

Laboratory Screening of Seeds of Some Cowpea Cultivars (*Vigna unguiculata*) for Tolerance to Cowpea Beetles (*Callosobruchus maculatus*) in a Hot Humid Environment

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Abstract: Seeds of five cowpea cultivars (Ife Brown, Ibadan Local, Ife BPC, Mala and Oloyin) were screened for tolerance to cowpea beetles (*Callosobruchus maculatus*). The screening was done in the Seed Testing Laboratory of the Institute of Agricultural Research and Training, Obafemi Awolowo University, Moor Plantation, Ibadan. Seed samples were inoculated with eggs of *Callosobruchus maculatus* while control experiments were treated with phostoxin to prevent infestation. Common storage methods adopted by the African cowpea farmers were also evaluated for their efficacy. The results showed that storage methods (S) Variety (V) and Treatment (T) were highly significant for all parameters assessed, while phostoxin treated seeds stores relatively better than non-phostoxin treated seeds. Similarly, cowpea seeds stored in plastic containers were better preserved than those stored in tins and earthen pots. In fact, storing cowpea in nylon bags should be discouraged. Absolute seed damaged was observed from seeds stored in nylon bags. Percentage pest tolerance was observed to vary directly with number of undamaged seed, remained seed weight and reduced weight loss, while Oloyin was able to combine gene for *C. maculatus* resistance with good storability. Implications of correlation coefficients (r) among seed characters for the tested cultivars were discussed.

Key words: Cowpea seed % *Callosobaruchus maculatus* % storage methods % tolerance level

INTRODUCTION

Cowpea (*Vigna unguiculata*) is a staple food crop of Nigeria and some tropical African countries. It is one of the major plant protein found in many African diets. It is consumed by people of different age bracket, cutting across religion and socio-economic classes. Its high protein content makes it extremely valuable in Tropical Africa where protein from meat and fish could not be afforded by the teeming population.

Substantial cowpea production is found in West and Central Africa, where its value lies not only in its protein content (25%), but also in its ability to tolerate drought and fixing of atmospheric nitrogen which enables it to grow on and improve poor soils [1]. Cowpea is adapted to a wide array of soils and moisture conditions [2]. One of the major pests of cowpea in storage is cowpea beetle (*Callosobuchus maculatus*) which was described by Drees and Jackman [3]. They described the adult as been found outdoors in flowers where eggs are laid by females and hatched in 5 to 20 days. Larvae do typically feed

inside the cowpea seed taking 2 to 6 weeks to develop before pupating. Six to seven generations of these insects may occur per year.

In Nigeria, Caswell [4] and Singh *et al.* [5], reported that about 4.5% of cowpea produced are lost to beetle, translating to about 30 USD million. Several attempts to preserve the seeds through chemical means sometimes results in poisoning of cowpea and environmental toxicity as experienced in Nigeria in 1996. This probably suggests the need for alternative method of preserving this important crop in storage.

Osekre and Ayertey [6] therefore suggested use of palm and coconut oils to control cowpea beetles. These oils were found to reduce oviposition and progeny emergence, but did not affect the longevity of the adult insects. For example, the highest dosage of 10 mlG¹ kg seed protected the seeds for 5 months. Both oils had no adverse effects on seed viability after five month-storage. Kenyan *et al.* [7] similarly reported that soybean cysteine protease inhibitor (soycystatin N) have defense functions against *Callosobruchus maculautus*. Botanical

insecticides have also been suggested for preserving cowpea seeds. These include *Nicotiana tabacum* (L), *Blumea aurita* (DC) and *Azadirachta indica* (Juss) among others as reported by Boeke *et al.* [8]. Other effective fumigants suggested for preserving cowpea seeds against *C. maculatus* are *Ocimum basilicum* (L) and *Ocimum gratissimum* (L) [9].

Since *C. maculatus* has been classified as a serious post harvest storage pest, loss of methyl bromide and possible restriction of phostoxin and their rising cost obviously suggests the need for non-chemical disinfestation treatment on cowpea seeds [10]. Although, the use of insecticides is discouraged around food materials [11], insecticides are supplementary to sanitation and proper storage. Perhaps the reason why Carlos [11] advocated for combination of good hygiene, aeration, drying, treating storages and equipment, as well as mixing chemical or dryacide with grains to prevent insect infestation.

The importance of cowpea in sub-Saharan Africa with respect to its production capacity and utilization potential therefore informed the need to breed insect pest resistance and high yielding genotypes for use as part of the farming system of the people. International Institute for Tropical Agriculture (IITA) in 1990 recognized this when she requested Gatsby to fund collaborative research project with UK's John Innes Centre to modify cowpea genetically for insect pests' resistance [12].

The objectives of this study therefore were to (1) screen the seeds of major cowpea cultivars in the laboratory for tolerance to *Callosobruchus maculatus* (2) identify possible source of gene for the development of *C. maculatus* tolerance and (3) to test the efficacy of some storage methods currently used by farmers.

MATERIALS AND METHODS

Five cowpea cultivars (Ife Brown, Ibadan Local, Ife BPC, Mala and Oloyin) were screened for tolerance to cowpea beetle (*Callosobruchus maculatus*). The screening was done in four storage containers namely: earthen pots, plastic containers, nylon bags and tins placed on the Seed Testing laboratory benches of the Institute of Agricultural Research and Training, Obafemi Awolowo University, Moor Plantation Ibadan, under room temperature (28-34°C) and humidity (85-100%). Each storage container consists of 100 clean seeds with 100 eggs of cowpea beetle (*Callosobruchus maculatus*) collected from previously infested cowpea seeds without insecticides. Control experiments were equally set up with phostoxin treatment according to the methods described

by Jackai and Singh [13]. The experiment was laid out in randomized complete blocks with four replicates, but was analyzed in form of split experiment, where variety, storage methods and phostoxin treatment were factors. The following code denotes each cultivar/treatment as indicated in the text.

- C B-Ife Brown cowpea variety
- C C-Ibadan Local (check)
- C I-Ife BPC cowpea variety
- C M-Mala cowpea cultivar
- C O-Oloyin cowpea cultivar
- C P^o-Treatment without phostoxin
- C P'-Treatment with phostoxin.
- C E-Earthen pots
- C N-Nylon bags
- C P-Plastic containers
- C T-Tin containers.
- C All of these gave a total of 40 treatments.

Data were collected from the experiment for 18 months. These include: Initial seed weight (g), number of seed damaged (No perforated by beetles), numbers of undamaged seed (No not perforated by beetles), remained seed weight (g), [weight of seed after the experiment], seed weight loss (g) [initial seed weight-Final seed weight], % weight loss [Initial seed weight-Final seed weight/Final seed weight×100] and emerged insect population [number of insect emerged out of the 100 eggs used] and % pest tolerance [No of undamaged seed-No of damaged seed/No of undamaged seed/100] Data from, the four replicates were pooled together and were statistically analyzed using SPSS soft ware package of 1999. Analysis of variance was used to compute F-values for the parameters considered (p<0. 05 and 0.01). First order interaction of Variety×Treatment, Storage×Treatment and Storage×Variety as well as the second order interaction of Variety×Storage×Treatment interaction were computed.

Significant interactive means were partitioned for pertinent means. Similarly, correlation coefficients (r) for the cowpea seed were computed, while graphs showing relationship between cowpea cultivars and % seed weight and emerged insect population were plotted for non phostoxin treated seeds.

RESULTS AND DISCUSSION

Results from the analysis of variance are presented in Table 1. The results showed that storage method (S) was highly (p<0.01) significant for all seed parameters taken. These parameters included: Number of seeds damaged,

Table 1: F-value for cowpea characters stored by various methods and treatments

Source of variation	Df	Initial seed weight	No seed damaged	No. of undamaged seed	Weight loss	Remained seed weight	Emerged insect population	Percentage (%) tolerance
Replicate	3	27.15	13.83	10.67	6.31	11.38	7.73	8.28
Storage (S)	3	6.11**	42.93**	37.70**	14.40**	29.87**	23.92**	25.84**
Treatment (T)	1	0.88	153.55**	114.62**	71.54**	103.58**	84.63**	90.02**
Variety (V)	4	231.87**	4.79**	3.98**	7.24**	28.30**	4.14**	4.40**
S×T interaction	3	6.13**	40.95**	27.57**	13.89**	17.34**	21.97**	23.84**
S×V interaction	12	4.29**	3.07**	2.68**	1.22	2.91**	1.51	1.63**
T×V interaction	4	1.75	2.52*	1.83	4.98**	9.33**	2.29	2.49**
S×T×V interaction	12	2.94**	4.84**	3.95**	2.36**	2.23**	2.93**	3.08**
Error	78	0.01	4.07	5.15	0.09	0.06	0.91	0.86
Total	120							

*, ** Significant at p<0.05 and 0.01 respectively

Table 2: Seed characters interactive means for storage×Treatment×variety interaction

Variety	Initial seed weight	No of seeds damaged	No of undamaged seeds	Weight loss (g)	Remained seed weight (g)	% weight loss	Emerged insect population	% pest tolerance
Bp°E	13.64	33.33	66.66	4.41	9.20	32.27	8.33	66.00
CP°E	15.31	66.00	34.00	8.29	7.02	53.87	38.66	34.00
IP°E	14.11	100.00	0.00	9.41	4.70	66.76	28.00	0.00
MP°E	19.64	26.67	73.33	8.05	11.59	40.04	7.33	73.30
OP°E	21.43	0.00	100.00	1.42	20.01	6.65	6.67	100.00
BP°E	13.93	0.00	100.00	0.49	13.45	3.53	0.00	100.00
CP°E	16.22	1.66	98.33	0.81	15.40	5.07	2.00	98.00
IP°E	13.77	0.00	100.00	0.75	13.02	5.36	1.00	100.00
MP°E	23.45	35.00	76.66	3.47	20.19	14.02	15.66	76.66
OP°E	21.43	0.00	100.00	1.56	19.86	7.31	0.00	100.00
BP°N	13.50	93.00	7.00	6.16	7.34	45.57	30.66	7.00
CP°N	15.44	100.00	0.00	9.81	5.63	63.73	46.66	0.00
IP°N	14.75	100.00	0.00	9.72	5.04	65.73	39.66	0.00
MP°N	19.39	100.00	0.00	13.14	6.25	67.72	34.33	0.00
OP°N	19.69	100.00	0.00	13.25	6.44	67.28	25.00	0.00
BP°N	13.97	0.00	100.00	1.58	12.39	11.31	0.00	100.00
CP°N	15.89	0.00	100.00	0.66	15.23	4.17	0.00	100.00
IP°N	12.47	6.00	96.00	0.53	11.94	4.24	1.33	96.00
MP°N	17.99	0.00	100.00	1.44	16.55	8.15	0.00	100.00
OP°N	19.49	0.00	100.00	1.45	18.04	7.45	0.00	100.00
BP°P	13.10	0.00	100.00	1.03	12.07	28.08	0.00	100.00
CP°P	16.51	0.00	100.00	2.16	14.35	13.13	0.00	100.00
IP°P	14.10	0.00	100.00	0.55	13.55	3.86	0.00	100.00
MP°P	19.49	33.33	66.67	9.84	9.65	51.07	17.00	66.66
OP°P	20.15	0.00	100.00	0.73	19.09	3.68	0.00	100.00
BP°P	14.29	0.00	100.00	0.45	13.84	3.19	0.00	100.00
CP°P	15.29	0.00	100.00	0.19	15.14	1.22	0.00	100.00
IP°P	13.60	0.00	100.00	0.40	13.19	2.96	0.00	100.00
MP°P	19.76	0.00	100.00	0.85	18.63	4.32	0.00	100.00
OP°P	19.68	0.00	100.00	1.05	18.91	5.31	0.00	100.00
BP°T	13.40	0.67	93.33	0.43	12.97	3.25	1.00	93.33
CP°T	15.80	0.00	100.00	0.65	15.16	4.08	0.00	100.00
IP°T	14.10	0.00	100.00	0.26	13.85	1.84	0.67	100.00
MP°T	20.18	6.00	33.33	9.32	10.86	45.47	21.66	93.33
OP°T	20.83	0.00	100.00	0.21	20.62	1.01	0.00	100.00
BP°T	13.64	0.00	100.00	0.35	13.28	2.59	0.00	100.00
CP°T	15.90	0.00	100.00	5.78	15.04	5.44	0.00	100.00
IP°T	14.92	0.00	100.00	0.75	14.11	4.95	0.00	100.00
MP°T	20.01	0.00	100.00	0.29	19.38	1.46	0.00	100.00
OP°T	20.45	0.00	100.00	0.44	20.04	72.17	0.00	100.00
SE (0.05)	0.57	10.29	11.67	1.59	1.35	8.15	4.92	4.81

numbers of undamaged seeds, initial seed weight, Remained Seed Weight (RSW), weight loss, % pest tolerance and emerged insect population. Similarly, all these parameters were equally highly significant for Treatment (T) and Variety (V) except initial seed weight for the treatments used. Storage×Treatment as well as Storage×Variety interactions was significantly different for the assessed parameters except weight loss and emerged insect population under Storage×Variety interaction. Treatment×variety interaction was only significant for number of undamaged seed, initial seed weight and emerged insect population at p<0.05 and/or 0.01 respectively. All parameters were significantly different for the second order interaction of Storage×Treatment×Variety interaction at p<0.05 Table 1.

Results from a case study of cowpea storage showed that cocoon was effective but significantly more expensive for farmers to adopt [14], hence the need for locally available, cheap and affordable storage materials. Such alternatives were highlighted by Boeke [15], when he discovered that farmers who were unable to treat their beans with insecticides depend mainly on use of plant materials. He extracted these plant materials from laboratory and tested them against cowpea beetle. They were found to be potent and can protect stored seeds against insect pests.

Table 2 presents character means for each cowpea cultivar under various treatment and storage methods. From these results, seed treated with phostoxin were generally better protected than those without phostoxin. For non-phostoxin-treated seeds, in the plastic containers, storability was very high with negligible % damage of<0.1% followed by tin storage containers with reduced seed damaged except for ‘Mala’ where 66% damage was recorded. Storing cowpea in the nylon bags was not the best without phostoxin. Percentage seed damage of between 93-100% was recorded under this condition.

Hence, should be discouraged. Seed tolerance of the cowpea cultivars were also presented in Table 2. This of course is best assessed under non-phostoxin-treated seed condition. Ife Brown suffered 33% seed damage in the earthen pots and no damage in other containers except nylon bags which was generally poor a storage method for all varieties. Absolute seed damage was experienced by Ibadan Local in earthen pot and nylon bag, but stores well in other storage containers with no seed damage. Ife BPC on the other hand recorded 33% seed damage in earthen pot and 100% damage in nylon bags as against 66.0% seed damage obtained in other storage containers. This suggests that this variety should not be stored in earthen pot without phostoxin treatment. Mala suffered 26.7% damage in earthen pots, 100.0% in nylon bags and less than 1.0% in tin containers. Oloyin stored better in all kinds of containers, but suffered 100% insect damage in nylon bags showing that the use of nylon bags for storing cowpea should be avoided by farmers and cowpea seed merchants.

Cowpea initial seed weight varied significantly from 13.64 g (Ife Brown) to 23.45 g (Mala). This variability may be tapped by plant breeders to enhance higher storability and seed quality. For the remained seed weight, it was observed that, those varieties and seed treatments that reduce seed damage, promotes higher remained seed weight, reduced weight loss and increased % pest tolerance. Consequently, % pest tolerance varied directly with number of undamaged seed, reduced weight loss and remained seed weight (Table 2). For example, Ife Brown stored in earthen pots without Phostoxin recorded 33.33% seed damage, 9.20 g remained seed weight and 66.0% tolerance level, while the same variety stored in nylon bags recorded 93.0% seed damage, 7.34 g remained seed weight and 0.00 pest tolerance (Table 2). Similar trend do exist in other treatment combinations. Cowpea cultivars varied significantly with respect to % weight

Table 3: Correlation coefficients (r) for cowpea characters, stored under *Callosobruchus maculatus* artificial infestation

	Initial seed weight	No seed damaged	No of undamaged seed	Weight loss	%Weight loss	Remained seed weight	Emerged insect population	%pest tolerance level
Initial seed weight	-	-0.02		0.14	0.04	0.48**	-0.02	0.02
No seed damaged		-	-0.79**	0.84**	0.89**	-0.80**	0.88**	-0.89**
No of undamaged seed			-	-0.81**	-0.86**	0.179**	-0.02	0.87**
Weight loss				-	0.91**	-0.76**	0.76**	-0.77**
% Weight loss					-	-0.82**	0.83**	-0.83**
Remained seed weight						-	-0.73**	0.73**
Emerged insect population							-	0.83**
%pest tolerance								-

**Significant at p<0.05

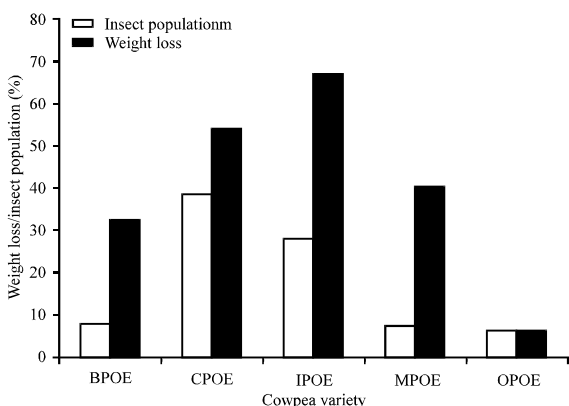


Fig. 1: Relationship of insect population and % weight loss of cowpea varieties, screened for *Callosobruchus maculatus* tolerance in earthen pots

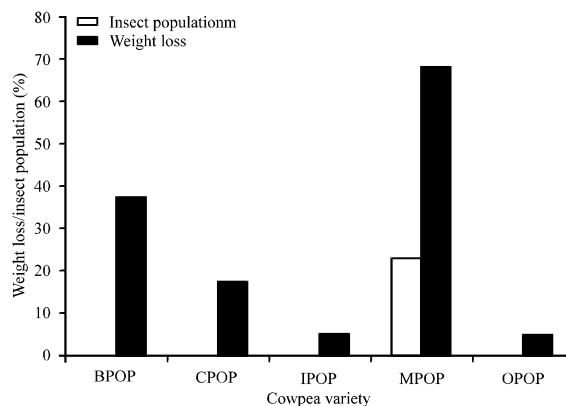


Fig. 3: Relationship of insect population and % weight loss of cowpea varieties, screened for *Callosobruchus maculatus* tolerance in plastic containers

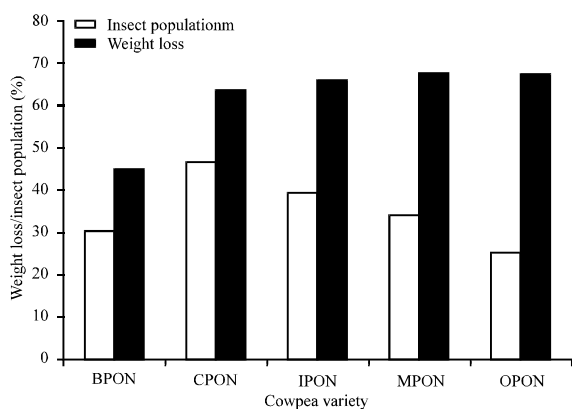


Fig. 2: Relationship of insect population and % weight loss of cowpea varieties, screened for *Callosobruchus maculatus* tolerance in nylon bags

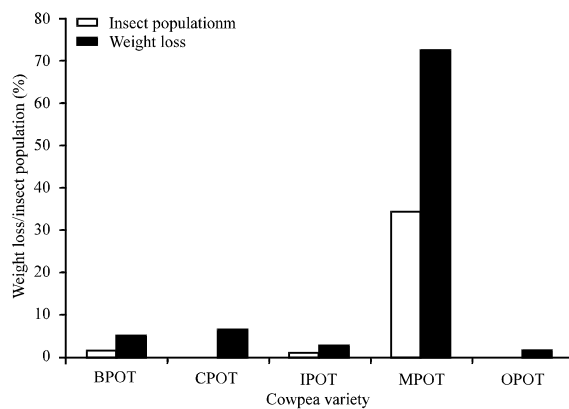


Fig. 4: Relationship of insect population and % weight loss of cowpea varieties, screened for *Callosobruchus maculatus* tolerance in tin containers

loss and emerged insect population. For instance, while Ife Brown suffered 32.2% weight loss from 8 insect pests, Ibadan local recorded 53.87% weight loss from 38 insect pests. *Mala* on the other hand recorded weight loss of 40.0% from only 7 insects showing differences in the tolerance levels of the cowpea to varied insect population.

Correlation coefficients (r) of cowpea parameters screened for *Callosobruchus maculatus* are presented in Table 3. Number of seed damaged was positive and significantly correlated with weight loss, % weight loss and emerged insect population, whereas this character (No of damaged seed) was negative and significantly correlated with number of undamaged seed, remained seed weight and % tolerance level at $p < 0.01$. In the same vein, Number of undamaged seed was positive and

significantly correlated with remained seed weight and % tolerance level, while it was negative and significantly correlated with seed weight loss, % weight loss and emerged insect population ($p < 0.01$).

Even though, initial weight was only positive and significantly correlated with remained seed weight, remained seed weight was negative and significantly correlated with weight loss, % seed weight loss and emerged insect population. This was however, positively correlated with % tolerance level. Weight loss was positive and significantly correlated with % weight loss ($r = 0.91^{**}$) and emerged insect population ($r = 0.76^{**}$), but, was negative and significantly correlated with % tolerance level. Seed weight loss was positive and significantly correlated with emerged insect pest ($r = 0.83^{**}$), while % tolerance level was

negative and significantly correlated with emerged insect population ($r = 0.99^{**}$).

Relationship between % weight loss and emerged insect population in stored cowpea are presented in Fig. 1-4. Cowpea seeds stored in earthen pots suffered proportionate weight loss with insect population (Fig. 1) except Oloyin which stores better in earthen pots with only 10.0% seed damage and reduced insect population. Similar trend was observed for cowpea stored in nylon bags where there was more insect damage and higher weight loss (Fig. 2). From Fig. 3, it was observed that plastic containers store seed better than other methods of cowpea storage for all varieties except *Mala* with about 10% emerged insect population. Although similar trend was obtained in Fig. 4 for cowpea stored in tins, there were slight weight losses even without insect population, probably these varieties (Ibadan local, Ife Brown and Oloyin) were not dried to constant moisture % as at the time the experiment was set up (Fig. 3 and 4).

CONCLUSIONS

Storing cowpea with phostoxin in any storage container will preserve the seeds, while storing cowpea in nylon bags should be discouraged as larger % are damaged by cowpea beetles. Nevertheless, storing cowpea in plastic containers appears to be generally suitable a method. Oloyin possessed *C.maculatus* tolerant gene thus lend itself for use in cowpea improvement for resistance to cowpea beetles.

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