COMPARATIVE EVALUATION OF COLD AND CONTAINER STORAGE PRESERVATION EFFICIENCIES ON COWPEA GRAINS AGAINST *CALLOSOBRUCHUS MACULATUS* FAB.

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Abstract. The need for efficient storage of cowpea grains against post-harvest loss by *Callosobruchus maculatus* has led subsistent farmers to adopt different storage methods. The methods adopted are influenced by cost, simplicity and availability of materials. The use of cold storage and containers amongst the adopted techniques are evaluated for their efficiency in this study. Twenty-five insects were introduced into 100 seed samples of four susceptible genotypes and stored in -20°C, 10°C, plastic, nylon, tin and earthen pot. The Cold treatment, duration of treatment and their interactions significantly affect insect activeness, reduction and mortality while container treatments significantly affect seed damage and insect emergence observed. However, the cold storage at -20°C was most effective affecting both oviposition and physiology of the insect pest. Although, none of the container storage was able to limit the insect oviposition, storage efficiencies varied among evaluated technologies with the use of plastic containers being most efficient.

Keywords: *Callosobruchus maculatus*, Cold Storage, Container, Cowpea, Storage Techniques

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp.) is an important legume in many developing countries grown mainly for its grains, as it is one of the cheapest source of plant protein in the diet of people that cannot afford protein foods such as meat and fish (Fatokun, 2002; Olakojo et al., 2007; Adam and Baidoo, 2008; Baidoo et al., 2010). Subsistent farmers in the semi-arid and sub-humid regions of Africa are the major producers and consumers of cowpea (Fery, 2003). In West Africa, post-harvest storage and losses of cowpea grain majorly in storage are recurrent constraints with as much as approximately 100% of grain lost due to infestations from pests for cowpea (Umeozor, 2005; Shazia et al., 2006; Sanon et al., 2011). The cowpea beetle, *Callosobruchus maculatus*, is regarded as the most important storage pest. Infestation by this insect pest starts on the field, but exceedingly high levels of infestation is generated when they pass only one or two generations on the host plant during storage, resulting in heavy damage of stored grains (Umeozor, 2005, Deshpande et al., 2011). Damage by this insect both in quality and quantity due to emergence holes have cause a reduction in market value for infested grains, reduction in quality seeds for planting and consumer consumption. The importance of storage and preservation of the grains employs means to better preserve the grains against the damaging effect of the weevils. Prolonging the time of cowpea stored can help reduce and/or alleviate poverty among farmers (Sanon et al., 2011).

Various research institutions have embarked on identifying and/or development of suitable storage techniques for cowpea grains. The techniques needed to be low cost, simple to use, and make use of materials readily available locally. One key value was that the technologies should be acceptable by potential users (Murdock et al., 2003). This have resulted in various storage methods which have been adopted by farmers depending on the purpose of storage and the quantity of cowpea produced or procured for storage (Yakubu et al., 2012). Some of which have been highlighted in literature (Murdock, 2003; Kananji, 2007; Beneke, 2010). However, there have been no means of comparing these methods in terms of their efficiency in preserving the cowpea seeds against infesting *C. maculatus*. Hence, the objective of the study was to evaluate the efficiency some of the control methods and practices recommended for efficient cowpea storage.

MATERIALS AND METHODS

The study was conducted at the laboratory of the Department of Cell Biology and Genetics, University of Lagos, Nigeria.

Insect culture

Infested cowpea seeds were collected from the field of Institute of Agricultural Research and Training (IAR&T), Ibadan, Oyo state (IAR&T) and kept in plastic transparent jars covered with mesh. Insects collected were identified at the Department of Zoology, University of Lagos, Lagos. Cultures of the insect were established according to Beck and Blumer, (2014). Twenty adult bruchids (10 males and 10 females) 1 – 2 day old were introduced into fresh culture jars. They were removed 5 days after introduction and the resulting colonies were used for the study.

Sample seeds used for study. Seeds used in this study were freshly harvested collected from the IAR&T, Ibadan, Oyo state (Figure 1). They were oven dried at 30 °C for 24 hrs to kill off any bruchid eggs or larvae that might have been in the seeds and dry to have uniform and safe moisture content (Amusa et al., 2014). 100 healthy seeds of each cowpea cultivars were weighed and infested with 25 adult (10 males and 15 females) newly emerged cowpea weevils. These infested cultivars were then subjected to different storage methods and setup in 4 replicates. The infested samples were wraped and subjected to two storage techniques.

(1.) The cold storage technique which include (a) storage at -20 °C and (b) storage at 10 °C. Insect activities were observed during the study period.

(2.) The container storage technique which include (a) plastic container (b) nylon bags (c) tin container (d) earthen pot. Containers were kept at room temperature (28-34 °C) and humidity (85-100%) all through the duration of the study.

White **Drum** **Oloyin** **Pelebe**

Figure 1. Sampled seeds used in the study

Data Collection and Analysis

Data collected during study experiment include: Initial seed weight (g), Numbers of seed damaged (number perforated by beetles), Numbers of undamaged seeds (numbers not perforated by beetles), Residual seed weight (g) (weight of seed after the experiment), Seed weight loss (g) (initial seed weight – final seed weight), Percentage(%) weight loss (initial seed weight – final seed weight / final seed weight x 100) and emerged insect population (number of new insect that emerged from the 25 weevils used) percentage(%) pest tolerance (number of undamaged seeds – number of damaged seeds / number of undamaged seeds / 100). Data was analysed using IBM SPSS v24.

RESULTS AND DISCUSSION

Cold storage performance on cowpea preservation against bruchid infestation

Temperature management is one of the most promising biorational insect management tools for farm stored grains and grain processing industries (Dosland et al., 2006; Phillips and Throne, 2010) as it is one of the most important environmental factors influencing insect physiology and behaviour (Gupta and Apte, 2016). There was a gradual decline in insect activeness with increase in duration of cold storage, earlier in -20 °C than 10 °C (Fig 2). The initiation of reduced activeness was observed in insects introduced into setups after 4 mins in -20 °C and 20 mins in 10 °C in the study. 100% inactiveness was observed after 45 mins in -20 °C compared to 95 mins in 10 °C (Fig 2). The rate of mortality of bruchid insects in cold storage setups showed an increase with duration in the study (Fig 3). There was 100% insect mortality by 45 mins at -20 °C while in 10 °C storage, insect mortality increased with duration. Insect mortality was 100% by 98 mins in 10 °C cold storage (Fig 3). There was a gradual increase in percentage adult insect reduction with increased duration in cold storage. Although, reduction in adult insect started at 5 and 15 mins in -20 and 10 °C storage respectively, 100% mortality was observed at 45 and 95 mins in -20 and 10 °C storage respectively (Fig 4). Both storage environments inhibit oviposition ability in adult insects introduced into setups as no egg was observed on seeds of any genotype after 24 h. Various studies have shown optimal temperature for cowpea weevil activity to be 28.5 ±2.0 °C (Beck and Blumer, 2014; Devi and Devi, 2014). The reduced temperature may have interfered with the insects' physiological nature, reducing its activeness thereby increasing their mortality. This also inhibited the oviposition ability of the insect pest. However, there was a delay in cold temperature effect at 10 °C when compared to -20 °C treatment. There is a concern, that gradual cooling may allow pest insect to acclimatize to freezing temperatures, extending the required treatment time for disinfestations (Johnson and Valero, 2000). Reichmuth et al. (2007) reported that the cowpea weevil is relatively cold-tolerant insect. The study showed that increasing duration of treatment temperatures was associated with increasing mortality of insect pest subsequently leading to increase in insect population reduction (Table 1 and 3). This is an indication that the duration of cold treatment is significant factor when this storage technique is employed. Cooling and freezing have been reported to have a great effect to disturb the rate of insect growth and development, speed of movement, instantaneous rate of oviposition, survival, fecundity to mention a few (Overgaard and Sorenson, 2008; Reznik et al., 2009; Karl et al. 2011; Khaliq et al., 2014). Oviposition of adult insects was disrupted in all genotypes with cold treatments before 24 hours in the study. High adult insect mortality was also experienced in the study. Some authors noted that insects could not bear the challenge against low thresh hold temperatures resulting in high mortality rates and somewhat developmental rate was affected (Karl et al., 2011; Regniere et al., 2012).

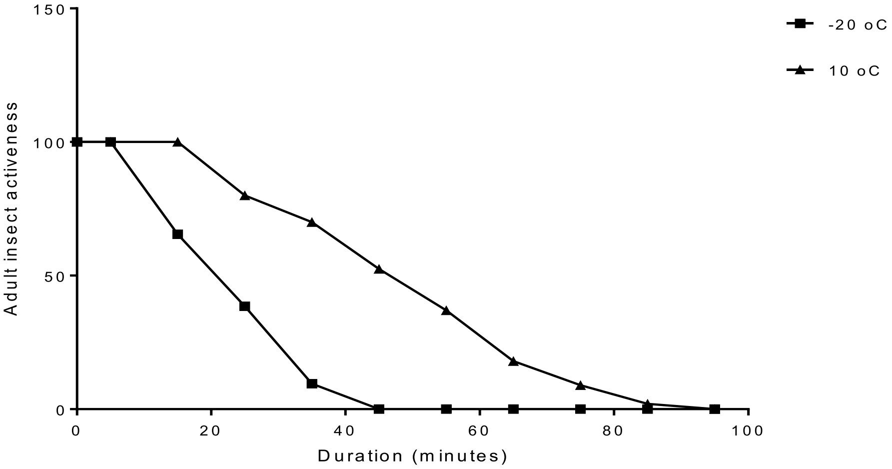

Figure 2. The rate of adult insect activity in cold treatment environments

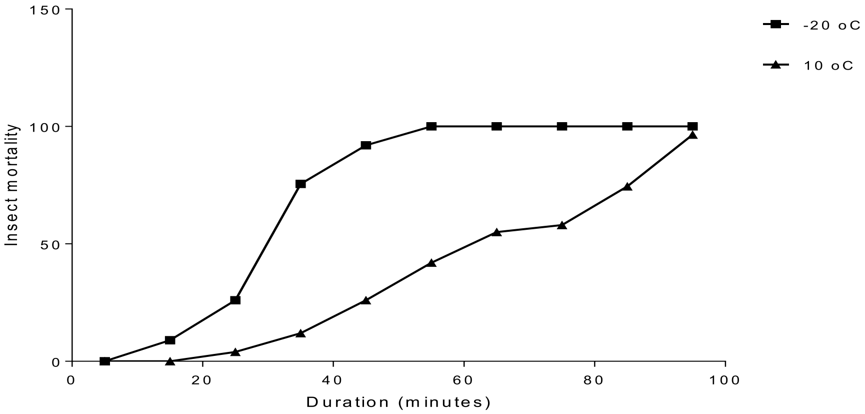

Figure 3. The rate of mortality of *C. maculatus* in cold treatment storages

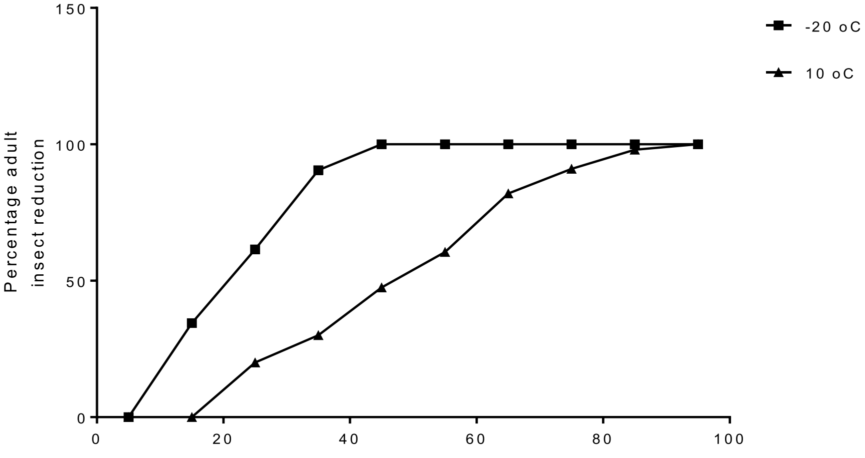

Figure 4. The rate of adult insect reduction in cold treatment environments

Association between performance parameters measured in cold storage

There was no significant correlation between the sample genotypes with evaluated parameters as storage performance in the study (Table 1). There was a significant positive correlation between storage used and activeness (r = 0.334, $p < 0.001$) and moribundity of introduced insects (r = 0.273, $p < 0.001$). Storage used showed a moderate negative correlation with insect mortality (r = -0.415, $p < 0.001$) and percentage insect reduction (r = -0.337, $p < 0.001$). On the other hand, significant negative correlation was observed between duration of storage with insect activeness (r = -0.843, $p < 0.001$) and moribundity (r = -0.080, $p < 0.001$) while a strong significant positive correlation was observed between duration of storage with both insect mortality (r = 0.82, $p < 0.001$) and percentage reduction of introduced insects (r = 0.841, $p < 0.001$). Insect actives showed weak and strong significantly negative correlation with insect mortality (r = -0.024, $p < 0.001$) and percentage insect reduction (r = -0.944, $p < 0.001$) respectively while insect mortality showed a strong significant negative correlation with moribundity of insects introduced (r = 0.994, $p < 0.001$) (Table 1). The study observed no genotype effects on seed damage. This might be due to the fact that all genotypes used were not resistant to *C. maculatus* infestation. The study observed a significant positive correlation between cold treatment duration and insect mortality which corroborates with the works of Mullen and Arbogast (1979) and Johnson and Valero (2003).

Table 1.

Parameters correlation in cold treatment environments

	Stor.	Dur.	Gen.	Act.	Mor.	Mort.	P.R.
Stor.	1.000	0.000	0.000	0.334***	0.273***	-0.415***	-0.337***
Dur.		1.000	0.000	-0.843***	-0.080	0.820***	0.841***
Gen.			1.000	0.001	0.024	-0.014	0.001
Act.				1.000	-0.024	-0.944***	-0.999***
Mor.					1.000	-0.303***	0.023
Mort.						1.000	0.944***
P.R.							1.000

*** Significant at $p < 0.001$; Stor.: Storage type; Dur.: Duration of storage; Gen.: Sample Genotype; Act.: Activeness of insect; Mor.: Moribund of insects; Mort.: Insect mortality; P.R.: Percentage insect reduction

Container storage performance on cowpea preservation against bruchid infestation. The performances of sampled genotypes in various container storages were illustrated in Table 2. The lowest percentage pest tolerance was observed in sampled genotypes with nylon storage, while the highest tolerance was observed in samples stored in plastic containers. The nylon storage also gave the highest insect emergence among sampled container storage techniques. The performance of the plastic container for storage use corroborate with the reports of Reznik et al., (2009) who stated that grains stored in plastic container performed best among evaluated techniques. Undamaged seeds were highest in storage in plastic containers in the study. There were no storage containers without oviposited eggs nor immune to infestation of the bruchid insect. This indicates that these storage techniques did not inhibit oviposition of the insects introduced, but reduction in insects emerged may be due to insect suffocation. The insects were able to oviposit before mortality. Oviposited eggs were also able to complete their cycles and emerge from seeds, hence the lost in seed weight observed among sample genotypes used in the study (Table 2). Results from this study corroborate with Gupta and Apte (2016) who reported descending trend storage preservation performance of plastic container > galvanised fin> polythene fertilizer bag > earthen pot > gunny bag and bamboo basket. They reported that seed damage was 100% in all the container after 60 days (Gupta and Apte, 2016) which differs from the study result. Minimal damage was experience in plastic storage and the highest seed damage was observed earthen pot. Only in nylon storage did we observed 100% seed damage with some genotypes.

Effect of storage on seed preservation performance. Both cold storage types (-20 °C and 4 °C), duration of storage and their interactions had significant effects on morbidity, insect mortality and percentage insect reduction ($p < 0.001$) in this study (Table 3). However, the genotype of samples used in the study did not significantly affect moribundity ($p = 0.719$), insect mortality ($p = 0.972$) and percentage reduction ($p = 0.969$) of insects introduced during the study. Both storage and duration of storage interactions with sample genotypes did not significantly affect moribundity, insect mortality and percentage reduction of introduced insects ($p > 0.05$) (Table 3). Both cold temperatures used in the study were effective on the storage preservation of cowpea for the duration of the study. The freezer (-20 °C) showing complete insect mortality with 50 mins while 100% insects mortality was attained by approximately 1 h 40 mins using the fridge at 10 °C storage preservation.

Table 2.

Various container storage performance against *C. maculatus* establishment.

Storage medium	Sample seeds	Initial seed weight	Remained seed weight (g)	Weight loss (g)	percentage weight loss (%)	Number of seeds damaged	Number of undamaged seeds	Emerged insect population	Percentage pest tolerance (%)
Plastic container	Drum	40.14	30.75	9.39	23.39	2	98	3	100
	Oloyin	36.41	20.11	16.30	44.77	8	92	8	90
	White	43.03	28.53	14.50	33.70	3	97	3	97
	Pelebe	32.64	18.69	13.95	42.74	2	98	3	98
Nylon bag	Drum	13.08	2.05	11.03	84.33	94	6	96	10
	Oloyin	15.23	0.55	14.68	96.39	100	0	102	0
	White	16.22	2.04	14.18	87.42	95	5	99	5
	Pelebe	15.57	0.89	14.68	94.28	100	0	107	0
Tin container	Drum	34.54	15.02	19.52	58.51	66	34	78	34
	Oloyin	30.48	10.01	20.47	67.16	54	36	69	36
	White	34.63	21.01	13.62	39.33	44	56	51	56
	Pelebe	24.62	10.92	13.70	55.65	63	37	81	37
Earthen pot	Drum	42.05	4.59	37.46	89.08	90	10	103	10
	Oloyin	40.01	12.48	27.53	68.81	69	31	75	31
	White	43.81	15.43	28.38	64.78	65	35	76	35
	Pelebe	28.48	5.08	23.40	82.16	83	17	97	17

In the container storage techniques, the genotypes of the sampled cowpea used in the study did not have significant effect on seed damage ($p = 0.336$), insect emergence ($p = 0.156$) and seed tolerance ($p = 0.336$) in the study (Table 4). This might be due to the susceptible response of these genotypes to bruchid infestation. The various container storage showed significant effect on seed damage, insect emergence and seed tolerance ($p < 0.001$). There was also interaction effect between storage and genotype on seed damage, insect emergence and seed tolerance ($p < 0.001$) (Table 4). The containers used as a storage techniques were not able to effectively protect seeds during the study period. These storage technologies did not also prevent insect oviposition, hence the incident of damage seeds at the end of the study. Although, significant differences were observed among the storage types use, the use of nylon and earthen pots are not recommended to cowpea storage. There were no genotype effects on seed damage. However, there was genotype by container interactions on the level of seed damage, emergence of adult insect and seed tolerance to cowpea weevil.

Table 3:.

Mean square values for storage, duration, genotypes and their interaction effects on storage performance

SoV	df	Moribund	Insect mortality	Percentage reduction
Stor.	1	131.406***	2797.256***	26522.50***
Dur.	9	58.867***	1282.77***	20277.433***
Gen.	3	5.023ns	7.773ns	124.633ns
Stor. * Dur.	9	97.962***	154.992***	2074.500***
Stor. * Gen.	3	0.306ns	6.773ns	10.043ns
Dur. * Gen.	27	1.949ns	6.694ns	74.078ns
Stor. * Dur. * Gen.	27	2.14ns	2.879ns	2.995ns
Error	80	1.156	2.381	16.100

*** Significant at $p < 0.001$; ns: Not significant; df; degree of freedom; SoV: Sourece of variation; Stor.: Storage; Dur.: Duration of storage; Gen.: Genotype

Table 4.

Mean square values for container and genotype effects on storage performance

SoV	df	Seed damage	Insect emergence	Seed tolerance
Stor.	3	19167.19***	14938.91***	19167.19***
Gen.	3	1809.69ns	2158.91ns	1809.69ns
Stor. * Gen.	9	1245.35***	797.35***	1245.35***
Error	32	0.750	0.33	0.75

*** Significant at $p < 0.001$; ns: Not significant; df; degree of freedom; Stor.: Storage; Gen.: Genotype

CONCLUSION

Comparative studies on simple and cheap storage techniques was evaluated in this study. Temperature may serve as a key factor regulating insects' cycles and thus indirectly influence various aspects of insect biology. The manipulation of storage temperature to significantly affect the fitness of infesting insect population can make tremendous difference in cowpea grain preservation against loss to the cowpea weevil. Although, plastic container preservation to some extent reduced seed damage from *C. maculatus* when compared to other containers' preservation performance, the presence of some damaged seeds is an indication that these techniques are not 100% full prove when compared to low temperature storage. The only limitation to continuous effective use of low temperature (-20 °C) technique is the constant supply of power.

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