

Research Note

Effect on germination and nutrient composition of storing cowpea [*Vigna unguiculata* (L.) Walp.] seeds in a hermetic atmosphere modified with essential oil vapour from leaves of sweet basil [*Ocimum basilicum* (L.)]

Olusegun A. Ojumoola^{1*}, Adeyinka A. Adesiyun² and Lamidi A. Usman³

^{1,2}Department of Crop Protection, University of Ilorin, Ilorin, Nigeria.

³Department of Chemistry, University of Ilorin, Ilorin, Nigeria

*Corresponding author email: ojumoolaolusegun@gmail.com

Cowpea represents an important staple crop in Sub Saharan Africa and can play significant roles in ensuring food security in that part of the world. During storage, the grains are attacked and decimated by the cowpea bruchid, *Callosobruchus maculatus* (F). Methods such as hermetic storage and treatment with botanical insecticides have been recommended as safer and cheaper protection alternatives for cowpea grain, than conventional insecticides. When used in combination, these two prove to be faster and more effective in killing resident *C. maculatus* adults at the commencement of storage and also adults that may later emerge from any parasitized seed in the course of storage. Grains so protected will however be of little value if the germination and nutrient composition of the cowpea seeds are adversely affected by the botanically modified storage atmosphere. In this study, an insecticidal atmosphere within hermetic plastic structures was created using essential oil from leaves of *Ocimum basilicum* (L.) on filter papers. Cowpea seeds stored hermetically for 30 and 60 days in the presence or absence of the botanically modified atmosphere were subjected to germination tests and proximate analyses. Results showed that the presence of the insecticidal atmosphere during storage did not negatively affect the germination and nutrient composition of the seeds.

Keywords: *Callosobruchus maculatus*, *Ocimum basilicum*, essential oil, hermetic storage, cowpea

Cowpea, *Vigna unguiculata* (L.) Walp, is the most important grain legume crop in sub Saharan Africa. Apart from being consumed as a source of plant protein, minerals and vitamins, it is also used as animal feed and as a source of cash income (Dugje et al. 2009). In the absence of proper preventive measures, as much as 70% of threshed cowpea grains could be lost in storage (Ntoukam et al. 2000). Though cowpea seed borne fungi have also been implicated in the deterioration of stored cowpea seed quality, the major culprit is the cowpea bruchid, *Callosobruchus maculatus* (F). This preharvest and postharvest insect pest has a short generation period of approximately 30 days and is capable of causing weight loss of up to 60% in infested stored cowpea seeds (Allotey and Oyewo 2004; Radha and Susheela 2014).

Several methods have been employed to

prevent serious damage to cowpea grains in storage. Conventional insecticides and fumigants like pirimiphos-methyl, malathion, aluminium or magnesium phosphide, methyl bromide and carbon disulfide have been used to control storage insect pests like *C. maculatus*. Their use has, despite their effectiveness, been associated with such problems as cost, environmental pollution, toxicity to non-target organisms as well as residual effects on seeds and seedlings. (<http://www.fao.org/docrep/>; Ntoukam et al. 2000; Mahmud et al. 2002; Ajayi et al. 2014).

Storage of grains in air tight structures like metal silos (Tefera et al. 2011), sealed drums (Seck et al. 1996), jerry cans (Walsh et al. 2014), oxygen impermeable plastic bags (De Bruin et al. 2012) and triple layer plastic bags (Baoua et al. 2012) is an environmentally innocuous alternative to conventional insecticides. Once

sealed, the atmosphere within such hermetic structures undergoes a type of biological modification as a result of the aerobic respiration of resident insect pests and or grains (Navarro and Navarro 2014). The progressive depletion in oxygen and increase in carbon dioxide inhibit the insects' growth, development and reproduction capabilities and eventually leads to their death presumably by desiccation (Murdock et al. 2012).

The biological process that leads to the death of insect pests within hermetic structures will be faster and more effective if an artificial insecticidal atmosphere is created within the system (Ofuya and Lale 2001). Gases such as pure nitrogen, carbon dioxide or fumigants like methyl bromide or phosphine gas have been used to create insecticidal atmospheres that hastened the control of insects within hermetic structures (Ofuya and Reichmuth 1992; Mbata et al. 1996; Ofuya and Reichmuth 1993a). Insecticides and fumigants of a botanical origin like plant essential oils can be used to achieve such insecticidal atmosphere within hermetic structures as effectively as their synthetic counterparts. According to Ojumoola et al. (2015), all adult *C. maculatus* in an insecticidal atmosphere created within a hermetic structure with vapour of essential oil from leaves of *O. basilicum* were killed within 48 hours of exposure. On the other hand, *C. maculatus* adults in a similar hermetic structure but without the oil vapour were found to be alive after the observation period. Apart from having great potential as eco-friendly alternatives to conventional insecticides or fumigants (Koul et al. 2008), plant essential oils are cheaper and easier to use within hermetic storages available to poor resource cowpea farmers in Sub Saharan Africa.

The adulticidal potential of essential oil vapour from leaves of the sweet basil against *C. maculatus* within a hermetic structure was reported by Ojumoola et al. (2016). The ineffectiveness of the oils in killing the larval and pupal stages of the pest after 3 days of fumigation however points to the need for

other methods or combination of methods. Exposing cowpea seeds to an essential oil based insecticidal atmosphere within a hermetic storage structure will not only help kill the egg and adult stages of *C. maculatus* present at initial seed treatment and storage but will also ensure the quick destruction of adults that may later emerge from any seed harbouring larvae or pupae at the time of initial storage.

Storing seeds for long periods under hermetic and botanical insecticidal conditions may however have significant consequences on seed germination and nutrient composition. This study thus investigated the effect of 30 days and 60 days of storage in a hermetic structure having an insecticidal atmosphere produced with *O. basilicum* leaf essential oils.

Materials and methods

Source and treatment of cowpea seeds

Seeds of the cowpea variety, SAMPEA-12, were used in this study. They are medium-to-large brown seeds with a rough seed coat and were obtained from the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria. In the Department of Crop Protection, University of Ilorin, Ilorin, Nigeria (8.50°N, 4.68°E), the seeds were sterilized by freezing for 7 days and thereafter kept in a polyethylene bag in the refrigerator (4°C, 70-75% relative humidity) until needed. Only unbroken seeds without holes and bruchid eggs were used in the experiment.

Mini hermetic storage structures and *O. basilicum* essential oil

A hermetic storage structure was made using a 100 ml plastic container with screw type plastic lid (4.5 cm in diameter) and a 200 ml transparent disposable cone-shaped cup (base diameter of 4 cm and top diameter of 6.5 cm) without lid. A round 2.6 cm diameter hole was cut in the center of the plastic lid and covered with fine muslin from the inside with glue. The

100 ml container was thereafter covered with its modified lid. To complete the assemblage, the 200 ml cup was inverted over the 100 ml container and the latter was then gently pushed upwards into the former until stuck and airtight. The space between the base of the 100 ml container and the top diameter of the 200 ml container was stuffed with polystyrene to further make the assemblage airtight. The essential oil used was extracted from freshly harvested *O. basilicum* leaves by hydrodistillation, using a Clevenger type apparatus. The extracted oils were stored in 2ml glass vials and kept in the refrigerator (4°C, 70-75% relative humidity) until needed. The terpenic and non terpenic compositions of the oil have been reported in Ojumoola et al. (2016).

Experimental procedure

The experiment was run for two periods; 30 days and 60 days. Similarly, there were two treatments: with the essential oil and without as a control. For each treatment, 3 replicates of the hermetic structure described above were set up under ambient laboratory conditions (25-30°C, 70-75% relative humidity). To test the effect of the essential oil on germination and nutrient composition, the cowpea seeds were first exposed to the oil vapour within the replicate hermetic structures. Into the 100 ml container of each structure, 40g of sterilized cowpea seeds with 6% moisture content (AOAC 2003) were placed. The containers were then firmly covered with their modified lid and a 4cm diameter filter paper soaked with a constant dosage of 30 µl using a micropipette (1000 µl capacity) was placed on the lid of the 100 ml container before the container was fitted hermetically into the disposable plastic cup as described above. The 30 µl dosage used has been found to cause 100% mortality of adult *C. maculatus* after two days within a similar set up (Ojumoola et al. 2016). After set up, the total space left above the surface of the

closely packed grains in the 100 ml container was calculated to be approximately 80 ml.

Seed germination test

Fifteen seeds of each treatment were planted in loamy soils (500 g), at a depth of about 1 cm, in plastic sieves placed on plastic trays. The soil and water used daily to moisten it were sterilized by autoclaving for 30 minutes. The germination of the differently treated seeds was assessed at 4 and 5 days after planting (DAP). Only seedlings with normal parts (ISTA 1985) were counted during the assessment of percentage germination. The following data on germination were taken:

- i. Percentage of seedlings with normal hypocotyl
- ii. Percentage of seedlings with normal epicotyl
- iii. Percentage of seedlings with normal primary leaves

Proximate analysis

Thirty seeds of each treatment were ground separately into a fine powder. The percentage moisture, fat, crude protein and ash contents were determined by the AOAC (2003) method. The sum of these was deducted from 100 percent to obtain the total percentage carbohydrate.

Data analysis

A 2 x 2 factorial experiment was laid out in a completely randomized design. Data were subjected to a two-way Analysis of Variance (ANOVA) using the Generalized Linear Model function of IBM SPSS version 21 software. Where treatment means were significant, separation of means was done with the Tukey's Honestly Significant Difference at 5% level of significance using the same software.

Results

Table 1 shows the effects of hermetic storage alone and in combination with *O. basilicum* essential oil on the germination of cowpea seeds. At 4 DAP, percentage emergence of seedling with hypocotyl, epicotyl and primary leaves was generally poor (≤ 20.0) in cowpea seeds stored for 60 days. Conversely, the best mean percentage germination (80.0, 60.0 and 57.78) was observed in cowpea seeds stored hermetically for 30 days without the insecticidal atmosphere. Cowpea seeds stored hermetically under the insecticidal atmosphere for 30 days had intermediate mean percentage germination values. There was a significant difference ($P < 0.05$) in percentage germination (4 DAP) of seeds stored hermetically for 30 and 60 days with or without the essential oil based insecticidal atmosphere. Cowpea seeds stored hermetically for 60 days under the insecticidal atmosphere had the lowest emergence (2.22, 0.00, and 0.00) of hypocotyl, epicotyl and primary leaves, respectively 5 DAP. The highest mean percentage emergence

(93.3, 86.7 and 91.1) of hypocotyl, epicotyl and primary leaves respectively occurred in seeds stored hermetically for 30 days under the insecticidal atmosphere. At 5 DAP, there was a significant difference ($P < 0.05$) between treatments stored for 30 and 60 days whether an insecticidal atmosphere was present or not.

The effects of hermetic storage and *O. basilicum* essential oil on the nutrient composition of cowpea seeds is presented in Table 2. Cowpea seeds stored hermetically for 60 days under the essential oil based insecticidal atmosphere had the highest percentage (23.86) crude protein content. This value was significantly different ($P < 0.05$) from the lowest (21.99) crude protein content recorded in seeds stored without the insecticidal atmosphere for the same duration. There was no significant difference ($P > 0.05$) in the crude protein content of seeds stored for 30 days and those stored for 60 days whether an insecticidal atmosphere was present or not. All the treatment combinations were however not significantly different ($P > 0.05$) in their mean percentage moisture, ash, fat and total carbohydrate contents.

Table 1: Effects of hermetic storage and *O. basilicum* essential oil on cowpea seed germination

Duration of Hermetic Storage	Essential Oil Based Insecticidal Atmosphere	Mean percentage germination					
		4 days after planting			5 days after planting		
		Hypocotyl	Epicotyl	Primary leaves	Hypocotyl	Epicotyl	Primary leaves
30 days	Absent	80.00a	60.00a	57.78a	88.89a	80.00a	84.44a
30 days	Present	73.33a	53.33a	55.56a	93.33a	86.67a	91.11a
60 days	Absent	0.00b	0.00b	0.00b	31.11b	2.22b	2.22b
60 days	Present	20.00b	0.00b	0.00b	2.22b	0.00b	0.00b

Values in a column followed by the same letter(s) are not significantly different at $P = 0.05$ according to Tukey's Honestly Significant Difference

Table 2: Effects of hermetic storage and *O. basilicum* essential oil on cowpea seed germination

Duration of Hermetic Storage	Essential Oil Based Insecticidal Atmosphere	Mean Percentage Nutrient composition				
		Moisture	Ash	Fat	Crude protein	Total Carbohydrate
30 days	Absent	5.75	4.25	1.00	23.08ab	65.92
30 days	Present	5.50	3.49	1.00	22.97ab	67.04
60 days	Absent	6.25	3.25	1.00	21.99b	67.77
60 days	Present	4.00	3.00	0.75	23.86a	68.15
		ns	ns	ns		ns

Values in a column followed by the same letter(s) are not significantly different at P=0.05 according to Tukey's Honestly Significant Difference

ns: not significant

Discussion

Seed germination involves the emergence of the radicle through the seed coat and the development of seedling structures that indicate the capacity of the seedling to develop further into a satisfactory plant under favourable conditions (Perry 1995; ISTA 2008). In this study, an increase in hermetic storage duration from 30 to 60 days resulted in a decrease in germination of the cowpea seeds 5 DAP. This is in agreement with Awurum et al. (2014) who also observed a decrease in germination of stored cowpea seeds with increasing duration of storage. They attributed the observed decrease to the toxins and metabolites deposited by associated seed borne fungi. Generally, germination was not negatively affected by storing cowpea seeds in a hermetic structure whose atmosphere had been modified with *O. basilicum* leaf essential oil. Based on the findings in this study, the presence of the oil vapour did not significantly improve or delay seed germination.

Cowpea seeds are mostly consumed as an important source of plant protein (Agazounon et al. 2004). It, therefore, follows that when it comes to cowpea, the percentage crude protein content is the main nutrient of interest. It was observed in the present study that seeds stored hermetically for 60 days without an insecticidal atmosphere had lower percentage

crude protein contents compared to their counterparts stored for 30 days. According to Amadi and Oso (1996), this depletion over time is due to the presence of seed borne mycoflora which make use of the protein and fat contents of their host seeds. The depletion in crude protein content was however not noticed in cowpea seeds stored hermetically and protected with the essential oil based insecticidal atmosphere. These latter seeds were not only better in protein content than the ones stored for 60 days without essential oil protection; they were also comparable to those stored for 30 days with or without the essential oil based insecticidal atmosphere.

The absorption of fumigants by commodities being fumigated has been described as one of the physical processes that occur within a fumigation system (FAO 1975). Cowpea stored for 60 days in the present study may have been able to absorb and adsorb a relatively higher quantity of the volatilized essential oil constituents. The presence of these constituents within and on the surface of the cowpea seeds coupled with their low moisture content may have drastically reduced the survival and proliferation of the seed borne mycoflora responsible for percentage crude protein content depletion within the seeds.

It is, therefore, concluded from this study that storing cowpea seeds hermetically under an atmosphere modified with *O. basilicum* leaf

essential oil vapour will not negatively affect their germination and nutrient composition. Further studies will however be required to identify simple and cost effective methods for generating insecticidal atmospheres, using plant essential oils, within the different small scale hermetic storage structures available to cowpea farmers.

References

- Agazounon, C., O. Coulibaly, and V. Houndekon. 2004. "Analyse des techniques de transformation de niébe au Bénin." *Bulletin de la recherche Agronomique du Bénin* **45**(1): 8.
- Ajayi, O.E., A.G. Appel, and H.Y. Fadamiro. 2014. "Phytotoxicity of Some Essential Oil Components to Cowpea (*Vigna unguiculata* (L.) Walp.) Seeds." *International Journal of Plant Biology Research* **2**(4): 1024.
- Allotey, J., and E.O. Oyewo. 2004. "Some aspects of the biology and control of *Callosobruchus maculatus* on some stored soyabean, *Glycine max* (L) Merr varieties." *African Journal of Food, Agriculture, Nutrition and Development* **4**(2): 1-13
- Amadi, J.E., and B.A. Oso. 1996. "Mycoflora of cowpea seeds (*Vigna unguiculata* L) and their effects on seed nutrient contents and germination." *Nigerian Journal of Science* **30**:63-69.
- AOAC, 2003. *Official methods of analysis of the association of official's analytical chemists*, 17th ed. Association of official analytical chemists, Arlington, Virginia.
- Awurum, A.N., D.N. Enyiukwu, and P.O. Ucheagwu. 2014. "Effectiveness of duration of storage and phytochemicals in the management of seed-borne mycoflora of stored cowpea (*Vigna unguiculata* (L.) Walp) seeds." *Greener Journal of Agronomy, Forestry and Horticulture*. **2**(2): 022-026.
- De Bruin, T., P. Villers, A. Wagh, and S. Navarro. 2012. "Worldwide use of hermetic storage for the preservation of agricultural products." In 9th International Controlled Atmosphere and Fumigation Conference. Antalya, Turkey, October 15-19.
- Dugje, I.Y., L.O. Omoigui, F. Ekeleme, A.Y. Kamara, and H. Ajeigbe. 2009. "Farmer's guide to cowpea production in West Africa." International Institute of Tropical Agriculture.
- FAO. 1975. "Recommended methods for the detection and measurement of resistance of agricultural pests to pesticides. Tentative method for adults of some major pest species of stored cereals with methyl bromide and phosphine." FAO Method No.16. *FAO Plant Protection Bulletin*. **23**: 12 - 25.
- <http://www.fao.org/docrep/x5042e/x5042E02.htm#Evaporation%20of%20fumigants>
- International Seed Testing Association, ISTA. 1985. International rules for seed testing. Rules 1985. *Seed Science and Technology* **13**: 299-355.
- International Seed Testing Association (ISTA). 2008. International rules for seed testing. *Seed Science and Technology* **13**:356-513.
- Koul, O., S. Wali, and G.S. Dhaliwal. 2008. Essential oils as green pesticides: potential and constraints. *Biopesticide International*. **4**(1): 63-84.
- Mahmud, M.K., M.M.H. Khan, M. Husain, M.I. Alam, and M.S.I. Afrad. 2002. "Toxic effects of different plant oils on pulse beetle *Callosobruchus chinensis* Linn. (Coleoptera: Bruchidae)." *Journal of Asiatic Society of Bangladesh Science* **28**:11-18.
- Mbata, G.N., C. Reichmuth, and T. Ofuya. 1996. "A comparative study on the toxicity of carbondioxide to the developmental stages of *Callosobruchus subitnnotatus* (Pic.)." *Postharvest Biology and Technology* **7**:271-276.
- Murdock, L.L., V. Margam, I. Baoua, S. Balfe, and R.E. Shade. 2012. "Death by desiccation: Effects of hermetic storage on cowpea bruchids." *Journal of Stored Products Research* **49**:166-170.

- Ntoukam, G., L.L. Murdock, and R.E. Shade. 2000. "Managing insect pests of cowpea in storage," Bean/Cowpea, Midcourse Research meeting Senegal, 3-4.
- Navarro, S. and Navarro, H. 2014. "The biological and physical aspects of hermetic storage: A critical review." *11th International Working Conference on Stored Product Protection*, Chiang Mai, Thailand, 337 – 354.
- Ofuya, T.I. and N.E.S. Lale. 2001. "Pests of Stored Cereals and Pulses in Nigeria." *Biology, Ecology and Control*, pp 26.
- Ofuya, T.I. and C. Reichmuth. 1992. "Mortality of the cowpea bruchid, *Callosobruchus maculatus* (Fabricius) in a highly elevated carbondioxide atmosphere." *Proceedings of 1st European Conference on Grain Legumes*, Angers, France, 365-366.
- Ofuya, T.I. and C. Reichmuth. 1993a. "Mortality of cowpea storage beetle, *Callosobruchus maculatus* (Fabricius) in some high carbon dioxide atmospheres." *Anzieger Schadlingskude Pflanzschutz und Umweltshutz* **66**:57-59
- Ojumoola, A.O., A.A. Adesiyun, and L.A. Usman. 2015. "Fumigant effect of sweet basil (*Ocimum basilicum* L) leaf essential oil on the longevity and fecundity of adult cowpea bruchid *Callosobruchus maculatus* (F) and on germination." *Agrosearch* **15**(2): 47-58. <http://dx.doi.org/10.4314/agrosh.v15i2.5>
- Ojumoola, O.A., A.A. Adesiyun, and L.A. Usman. 2016. "Susceptibility of the life stages *Callosobruchus maculatus* (Fabr.) in stored cowpea grains to fumigation with essential oil vapour of *Ocimum basilicum* L. leaves." *Tropical Agriculture* (Trinidad) **93**(3):178-184.
- Perry, D.A. 1995. *Handbook of vigour test method*. 3rd ed. International Seed Testing Association. Zurich. Switzerland.
- Radha, R. and P. Susheela. 2014. "Studies on the Life History and Ovipositional Preference of *Callosobruchus maculatus* Reared on different Pulses." *Research Journal of Animal, Veterinary and Fishery Sciences* **2**(6): 1-5.
- Seck, D., G. Haubruge, E. Marlier, and C. Gaspar. 1996. "Alternative protection of cowpea seeds against *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) using hermetic storage alone or in combination with *Boscia senegalensis* (Pers) Lam ex Poir." *Journal of Stored Products Research* **32**:39-44.
- Tefera, T., F. Kanampiu, H. De Groote, J. Hellin, J. Mugo, S. Kimenju, Y. Beyene, M. Bodupalli, B. Shiferaw, and M. Banziger. 2011. "The metal silo: An effective grain storage technology for reducing post-harvest insect and pathogen losses in maize while improving small holder farmers food security in developing countries." *Crop Protection* **30**:240-245.
- Walsh, S., D. Baributsa, T. Remington, and L. Sperling. 2014. *Seed Storage Brief #2: Hermetic Seed Storage Technology: Principles, Use, and Economics – a Practitioner's Guide*. Nairobi: Catholic Relief Services.