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Determination of appropriate packaging materials for cowpea seed storage

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Quality seed is an important resource for any seed-propagated crop. Cowpea *(Vigna unguiculata)* seed is prone to cowpea weevil (*Callosobruchus maculatus*) infestation during storage. This can result in 100% seed loss when stored without treatment. Smallholder farmers in Kenya use traditional methods of storage and occasionally accompanied by pesticides treatment. Due to health concerns, pesticide use is discouraged. Therefore, this study was conducted to determine effectiveness of packaging materials on reducing cowpea weevil damage during seed storage. A repeated experiment was carried out at the entomology laboratory using a single cowpea line each time. Packaging materials evaluated were; cellulose paper bags, gourds, grain storage bags, polyethylene bags and glass bottles. Completely randomized design was used and each treatment was replicated thrice. Data were obtained on number of seeds with holes, mean number of holes per seed, seed weight loss, damage score, and seed germination. Quantitative data were subjected to ANOVA and correlation analysis. Glass bottles, polyethylene bags, and hermetic grain storage bags showed significantly less seed damage than gourd and cellulose bags in all the parameters tested except germination ($p \leq 0.05$). The parameters used to evaluate damage were positively correlated except germination. Only seed stored in glass bottles showed high germination rates; whereas, germination rates were generally less than 50% for the other seed packaging materials. Glass bottles were ranked the best packaging material, followed by polyethylene bags and grain storage bags respectively. Hermetic seed packaging was effective in reducing weevil damage and preserving seed quality.

Key words: *Callosobruchus maculatus*, seed quality, hermetic seed storage, smallholder growers.

INTRODUCTION

Cowpea production is wholly dependent on seed as propagation material. The quality of cowpea seed is therefore, an essential determinant of final quantity and quality of cowpea leaves and grain (Abukutsa-Onyango, 2011). As a legume and mostly self-fertilizing crop, farmers usually produce and store their own seed (Francis and Waithaka, 2015); only purchasing occasionally commercial seed or procuring from the development agencies during periods of crop failure. Farmers use traditional forms of seed storage like

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hanging pods in the kitchen, use of gourds and pots and storage in sisal bags. The cowpea seed, if stored and not treated well, will be vulnerable to cowpea weevil damage. Damaged cowpea seed will result in poor germination, poor establishment and poor crop stand density when used as planting material in subsequent seasons (Olasoji et al., 2013). Farmers' seed treatment methods against the cowpea weevil, packaging and storage methods can only be applied on small scale (Ilesanmi and Gungula, 2013) which calls for a better method that can be adopted widely by large scale farmers for storage of cowpea seed.

Seed treatment is one of the alternative solutions to reducing cowpea seed loss in storage due to *C. maculatus* infestation. Some of the indigenous methods of seed treatment used by cowpea farmers prior to seed storage include; use of soot and ash, botanical components like garlic, neem and moringa (Bamphitilhi et al., 2015). The adoption of these methods is however low because of the bitter taste, limitation to small-scale production and the uneconomical method of extraction (Ilesanmi and Gungula, 2013). The use of plastics as packaging material for seed storage has to be accompanied by a combination of synthetic seed treatments to increase efficiency; polymer coat, ferrous sulphate, and mancozeb (Susmithar and Rai, 2017). Though the commercial products significantly reduce pest infestation, their utilization is also limited due to stringent measures on minimum residue levels in food products and emerging health and environmental concerns of pesticide use (Muhammad, 2015). The super bags like Purdue Improved Cowpea Storage (PICS) have been tried and well adopted in West Africa and several other countries for cowpea storage (Divekar and Sharma, 2016) but not in Kenya. The hermetic storage conditions have been applied in Kenya mostly for maize storage against the maize weevil and large grain borer (Groote et al., 2013). It is therefore important to obtain proper storage techniques to minimize seed loss (Adetumbi et al., 2010).

The objective of the study is therefore to determine effectiveness of packaging materials on reducing cowpea weevil damage during seed storage and thereafter determine alternative storage mechanisms in order to alleviate the challenges of cowpea seed loss and availability of seed to the smallholder cowpea farmers.

MATERIALS AND METHODS

Two laboratory experiments were carried out. A freshly harvested cowpea line was used each time; it was produced at JKUAT farm and procured from a farmer. The seed was used without any pesticide treatment. The packaging materials used for seed storage were; polyethylene bags, cellulose paper bag (size 14) and glass bottles from (Nairobi plastics and packaging material), hermetic grain storage bags (AgroZ®) and gourds (Maasai market) (Figure 1).

The two experiments were carried out at the entomology laboratory. The freshly harvested non-treated cowpea seed was cleaned off plant debris and broken seed. Each of the storage packaging material treatments received 80 grams of the seed. The packaging material treatments were arranged in completely randomized design with three replications. Inoculum was based on inherent *C.maculatus* in the freshly harvested seed and infested seed available in the laboratory from untreated cowpea harvested in the previous season. The daily laboratory temperature ranged between 12-27°C. The seeds were stored for a period of 5 months. Data were obtained on the seed for presence of cowpea weevil eggs, average number of emergence holes on seeds, damage score based on severity, cumulative seed weight loss and germination.

Data collection and analysis

Presence of eggs was evaluated by closely examining the stored seed using a hand lens and recorded as present or absent. Number of seeds with emergence holes was obtained as the number of seeds with holes from a random subsample of 100 seeds. The mean number of emergence holes per seed was also determined for a subsample of 10 seeds. The damage score was evaluated as the proportion of damaged seed to total number of seed in a sample expressed on a scale of 1-5. The weight loss was assessed after storing the seed for five months. The seeds were initially sieved to eliminate dust or chaff, completely broken seed, and dead or alive weevils. The seeds were then weighed to obtain the weight of seed at the end of the storage period. The cumulative weight loss was the difference between the initial weight and final seed weight. Number of seeds with emergence holes, number of emergence holes and weight loss data were subjected to Analysis of Variance (ANOVA) using GenStat software 17th edition and means separated at p≤0.05 to determine variation among the storage materials. Pearson Correlations analysis was carried out to establish the relationships among the variables used to measure seed damage.

Germination tests were carried out at the end of the second trial according to Sikinyi (2010). A sample comprising 150-300 seeds from each of the packaging storage materials was soaked in distilled water for 24 h. The seeds from each treatment were divided into three subsamples and placed on Petri dishes lined with wet filter papers. The seed samples were incubated in a growth chamber at 25ºC for seven days. Germination data were collected after every 24 h. The data collected were used to determine speed of germination, mean germination time and total germination rate. Seed germination was calculated as the proportion of germinated seeds divided by the total number of seeds planted, expressed as a percentage. Speed of germination was calculated by the following formula given by Czabator (1962).

$$\text{Speed of Germination} = \frac{\text{n1}}{\text{d1}} + \frac{\text{n2}}{\text{d2}} + \frac{\text{n3}}{\text{d3}} + \cdots$$

Where, n = number of germinated seeds, d= number of days, Mean Germination Time (MGT) was estimated according to Ellis and Roberts (1981).

$$\text{MGT} = \frac{\text{n1d1} + \text{n2d2} + \text{n3d3} + \cdots}{\text{Total number of days}}$$

Where n= number of germinated seed; d = number of days.

RESULTS

At the end of the experiments, there were no eggs present in the glass bottle and the same applied to the hermetic grain storage bag in experiment one. Eggs were present on seeds stored in hermetic grain storage bag in

Figure 1. Storage materials; (a) glass bottles, (b) cellulose paper bag; (c) polyethylene bags; (d) hermetic grain storage bags; (e) gourds.

experiment two; polyethylene bags, cellulose paper bags, and gourds in both experiments (Table 1).

The greatest damage was observed on seeds stored in cellulose bags and gourds compared to hermetic grain storage bags, polyethylene bags and glass bottles as measured by weight loss, number of holes per seed, number of seeds with holes in a sample, and damage score (Table 1). Seeds stored in glass bottles showed the least damage for the traits measured. Germination rate was highest for seeds stored in the glass bottles. Germination rate was generally less than 50% for the other treatments. Although grain storage bags and polyethylene bags showed similar seed damage, seed germination rate was significantly lower for the grain storage bag treatment ($p \leq 0.05$). Seeds stored in glass bottles also showed significantly ($p \leq 0.05$) more rapid germination compared to other treatments (Figure 2). There was strong correlation between weight loss and number of holes per seed, proportion of seeds with holes, damage score, germination rate and germination speed. Significant positive correlation was observed between weight loss, number of holes per seed, damage score and number of seeds with holes ($p \leq 0.05$); however, the germination traits were not as highly correlated with seed damage traits (Table 2). Seed germination rate cannot be accurately estimated by the number of holes per seed and the damage score. Mean germination time is closely related to germination rate and not to the other measures of seed damage.

Five different packaging materials were evaluated for efficacy in storage of cowpea seed. Damage on the stored seed was determined through weight loss, number of holes on 10 seeds, proportion of seeds that had holes, general damage score and presence of eggs. The experiment was repeated twice. Germination tests were carried out in experiment two. A column with similar letters indicates not significant.

DISCUSSION

Cowpea seeds stored in glass bottle, hermetic grain storage bags and polyethylene bags from this study typically showed less weevil damage with the highest seed quality obtained from glass bottle storage. A glass

Table 1. Influence of packaging material on cowpea seed damage due to *C. maculatus* infestation.

Packaging material	Experiment one					Experiment two					
	Weight loss(g)	No of holes /10 seeds	Seeds with holes in 100 sample	Damage score	Eggs present	Weight loss(g)	No of holes /10 seeds	Seeds with holes in 100 sample	Damage score	Germination rate	Eggs present
Cellulose paper bag	38^{a}	23^{a}	99^{a}	5	Yes	17.6^{b}	12^{b}	70^{c}	5	49.3^{b}	Yes
Gourd	30^{a}	25^{a}	66^{a}	4	Yes	18.5^{b}	11^{b}	72^{c}	5	40.7^{ab}	Yes
Grain storage bags	3^{b}	1^{b}	14^{b}	2	No	7.5^{a}	5^{a}	41^{b}	2	29.3^{a}	Yes
Polyethylene bag	0.25^{b}	0^{b}	0^{b}	2	Yes	7.5^{a}	5^{a}	42^{b}	2	51.3^{b}	Yes
Glass bottle	0.01^{b}	0^{b}	0^{b}	1	No	0.04^{a}	0.2^{a}	2^{a}	1	92.7^{c}	No

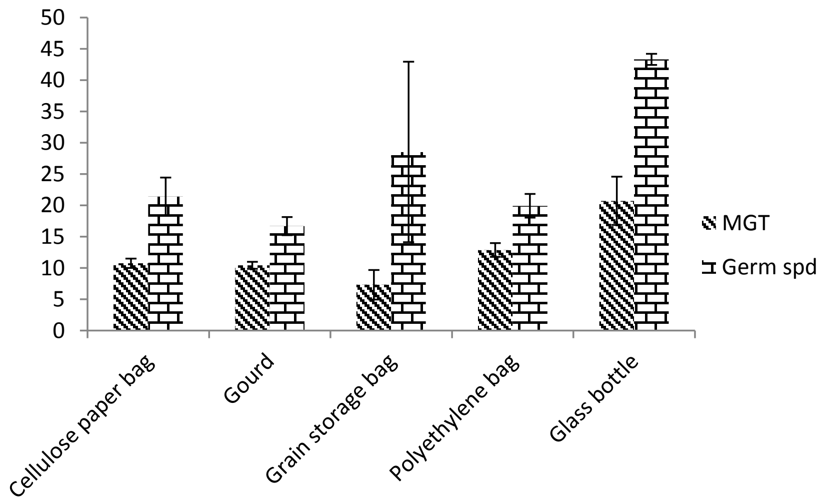

Figure 2. Evaluation of germination attributes of seed stored in cellulose paper bags, gourds, hermetic grain storage bags, polyethylene bags, glass bottles (MGT- mean germination time, Germ spd- Germination speed Error bars represent standard error.

bottle is an airtight container which prevents oxygen intake. Similarly, the grain storage bags with double heavy gauge polyethylene do not allow exchange of gases. Insects present during the storage period will survive on limited oxygen in the early stages of seed storage but later on suffocate (Divekar and Sharma, 2016). In a related study carried out in Ghana on influence of seed storage technique on germinability, it was found that seed stored in glass bottles and polythene bags recorded higher germination rate and the seeds were stored for longer periods as opposed to those stored in the cotton cloth (Bortey et al., 2016). Use of Purdue Improved Cowpea storage bags in West Africa has helped

Table 2. Correlation between measures of seed damage caused by **C. maculatus.**

Variable	Weight loss	No. of holes/seed	Seeds with holes	Damage score	Germination rate	Germination Speed	MGT
Weight loss	-						
No. holes/seed	0.934**						
Seeds with holes	0.878**	0.969**					
Damage Score	0.933**	0.842**	0.811**				
Germination rate	-0.508*	-0.498 ^{ns}	-0.575*	-0.457 ^{ns}			
Germ speed	-0.562*	-0.604*	-0.694*	-0.580*	0.4235 ^{ns}		
MGT	-0.471 ^{ns}	-0.438 ^{ns}	-0.489 ^{ns}	-0.429 ^{ns}	0.8763**	0.299 ^{ns}	-

MGT-Mean germination time *-significant **- highly significant ns- not significant.

to reduce cowpea weevil infestation; the bags are double layered and cause insulation against the weevils. When the grain and insects respire, the inter-granular oxygen levels are greatly reduced from 21 to 5% resulting in reduced insect activity and eventually causing their mortality (Groote et al., 2013). This explains the high rate of insect mortality in the glass bottle for both experiments and for the grain storage bags in the first experiment. The grain storage bags infestation was higher in the second experiment compared to the first experiment. This could be attributed to the cowpea line used in the second experiment that was procured from the farmer. The seeds were already infested prior to packaging. The seeds in the second experiment could not have gone through appropriate seed handling procedure like drying and sorting as these can lead to faster seed deterioration (Houssou et al., 2010). Hermetic storage system for food products has been exploited in India to allow food longevity because the system prevents oxygen uptake and maintain high carbon dioxide; a controlled atmosphere that limits the lifespan of insects (Divekar and Sharma, 2016). The polyethylene bags on the other hand ranked second as these bags are thin walled and partially hermetically sealed or could allow gaseous exchange. Even though polyethylene bags allow some gaseous exchange, it prevents direct entry and infestation by the weevils. The effectiveness of polyethylene is dependent on the environment for storage and inherent insect pests in the seed lot (Malarkodi and Ananthi, 2017); packaging in polyethylene accompanied by seed treatment increases its effectiveness. Use of polyethylene bags in storage of cowpea is better than unpackaged seeds or storage in cloth bags (Susmithar and Rai, 2017). In East Africa, many smallholder farmers depend on traditional methods of seed storage like gourds (Francis et al., 2015). The gourd is a traditional seed storage technique used by farmers in Eastern and Western Kenya; it provides a physical barrier to weevil infestation but does not provide a hermetic environment. The lid made from animal skin provides avenues for gaseous exchange and there are other secondary holes on the gourd itself. Because of this, the weevils are able to access and infest the seeds. Presence of air circulation and moisture allows weevils inherent in the seed lot to proliferate and lay eggs continuously as it is the case with use of cloth bags (Malarkodi and Ananthi, 2017). To improve utilization of gourd in seed storage, farmers have to dress the seeds with an insecticide, or a biological agents or plant products to kill any inherent weevils. It is important to use gourds that do not allow entry of the weevil (Nyamandi and Maphosa, 2013). The cellulose paper bag which represents minimum or no packaging at all showed high levels of seed damage. The weevils easily accessed the seed in the cellulose paper. With uncontrolled moisture and air circulation, weevils are able to aerate and reproduce at high rate. The storage conditions of 12-27°C favored cowpea weevil infestation. The infestation within the cellulose paper bag was continuous throughout the entire storage period. The seed lot stored under these conditions is not suitable for consumption because it has holes, its broken, and flour dust, and loses its aesthetic value. The germination rate similarly declines hence not suitable (Cruz et al., 2015).

Seed packaging materials affect germination of seeds (Yakubu, 2014). This was demonstrated clearly in the glass bottles. In a similar study, Olasoji et al (2013) obtained cowpea seed germination rate of 66- 92%. Ilesanmi and Gungula (2013) established that cowpea germination rate reduced to 35% after a period of storage even with moringa treatment. The low germination rates of stored seed result from deterioration that occurs during storage due to weevil damage and nutrient exhaustion (Susmithar and Rai, 2017). Seeds that are stored using appropriate packaging material retain higher germination capacity. Glass bottle therefore offered the best conditions for storage of cowpea seed. Seed that is poorly packaged for storage has potential to reduce its germination potential as the case in gourds and cellulose bags. Poor quality seed will lead to a rapid loss of viability (Morad, 2013). Treatment using plant biological products provides a short term solution against the cowpea weevils. That makes hermetic storage a better option for cowpea storage.

The strong correlation among measures of damage indicates that any of these measures could be used for determination of seed damage by the cowpea weevil. This is because high weight loss indicates a high degree damage of the seeds thus leading to poor germination in the field (Morad, 2013). When the degree of the measures of damage increased the germination performance also reduced. This implies that seed damage has a direct negative influence on the overall seed quality of cowpea (Olasoji et al., 2013).

CONCLUSION AND RECOMMENDATION

To better manage cowpea weevil infestation, application of hermetic packaging techniques sustains cowpea seed quality under storage conditions. Hermetically sealed glass bottle is the best seed packaging method followed by polyethylene and hermetic grain storage bags. Use of the grain storage bags that provide hermetic conditions is equally encouraged because with proper sealing, the seeds can be kept for a long time with minimum damage from weevils.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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