Evaluation of Selected Cowpea Lines and Cultivars for Inherent Resistance against Cowpea Seed Beetle, Callosobruchus maculatus Fabricius (Coleoptera: Chrysomelidae: Bruchinae)

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Abstract
Four cowpea lines (IT99K-494-6, IT97K-390-2, IT84S-2246-4 and IT06K-141) obtained from the Genetic Resources Centre, International Institute of Tropical Agriculture (IITA), Ibadan Nigeria, one cultivar (Ife Brown) obtained from Institute of Agricultural Research and Training (IAR&T), Obafemi Awolowo University, Ibadan and two cultivars (Drum and Oloyin) obtained from Wazobia Market, Ogbomoso, Nigeria were screened for comparative susceptibility to the infestation of cowpea seed bruchid, *Callosobruchus maculatus.* The number of eggs laid on IT99K-494-6 (20.05) was significantly ($p < 0.05$) lower than the number laid on all the local cultivars (56.33-78.83). First filial progeny emergence (0.71) observed in IT99K-494-6 was significantly ($p < 0.05$) lower than 2.06 and 1.65 observed in Ife Brown and Drum cowpea cultivars, respectively. Percentage seed damage for 1T99k-49-6 (10.14) was significantly lower than the value obtained in 1T06K-141 (49.93) and Drum (47.74). Alkaloids content was significantly higher in IT06K–141 (31.67 mg/100 g) than in other lines and cultivars. Oloyin had the highest flavonoid (51.7 mg/100 g), tannins (43.3 mg/100 g) whereas 1T84S-2246-4 had the highest saponins (61.7 mg/100 g) Drum had the highest terpenoid (33.33 mg/100 g). Steroid was highest in Oloyin and 1T84S-2246-4 (11.67 mg/100 g). Reducing sugar was highest in Oloyin (5.33 mg/100 g) followed by 1T84S-2246–4 (4.3 mg/100 g) and IT06K–141 (4.0 mg/100 g). Significant and positive relationship exists between saponins content and the number of grains without exit holes ($r = 0.46$, $p = 0.04$), suggesting that high saponins was contributory to cowpea host plant resistance to the cowpea seed bruchid, *C. maculatus.*
Keywords: cowpea cultivars, Drum, Oloyin, host plant resistance

INTRODUCTION

Pulses are major staple arable crops in many developing countries due to their ease of cultivation and their nutritional benefits. Cowpea, a major source of protein belongs to this group of crop and it is been consumed by both resource-poor and the rich. Incidentally, its all year round supply is being threatened by the menace of pest infestation and the cowpea seed bruchid, *Callosobruchus maculatus* is a major pest attacking it on the field and at postharvest level. The damage symptoms of the pest as highlighted by Babarinde *et al.* (2015) include weight loss, reduction in marketability value and loss of germination potentials.

In times of emergency, its effective control centres mainly on the use of synthetic pesticides. Several problems like food poisoning, pesticide resistance (Odeyemi *et al.*, 2006) ecological toxicity (Adedire, 2002) and emergence of secondary pests are, however, associated with abuse of synthetic pesticides. In Nigeria, the Africa's major producer of cowpea with 1.5 million tons annually, the net gains from not using chemicals have been estimated to be about US$ 500 million (Bafana, 2010). Although these net gains include the gains of not using pesticides on the field and at postharvest, it is an index of high cost implication of pesticide usage in cowpea production in Nigeria. Therefore, investigating other non-chemical basis of controlling the pest cannot be out of order, if food security should be prioritized for the teaming population in any developing country.

The concept of host plant resistance (HPR) in pest control is an ancient strategy that has been identified, even by resource-poor farmers. Although, they lacked the knowledge of the scientific basis for the concept, ancient farmers exchange information on observed inherent crop resistance against pests among different varieties but they lacked the knowledge of how such characteristics could be transferred into their chosen cultivars. The major advantages of HPR include the following. Unlike chemical control strategy, it has no negative ecological impacts or the risk of food poison. It add no extra cost to production because farmers only need to pay for seed but not any extra inputs to achieve the pest control goal. There has been some works on host plant resistance against *C. maculatus.* For instance, Swella and Mushology (2009) reported the comparative susceptibility of different legume seeds to infestation of *C. maculatus.* However, Sawar (2012) asserted different chick pea genotypes for resistance to the attack of C. *maculatus.* Badri *et al.* (2013) reported varietal susceptibility of selected elite lines and improved cultivars of cowpea to *C. maculatus* in Ghana. Despite the reported inherent resistance of cowpea lines/cultivars against bruchid infetation, there is the tendency for development of biotypes after a shift in the plant-insect relationship as a result of selection pressure, which can render a previously declared resistant line to become susceptible (Lima *et al.* 2004). That is the reason why periodic studies on evaluation of cowpea cultivars/lines for inherent resistance against *C. maculatus* should be promoted.

In this study, improved cultivars obtained from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria and Institute of Agricultural Research and Training (IAR&T), Obafemi Awolowo University, Ibadan were compared with local cultivated varieties obtained from local market in Ogbomoso, Nigeria. A criterion for selection of the studied number of cultivars/varieties was the tendency to achieve ease of handling and to allow comparison of the local with the improved lines. Therefore, this study was designed with the aim of evaluating the comparative resistance of selected cowpea lines/cultivars to infestation by cowpea seed beetle, *Callosobruchus maculatus.*

MATERIALS AND METHODS

Experimental site

The research was carried out in the laboratory of Crop and Environmental Protection (CEP) Ladoke Akintola University of Technology, Ogbomoso, Nigeria under ambient conditions.

Insect Culture

The original *Callosobruchus maculatus* used for this experiment was obtained from Entomology Unit of the CEP Departmental Laboratory, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. The culture was raised according to the method described by Babarinde and Ewete (2008).

Procurement of cowpea line/cultivars

Four cowpea lines used for the experiment (IT99K-494-6, IT97K-390-2, IT84S-2246-4 and IT06K-141) were obtained from the Genetic Resources Centre, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria; one cultivar (Ife Brown) was obtained from Institute of Agricultural Research and Training (IAR&T), Obafemi Awolowo University, Ibadan, Nigeria and two cultivars (Drum and Oloyin) were obtained from Wazobia Market, Ogbomoso, Nigeria. Each of the lines/cultivars was sorted to remove any exogenous material or broken seed prior to use for bioassay.

Screening of cowpea cultivars/lines for relative susceptibility to *Callosobruchus maculatus*

Three pairs (1:1 sex ratio) teneral adult of *C. maculatus* were added to 30 g of each cowpea line or cultivar inside Kilner jars covered with netted lids as described by Babarinde *et al*. (2015). Data on the number of eggs were collected after 3 days post infestation (DPI). The set up was kept for another 25 days after which the following additional data were collected.

(i) Number of grains with exit holes (GWH)
(ii) Number of grains without exist holes (GWoH)
(iii) Percentage Seed Damage (PSD) was determined using the formula:

$$\%PSD = \frac{GWH}{GW_0H + GWH} \text{ X } 100$$

(iv) Reproductive efficiency was calculated according to Babarinde and Ewete (2008) as

$$\text{RE} = \frac{\text{NO of F1 adult}}{\text{No of Egg}} X \frac{100}{1}$$

Evaluation of secondary metabolites and reducing sugars in cowpea lines and cultivars

The abundance of the following secondary metabolites: tannins, saponins, steroids, terpenoids, alkaloids, flavonoids, and reducing sugars were determined according to the methods described by Marcano and Hasenawa (1991).

Statistical analysis

Count data were square root-transformed while percentage data were arcsine-transformed prior to analysis. Data were thereafter subjected to analysis of variance (ANOVA). Means were separated using Duncan Multiple Range Test (DMRT) at 5% level of probability. The secondary metabolites and reducing sugar values were correlated with the biological parameters of *C. maculatus* in the cowpea lines/cultivars.

RESULTS

The number of eggs laid on IT99K-494-6 (20.05) was significantly (p =0.0057) lower than the number laid on all local cultivars (56.33 – 78.33). The F_1 progeny observed in IT99k-494-6 (0.71) was significantly lower than 2.06 and 1.65 obtained in Ife Brown and Drum local varieties respectively (Table 1).

The number of grain with characteristics holes was not significantly affected by variety. However, grain without exist hole (GWoH) which was an index of seed damage was significantly however in Drum (22.05), IT06K-141 (21.33), than what was observed in other improved varieties. Percentage seed damage obtained in

IT99K-494-6 (10.14%) and IT845-2246-4 (16.77%) was significantly lower ($p < 0.05$) than 49.93% and 47.74% obtained in IT06K–141 and Drum respectively (Table 2). There was significant effect of cowpea line/cultivars on all secondary metabolites and reducing sugars (Table 3). There was positive correlation ($r=0.58$, $p<0.01$) between number of eggs laid (EGGN) and the number of grains with *C. maculatus* exist holes (GRWH); and positive correlation ($r=0.65$, $r<0.01$) between number of eggs laid and percentage seed damage. Also there was a positive correlation ($r=0.46$, $r=0.04$) between saponin levels the studied cowpea lines/cultivar and number of grain without *C. maculatus* exist holes (Table 4).

Table 1: Oviposition and first filial progeny emergence of *Callosobruchus maculatus* in selected cowpea varieties

Cowpea line/ cultivars	Number of eggs	F_1 adult emergence
IT99K-494-6	20.05 ± 6.9 c	0.71 ± 0.0 c
IT97K-390-2	67.12 ± 14.0 a	1.26 ± 0.2 b c
IT84S-2246-4	29.17 ± 8.1 b c	0.79 ± 0.1 c
IT06K-141	77.33 ± 14.1 a	0.71 ± 0.0 c
Ife Brown	56.33 ± 9.7 a b	2.06 ± 0.5 a
Oloyin	59.00 ± 7.5 a b	0.71 ± 0.0 a
Drum	78.83 ± 13.6 a	1.65 ± 0.2 a b
ANOVA Result	df = 6, 41; p = 0.0057	df = 6, 41; p = 0.008

Means with the same letters along the column are not significantly different using DMRT at 5% probability level.

Table 2: Damage parameters of selected cowpea varieties due to infestation of *Callosobruchus maculatus*

Cowpea line/cultivar	GWEH	GWoEH	PSD	RE
IT99K-494-6	6.17 ± 2.9	53.67 ± 3.4 a	10.14 ± 4.6 b	47.37 ± 13.0
IT97K-390-2	18.50 ± 7.4	42.50 ± 7.2 a b	26.71 ± 9.2 a b	61.03 ± 4.8
IT84S-2246-4	12.00 ± 4.4	55.5 ± 2.4 a	16.77 ± 5.9 b	38.80 ± 14.0
IT06K-141	22.00 ± 7.2	21.33 ± 6.8 c	49.93 ± 15.5 a	44.40 ± 4.7
Ife Brown	24.00 ± 6.6	41.00 ± 6.5 a b	36.80 ± 10.1 a b	55.03 ± 10.6
Oloyin	13.50 ± 2.4	29.67 ± 1.9 b c	29.02 ± 5.4 a b	37.71 ± 8.1
Drum	20.50 ± 3.6	22.00 ± 2.9 c	47.74 ± 7.8 a	52.13 ± 4.6
ANOVA Result	df = 6, 41; p = 0.28	df = 6, 41; p < 0.001	df = 6, 41; p = 0.05	df = 6, 41; p = 0.54

GWEH= Grain with exit holes; GWoEH= Grain without exit holes; PSD= Percentage seed damage; RE= Reproductive efficiency

Means with the same letters along the column are not significantly different using DMRT at 5% probability level.

Table 3: Secondary metabolites and reducing sugar contents of selected cowpea varieties

Cowpea varieties	**Alkaloid (mg/100g)**	**Flavonoid (mg/100g)**	**Tannins (mg/100g)**	**Saponins (mg/100g)**	**Terpenoids (mg/100g)**	**Steroids (mg/100g)**	**Reducing Sugar (mg/100g)**
IT99K-494-6	21.69 ± 1.7 b	30.33 ± 2.9 c	5.00 ± 0.0 d	41.67 ± 1.7 b	16.67 ± 1.7 c d	5.00 ± 0.0 b	3.17 ± 0.1 e
IT97K-390-2	5.00 ± 0.0 d	13.33 ± 1.7 d	28.33 ± 1.7 c	26.67 ± 1.7 c d	13.67 ± 0.9 d	6.67 ± 1.7 b	3.47 ± 0.1 d
IT84S-2246-4	11.67 ± 1.7 c	28.33 ± 1.7 c	5.00 ± 0.0 d	61.67 ± 1.7 a	23.33 ± 1.7 b	11.67 ± 1.7 a	4.27 ± 0.1 b
IT06K-141	31.67 ±3.3 a	31.67 ± 1.7 c	38.33 ± 1.7 b	16.67 ± 1.7 e	25.00 ± 2.9 b	5.00 ± 0.0 b	4.03 ± 0.0 b
Ife Brown	21.67 ± 1.7 b	33.33 ± 1.7 c	5.00 ± 0.0 d	25.00 ± 2.9 d	23.33 ± 1.7 b	8.33 ± 1.7 a b	2.83 ± 0.1 f
Oloyin	8.33 ± 1.7 c d	51.67 ± 1.7 a	43.33 ± 1.7 a	38.33 ± 1.7 b	21.67 ± 1.7 b c	11.67 ± 1.7 a	5.33 ± 0.1 a
Drum	13.33 ± 1.7 d	41.67 ± 1.7 b	5.00 ± 0.0 d	31.67 1.7 d	33.33 ± 1.7 a	6.67 ± 1.7 b	3.77 ± 0.1 c
ANOVA Result	df = 6,20; p<0.0001	df = 6,20; p<0.0001	df = 6,20; p<0.0001	df = 6,20; p<0.0001	df = 6,20; p<0.0002	df = 6,20; p<0.0235	df = 6,20; p<0.0001

Means with the same letters along the column are not significantly different using DMRT at 5% probability level.

Table 4: Correlation between selected biological properties and damage potentials of *Callosobruchus maculatus* and secondary metabolites of selected cowpea lines

	EGGN	EMGADT	GRWH	GRWOH	PSDD	RE	ALK	FLA	TANN	SAP	TERP	STER	REDSG
EGGN	1												
EMGADT	0.27	1											
	0.08												
GRWH	0.58	0.48	1										
	<.01	0.001											
GRWOH	-0.66	-0.27	-0.76	1									
	<.01	0.09	<.01										
PSDD	0.65	0.34	0.93	-0.89	1								
	<0.01	0.01	<0.01	<0.01									
RE	0.28	0.45	0.45	-0.25	0.35	1							
	0.07	<0.01	<0.01	0.11	0.02								
ALK	-0.23	-0.24	-0.42	0.18	-0.31	-0.05	1						
	0.32	0.29	0.06	0.43	0.17	0.83							
FLA	0.17	-0.01	-0.32	-0.17	-0.04	-0.13	0.05	1					
	0.46	0.97	0.16	0.47	0.85	0.59	0.83						
TANN	0.32	-0.31	0.006	-0.43	0.02	-0.11	-0.03	0.18	1				
	0.16	0.17	0.98	0.05	0.93	0.63	0.9	0.44					
SAP	-0.29	-0.17	-0.14	0.46	-0.22	-0.08	-0.42	0.07	-0.41	1			
	0.2	0.45	0.55	0.04	0.35	0.75	0.06	0.76	0.07				
TERP	0.41	0.51	-0.05	-0.28	0.22	-0.09	0.15	0.47	-0.22	-0.05	1		
	0.07	0.02	0.83	0.21	0.35	0.7	0.52	0.03	0.34	0.81			
STER	-0.09	0.07	0.06	0.06	-0.07	-0.32	-0.48	0.29	0.05	0.45	0.12	1	
	0.68	0.77	0.79	0.81	0.77	0.18	0.03	0.19	0.82	0.04	0.59		
REDSG	0.31	-0.19	-0.08	-0.28	0.02	-0.21	-0.31	0.56	0.63	0.29	0.15	0.47	1
	0.17	0.39	0.73	0.22	0.94	0.36	0.17	0.01	0.002	0.19	0.52	0.03	

EGGN = number of eggs; EMGADT = emerged adult; GRWH = grain with exit holes; GRWOH = grain without exit holes
PSDD = percentage seed damage; RE = reproductive efficiency; ALK = alkaloids; FLA = flavonoids; TANN= tannins
SAP = saponins; TERP = terpenoids; STER = steroids; REDSG =reducing sugars

DISCUSSION

The parameters used in this study to evaluate inherent resistance of the cowpea lines/cultivars to the infestation of *C. maculatus* are similar to those reported by early authors on related study. For instance, Lima *et al.* (2004) reported the use of female fecundity (number of eggs laid) and percentage of emerged adults; Sharma and Thakur (2014) used adult emergence and percentage weight loss in seed weight; de Castro *et al.* (2013) used oviposition and number of emerged adults. Sawar (2012) used percentage weight loss in seed weight and adult emergence, while Badii *et al.* (2013) also used oviposition, adult emergence and percentage weight loss in seed weight. These variables have been reported as reliable indicators for resistance of cowpea to damage by *C. maculatus* (Jackai and Asante, 2003). The variability in oviposition rate on different hosts has been associated with the surface area of the seeds (Fitzner *et al.,* 1985). Although the surface area and seed coat texture were not determined in the present study, they may be contributory to the observed variability in the number of eggs deposited on the seed coats of the studied lines/cultivars. The numbers of eggs laid on and F_1 progeny obtained from IT99K-494-6 were significantly lower than the numbers observed on Ife Brown and Drum Local cultivars. The number of emergent F_1 progeny has been attributed to the biochemical properties of the host.

Cai *et al.* (2016) reported that tea saponins represented an economically feasible method of hindering the development of *Plutella Xylopia.* Of the seven lines/cultivars screened, IT99K-494-6 was more resistant to the infestation of *C. maculatus* than others. However on a general note, high saponins content of the experimented lines/cultivars was contributory to cowpea host plant resistance to *C. maculatus.* Hence, the mechanism of resistance of the variety could be partly antibiosis and the inherent levels of saponins can be maneuvered in cowpea breeding for resistance against the cowpea bruchid, *C. maculatus.*

In conclusion, the result of the research shows that IT99K-494-6 can be incorporated into Integrated Pest Management Scheme on HPR basis. It is recommended that future studies should attempt the identification/characterization of the genes responsible for the inherent resistance against *C. maculatus.*

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