	0.05	75	35.63	12.69	Susceptible
3	IT07K-284-1-2	0.02	72.5	10	1.41	Resistant
4	IT07K-292-10	0.44	50	20.26	11.8	Susceptible
5	IT07K-298-9	0.31	95	33.33	3.43	Resistant
6	IT07K-318-33	0.22	80	12.98	11.86	Susceptible
7	IT07K-125-107	0	100	0	0	Resistant
8	IT107K-298-15	0.29	50	15.01	15.46	Susceptible
9	IT10K-815-5-A	0.29	72.5	15.96	10.96	Susceptible
10	IT10K-815-5-B	0.01	62.5	13.35	13.09	Susceptible
11	IT10K-836-3	0.4	67.5	15.77	9.62	Susceptible
12	IT10K-836-4	0.07	72.5	20.03	11.19	Susceptible
13	IT10K-973-1	0.32	37.5	7.33	3.2	Resistant
14	IT13K-1000-3	0.27	97.5	30.15	11.28	Susceptible
15	IT13K-1329-8	0.45	75	40.41	14.79	Susceptible
16	IT13K-1424-12	0.13	62.5	7.72	9.21	Susceptible
17	IT13K-1427-3	0.34	47.5	21.88	14.66	Susceptible
18	IT13K-993-6	0.12	92.5	7.78	11.7	Susceptible
19	IT98K-573-2-1	0.07	95	33.86	13.04	Susceptible
20	OLOYIN	0.03	50	29.47	7.8	Moderately Resistant

21	SAMPEA-11	0.3	97.5	9.89	9.77	Moderately Resistant
22	SAMPEA-14	0.24	72.5	14.67	0.32	Resistant
23	SAMPEA-15	0.03	97	16.36	14.96	Susceptible
	MEAN	0.18	73.76	15.98		
	LSD	0.03**	0.06**	4.48		
	CV%	9.4	0.6	19.90		

SWL: Seed weight loss, Percentage Tolerance, RE: Reproductive efficiency, SI: Susceptibility index

Table 5: Percentage nutritional composition of twenty-three cowpea genotype

S/N	Genotypes	Moisture Content	Ash	Crude protein	Crude fat	Crude fibre	Carbohydrate
1	SAMPEA-15	6.82	2.40	28.26	1.52	3.01	57.99
2	SAMPEA-14	7.05	2.04	29.30	1.91	3.11	56.59
3	SAMPEA-11	7.11	2.39	31.67	1.17	2.78	55.71
4	IT98K-573-2-1	7.05	2.45	29.59	1.79	2.98	56.15
5	IT13K-993-6	6.86	2.90	30.37	1.61	2.47	55.79
6	IT13K-1427-3	6.26	2.49	30.97	1.11	3.10	56.38
7	IT13K-1424-12	7.05	2.08	29.33	1.21	2.52	57.82
8	IT13K8-1329-8	7.32	2.08	26.62	1.66	2.35	59.54
9	IT13K-1000-3	6.93	2.53	28.99	1.63	2.39	57.63
10	IT10K-973-1	6.92	2.45	27.66	1.78	2.04	58.88
11	IT10K-836-4	7.41	2.75	29.25	1.99	1.99	56.76
12	IT10K-836-3	7.09	2.61	27.43	1.25	3.00	58.84
13	IT10K-815-5-B	7.04	2.39	28.18	1.29	3.30	57.77
14	IT10K-815-5-A	7.09	2.42	31.63	1.64	2.82	54.66
15	ITI0K-292-10	6.99	2.17	26.88	1.86	1.83	59.97
16	IT07K-298-15	6.98	2.48	28.37	1.66	2.33	57.71
17	IT107K-125-107	7.11	2.97	31.24	1.12	2.02	55.80
18	IT07K-318-33	7.07	2.72	29.16	1.22	3.08	56.83
19	IT07K-298-9	7.11	2.66	26.49	1.64	2.88	59.35
20	IT07K-284-1-2	6.94	2.54	26.53	1.52	2.99	59.56
21	IT07K-210-1-1	7.03	2.47	27.10	1.38	3.07	59.26
22	IFE-BROWN	6.26	2.12	29.98	1.29	3.19	57.17
23	OLOYIN	6.92	2.47	27.56	1.91	2.85	58.30
	MEAN	6.97	2.45	28.81	1.53	2.70	57.58
	LSD	0.58	0.21**	2.86*	0.08**	0.15**	8.11
	C.V. (%)	1.36	1.96	1.49	2.3	2.38	0.35

Table 6: Biochemical composition of twenty-three cowpea genotypes

S/N	Genotypes	Phenolic compound mg/kg	Oxalate mg/kg	Phytate mol/kg	Flavonoid mg/kg	Trypsin inhibitor mg/kg
1	SAMPEA-15	3.05	0.64	0.04	2.96	5.18
2	SAMPEA-14	2.01	0.49	0.02	2.33	4.27
3	SAMPEA-11	2.87	0.16	0.041	2.26	5.76
4	IT98K-573-2-1	3.02	0.91	0.06	2.55	4.36
5	IT13K-993-6	3.72	1.01	0.04	10.01	2.55
6	IT13K-1427-3	3.02	0.28	0.02	3.49	4.93
7	IT13K-1424-12	2.67	0.54	0.04	2.85	5.31
8	IT13K8-1329-8	3.16	0.65	0.06	1.94	4.74
9	IT13K-1000-3	4.02	1.30	0.06	9.31	3.26
10	IT10K-973-1	2.85	0.68	0.06	4.30	2.81
11	IT10K-836-4	3.80	0.57	0.03	3.32	3.05
12	IT10K-836-3	1.96	0.91	0.04	2.77	3.77
13	IT10K-815-5-B	4.68	0.97	0.05	8.65	3.59
14	IT10K-815-5-A	1.99	0.28	0.07	10.94	4.85
15	ITI0K-292-10	2.65	0.54	0.02	3.45	2.25
16	IT07K-318-33	4.93	0.96	0.06	6.56	2.15
17	IT107K-289-9	2.71	0.94	0.06	2.68	3.55
18	IT07K-298-15	3.85	0.96	0.04	3.41	3.00
19	IT07K-1284-1-2	2.99	0.36	0.05	2.87	3.56
20	IT07K-210-1-1	3.08	0.57	0.06	3.21	1.64
21	IT07K-125-107	2.95	0.21	0.07	3.17	4.04
22	IFE-BROWN	4.16	1.06	0.06	7.95	3.09
23	OLOYIN	4.80	1.21	0.07	12.91	3.81
	Mean	3.26	0.70	0.05	4.95	3.71
	LSD	0.15**	0.06**	0.002*	0.08**	0.61**
	C.V. (%)	26.4	47.10	35.10	65.60	29.31

Table 7: Pearson's correlation between cowpea seeds biochemical attributes and *Callosobruchus maculatus* susceptibility parameters of twenty-three cowpea genotypes

	RSW	PWL	PT	RE	MDP	SI	PC	O	FL	TI	MC	ASH	CP	CF	CHO
RSW															
PWL	-0.72**														
PT	-0.01	-0.08													
RE	-0.35	0.35	0.18												
MDP	-0.23	0.26	-0.20	0.30											
SI	-0.08	0.16	-0.79**	-0.04	0.24										
PC	0.21	-0.39	-0.25	-0.07	0.02	0.24									
O	-0.05	-0.08	-0.29	0.19	0.33	0.17	0.61**								
FL	0.27	-0.28	-0.11	-0.07	0.09	0.07	0.54**	0.50							
TI	0.02	0.10	-0.11	-0.20	-0.12	0.06	-0.39	-0.43*	-0.21						
MC	0.01	0.15	0.32	0.73**	0.14	-0.38	-0.10	-0.02	-0.04	-0.18					
ASH	0.36	-0.29	0.06	-0.13	-0.46*	-0.05	0.22	0.08	0.16	-0.31	-0.01				
CP	0.36	-0.18	0.01	-0.46*	-0.33	0.02	-0.05	-0.35	0.21	0.40	-0.15	0.19			
CF	-0.15	0.12	0.20	0.45*	0.31	-0.05	-0.03	0.25	0.12	-0.23	0.20	-0.14	-0.36		
CHO	-0.36	0.23	-0.01	0.43*	0.28	-0.06	-0.01	0.23	-0.28	-0.35	0.19	-0.27	-0.95**	0.19	

Discussion

Cowpea breeding and selection activities aimed at developing high yielding and *C. maculatus*-resistant genotypes need to also consider improvement of populations for desirable biochemical traits. The result of the study showed that the genotypes exhibited varying levels of *C. maculatus* resistance/susceptibility as well as in some biochemical composition. The difference in the resistance to *C. maculatus* is in consonance with the findings of Mogbo *et al*., 2014 and could be exploited to develop new cowpea genotypes with combined genes for superior grain yield, biochemical attributes and resistance to *C. maculatus*. This is attested to by the significant differences among the genotypes for traits such as initial seed weight, residual seed weight, number of damaged seeds, seed weight loss and the weight of undamaged seeds. Thus, the variability among the cowpea genotypes will aid selection of superior progenies that could be utilized for development of improved cowpea genotypes. Various parameters such as the number of emerged adults, the median developmental period, reduced loss of grain weight have also been used as indicators for classifying cowpea genotypes either as resistant or susceptible to stored insect pests (Sharma and Thakur, 2014, Adebayo *et al*., 2016, Meisho *et al*., 2018). However, the lack of significant differences among the genotypes for population of emerged adults and number of eggs laid suggests that the genotypes' capacity to discourage oviposition by the bruchid were similar.

The means of various cowpea physical attributes and *C. maculatus* resistance parameters revealed that the cowpea genotypes with varying initial seed weight had varying levels of susceptibility indices revealing that susceptibility/resistance to the bruchid are not dependent on the weight or sizes of the grain. This finding corroborates earlier report of Kpoviessi *et al*. (2020) that the size/weight of the grains did not influence resistance to attack by the bruchid. This however is not in line with the findings of Jackai and Asante (2003) who reported that the number and the weight of damaged cowpea seeds is an important factor when classifying cowpea genotypes either as susceptible or resistant, and that variables such as reduction in weight of seed, and growth indices are reliable pointers for bruchid resistance. The result of analysis of the biochemical composition revealed that the moisture content of the seeds in the genotypes was low which is an indication that the seeds will have a long shelf life and will not deteriorate easily under storage conditions. The values obtained were lower than 9.69% reported for brown cowpea but higher than those reported for the white (Aletan, 2018).The ash content which is an indication of mineral content of the seeds in the genotypes evaluated is slightly lower than those reported by Gondwe *et al*. (2019) IT07K-125-107 contained the highest ash content among the genotypes which could be the cause of the resistance to the *C.maculatus* infestation. Insecticidal properties have been reported to be possessed by ash which prevents attack by store pests (Giga, 1995, Swella and Moshobozy, 2007). Although, the crude protein content obtained in this study was higher than those reported by Ajeigbe *et al*., (2008), it is still within the range reported by Mamiro *et al*., (2011). The slightly low mineral content of the cowpea genotypes can be attributed to the presence of other compounds such as phytic acid and tannins which were moderate while the protein content in some of the genotypes can be utilized where the breeding focus is in developing varieties that will serve as cheap source of plant protein to alleviate protein deficiency in many countries in Africa. However, the carbohydrate and crude fat content fell within the range of values reported by Sharma and Thakur (2014). The crude fibre content contains pectic, cellulose, hemicellulose, lignin and cutin substances. The values obtained in this study were slightly higher than those previously reported by Ajeigbe *et al*., (2008). This implies that they are excellent sources of dietary fibre which aids digestion and reduces cholesterol level. Consequently, these genotypes can be used as parents in breeding for high fibre content in cowpea. The total flavonoid and phenolic content values obtained in the genotypes were lower than those reported in previous study by Meisho *et al*., (2017) while values obtained for trypsin inhibitors were also lower than those reported by Sharma and Thakur (2014). The differences in values can be attributed to the differences in both the inherent genetic properties of the genotypes and the method used for the analysis. Cowpea like many legumes have been known to contain anti-nutritional factors and while they have been selected against because of the negative effect they have on the quality of food, several studies have shown that they play a key role in conferring resistance against insect (Ajeigbe *et al.,* 2008, Shama and Kumar, 2014).

Association of SI parameters with nutritional and anti-nutritional factors which was not significant in the cowpea genotypes evaluated implies that susceptibility to *C. maculatus* may be independent of the biochemical attributes. This observation partially corroborates the findings of Sharma and Thakur (2014) who hypothesized that nutritional factors are not responsible for conferring resistance to *C. maculatus* in cowpea. In other words, breeding for increased biochemical traits may not necessarily increase the level of resistance to bruchids. Our results however contradict earlier reports (Ajeigbe *et al*., 2008, Miesho *et al.,* 2018) which provided evidence that some biochemical traits inherent in plants are able to confer tolerance to pest attack. The positive and significant correlation of carbohydrate content, crude fibre and moisture content with reproductive efficiency of the bruchids suggests that high moisture content favours the development of *C. maculatus* in cowpea. Consequently, increased carbohydrate, crude fibre and reduced crude protein can increase the reproductive efficiency and intensity of the infestation of the insect. Also, the negative but significant correlation of ash content with the median developmental period suggests that the higher the ash content in the cowpea, the faster the emergence of F_1 progenies and subsequently the intensity of infestation of the cowpea genotypes by *C. maculatus*. This observation agrees with the findings of Demissie *et al*., (2015) who reported negative significant correlation between the ash content and median developmental period in different varieties of maize.

Conclusions

Among the accessions evaluated, IT08K-125-107 and five other cowpea genotypes including SAMPEA-14 were classified as resistant and could serve as sources of genes for development of *C. maculatus* resistant cowpea genotypes. Also genotypes SAMPEA-11 and IT10K-815-5-A can serve as parental materials if the breeding interest is in increasing the protein content in the test crop while IT08K-125-107 that exhibited resistance to *C. maculatus* can be used as parents for high mineral or vitamin content because of the high ash content.

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