



## The Effect of Storage on the Efficacy of *Eugenia Aromatica* (Baill.) in the control of *Callosobruchus Maculatus* (Fabricius) (Coleoptera: Bruchidae) Pest.

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**ABSTRACT:** The effect of storage on the efficacy of powders from *Eugenia aromatica* (Baill.) of different ages in the control of *Callosobruchus maculatus* (Fabricius), a major pest of stored cowpea seeds, were investigated in the laboratory. Powders stored for periods of < 1 month, 24, 36 and 48 months with particle size of 300 µm were tested. Contact toxicity was tested for the differently aged powders at 0.4 g of material per 20 g of seed in separate plastic Petri plates. In fumigant toxicity tests 20 g of infested cowpea seeds was suspended in a piece of muslin cloth, over 0.4 g of powder in a container. In the contact toxicity tests, all the differently aged powders killed *C. maculatus* adult within 48 hours. The differently aged powders significantly ( $P < 0.05$ ) reduced oviposition by the introduced *C. maculatus* adults and adults did not emerge from eggs laid. In the fumigant toxicity test, first use of the differently aged powders prevented emergence of adult *C. maculatus* from fumigated eggs. In a consecutive reuse of powders, adults emerged from fumigated eggs but the numbers were significantly ( $P < 0.05$ ) fewer than in the control. In conclusion, the shelf-life of *E. aromatica* powder as a bruchicide is at least four years. @JASEM

The cowpea seed beetle, *Callosobruchus maculatus* (F.) is a cosmopolitan pest of stored grain legumes, especially cowpeas, *Vigna unguiculata* (L.) Walp., in the tropics and subtropics (Ofuya, 2001). Severely damaged seeds are disfigured with egg covers and riddled with adult exit holes, and consequently have reduced weight and poor germiability. Often, after six months in storage 100% seed infestation may be recorded (Alabeek, 1996, Ogunkoya and Ofuya, 2001). A substantial part of world cowpea production comes from Nigeria with about 4 million hectares and approximately 1.7 million tonnes of beans annually (Ofuya, 2003). Cowpea seeds contain a high amount of protein and B-vitamins (Phillips and M. C. Waiters, 1991) and help to prevent starvation among low resource farmers and poor urban population (Quin, 1997). Many conventional insecticidal dusts such as pirimiphos methyl, and permethrin, and fumigants such as Aluminium phosphide had been reported to be effective against *C. maculatus* in storage. However rising prices and infrastructural problems impose considerable restrictions to their use, moreover, synthetic insecticides involve risks for human health and the environment especially when improperly used which may be common among uneducated rural farmers in Africa (Ofuya, 2003). Since the last three decades, plant-derived insecticides have been vigorously investigated worldwide, as possible replacement for the synthetic insecticides in stored products protection (Lale, 2001). Plant products such as vegetable oils, essential oils, volatile oils, crude extracts and powders have been tested against *C. maculatus*, (Lale, 1995, Dales, 1996, Golob *et al.*, 1999, Boeke *et al.* 2001). Dry powder made from neem seeds, *Azadirachta indica*, A. Juss, buds of Clove tree, *Eugenia aromatica*, Baill., fruits of West African brown Pepper, *Piper*

*guineense*, Schum and Thonn., Seeds of "pepper fruit" tree, *Dennetia tripetala* Baker and root bark of the "tooth ache plant", *Zanthozylum zanthozyloides* (Lam.) Waterm., applied at 2% of the weight of stored beans will effectively control the cowpea seed beetle in storage (Lale, 1994, Ogunwolu and Idowu, 1994; Ogunwolu and Odunlami, 1996; Adedire and Lajide, 2001, Ofuya and Salami, 2002). It is not known for how long these insecticidal powders will remain effective after preparation. These study investigated aspects of the shelf-life of *E. aromatica* dry flower buds powder against *C. maculatus*.

### MATERIALS AND METHODS

*C. maculatus* culture and experimental conditions: The *C. maculatus* used was derived from a colony originating from infested cowpea seeds collected from a local market in Akure, Nigeria. The colony has been maintained in Kilner jars in an open laboratory (28 ± 30 C and 70 ± 5% relative humidity) for more than 60 generations, using Ife Brown cowpea as substrate. All experiments were carried out in the open laboratory.

*Preparation of E. aromatica powder:* *E. aromatica* dry flower buds were purchased in local herbal stores in markets in Ondo State, Nigeria. The identity was confirmed at The Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. The flower buds were further oven dried to a constant weight at a temperature of 400°C. Thereafter, the dried buds were ground in 1.5 HP kitchen mill (model - KOAHLBACH). The resulting powder was then sieved to a particle size of 300 µm with a British laboratory test standard sieve (serial number 133032). *E. aromatica* powder had been prepared

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at different times in this manner since January 2000. The powders prepared at different times of a given particle size were kept in separate plastic containers with lightly fitted lids and placed in a wooden cupboard in the open laboratory.

**Contact toxicity of differently aged *E. aromatica* powders to *C. maculatus*:** The differently aged powders of *E. aromatica* were tested at 0.4 g of material per 20 g of seed in separate plastic Petri plates (8.5 cm diameter). The selected rate of application has been generally recommended for insecticidal plant powders (Lale, 1995) and has been found to be effective against *C. maculatus* (Adedire and Lajide, 2001). Ten couples (twenty individuals) of *C. maculatus* (aged 1-2 days old) were introduced into each plate which was shaken. Adult mortality was monitored in 48 hours and thereafter all insects were removed. The numbers of eggs laid by the female beetles on the seeds were then counted. The numbers of adults which emerged from these eggs were also counted as from three weeks after introducing the beetle couples unto the seeds. There was a control treatment involving no addition of plant powder onto seeds. Each treatment including the control was replicated three times.

**Fumigant toxicity of differently aged *E. aromatica* powders to *C. maculatus*:** Fumigant effect of each of the differently aged *E. aromatica* powders on freshly laid eggs (< 24 hours) and larvae of *C. maculatus* was determined. Each powder was tested at 0.4 g against 300 fresh eggs contained in 20 g of seed. The selected rate of application of *E. aromatica* has been found to be effective for fumigating *C. maculatus* eggs (Longe, 2004). Infested cowpea seeds were suspended in a piece of muslin cloth, over the powder in a container. The container lid was properly screwed up to hold the

muslin cloth and the cowpea seeds in space, and also make the set up airtight. There was a control with no powder in the container. All treatments were replicated thrice. Number of adults that emerged from treated eggs and larvae was counted. In another experiment, the same *E. aromatica* powders which had been used to fumigate *C. maculatus* eggs were immediately reused to fumigate 20 g of seed bearing 300 *C. maculatus* eggs as described previously. Number of adults that emerged from the second set of treated eggs was similarly counted.

**Data analysis:** Data collected were subjected to analysis of variance (ANOVA). Whilst egg counts were subjected to square root transformation, percentages were arcsine transformed, before analysis.

## RESULTS AND DISCUSSION

Summary of data recorded and analyzed when ten couples of *C. maculatus* were introduced unto 20 g of Ife Brown cowpea seeds and shaken with 0.4 g of *E. aromatica* powder of different ages is presented in Table 1. Irrespective of age of powder, all introduced adult beetles were killed in 48 hours. No adults were killed within 48 hours in the control treatment. Significantly greater ( $P < 0.05$ ) numbers of eggs were laid by the beetles in the control within 48 hours than in other treatments. Number of eggs laid by beetles in treatments involving use of the differently aged *E. aromatica* powders was not significantly different ( $P > 0.05$ ) from each other. Table I also shows that percentage adult emergence from eggs laid by the introduced beetles was significantly higher ( $P < 0.05$ ) in the control than in other treatments. No adults emerged from eggs in treatments involving use of the differently aged powders.

**Table 1:** Parameters measured when ten couples of *C. maculatus* were introduced into 20 g of cowpea seeds and shaken with 0.4 g of *E. aromatica* powder of different ages.

Age of <i>E. aromatica</i> powder	% <i>C. maculatus</i> adult mortality in 48 hours $\pm$ SE	Number of eggs laid by introduced females $\pm$ SE	% adult emergence from eggs laid $\pm$ SE
Less than 1 month	100.0 $\pm$ 0.00 (90.0)	5.3 $\pm$ 0.33 (2.6)	0.0 $\pm$ 0.00 (0)
24 months	100.0 $\pm$ 0.00 (90.0)	7.3 $\pm$ 0.33 (2.9)	0.0 $\pm$ 0.00 (0)
36 months	100.0 $\pm$ 0.00 (90.0)	9.3 $\pm$ 2.40 (3.2)	0.0 $\pm$ 0.00 (0)
48 months	100.0 $\pm$ 0.00 (90.0)	6.0 $\pm$ 0.00 (2.7)	0.0 $\pm$ 0.00 (0)
Control (no powder)	0.0 0.00 (0)	301.3 $\pm$ 32.39 (17.3)	65.5 $\pm$ 3.96 (54.0)
LSD (0.05)		37.18	4.53

Figures in parentheses are transformed values

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**Table 2:** Percentage of *C. maculatus* adults that emerged from two batches of seeds bearing beetle eggs and fumigated with 0.4 g of differently aged *E. aromatica* powders on two successive occasions

Age of <i>E. aromatica</i> powder	% adult emergence from eggs first fumigated with powder $\pm$ SE	% adult emergence from eggs fumigated with previously used powder $\pm$ SE
Less than 1 month	0.0 $\pm$ 0.00 (0)	5.6 $\pm$ 0.70 (13.6)
24 months	0.0 $\pm$ 0.00 (0)	20.5 $\pm$ 1.47 (26.9)
36 months	0.0 $\pm$ 0.00 (0)	23.6 $\pm$ 1.45 (29.1)
48 months	0.0 $\pm$ 0.00 (0)	33.1 $\pm$ 2.06 (35.1)
Control (no powder)	79.1 $\pm$ 0.77 (62.8)	63.8 $\pm$ 1.82 (53.0)
LSD (0.05)	0.88	4.02

Figures in parentheses are arcsine transformed values

Table 2 shows percentage of *C. maculatus* adults that emerged from two batches of seeds bearing beetle eggs and fumigated with 0.4 g of differently aged *E. aromatica* powders on two successive occasions. In first fumigation percentage adult emergence was significantly higher ( $P < 0.05$ ) in the control treatment than in other treatments involving the differently aged powders. No adults emerged from eggs in treatments involving use of the differently aged powders. In the second fumigation, percentage adult emergence was significantly higher ( $P < 0.05$ ) in the control treatment than in other treatments involving the immediate reuse of the differently aged powders. The less than one month old *E. aromatica* powder reduced percentage adult emergence more significantly during reuse than other powders.

The results of this study have corroborated reports of previous workers (Adedire and Lajide, 2001; Longe, 2004) that *E. aromatica* powder has significant contact and fumigant action against *C. maculatus*. The mechanisms of its protective action against the cowpea seed beetle include direct toxicity to adults and eggs, and inhibition of oviposition by female beetles. More importantly, it was observed that *E. aromatica* powder still manifested significant contact and fumigant insecticidal activity against the cowpea seed beetle four years after the dry flower buds was powdered. An important factor in the retention of insecticidal action of these powders may have been proper packaging (plastic containers with tightly fitted lids) which may have prevented escape of the active ingredients. Good packaging and storage have long been known to be vital in preserving and maintaining activity of chemical insecticides (Pedigo, 1991). The possibility of reuse of a given quantity of *E. aromatica* powder for fumigation of *C. maculatus* eggs is an added advantage this

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botanical. It was observed that the same freshly prepared powder (less than one month old) can be reused immediately after first use and still cause significant mortality of *C. maculatus* eggs and consequently reduction in adult emergence and damage to seeds. The effectiveness of reuse of powder however decreased significantly with the age of powder. The possibility of reuse of a given quantity of *E. aromatica* powder for fumigation suggests that the toxic vapours may be released slowly. The most widely used chemical fumigant for infested cowpeas in Nigeria is perhaps aluminium phosphide. The fumigation tablets emit toxic phosphine gas ( $\text{PH}_3$ ) on contact with the moist atmosphere in a sealed grain store (Hassall, 1990). The same tablet cannot be reused a second time. Information that is usually provided in pesticide and in fact all drug labels is time of production or manufacture and time in which the chemical will become ineffective in performing its normal function. The sale and use of adulterated and expired pesticides are part of the problems militating against pesticide use in Nigeria (Ogunwolu *et al.*, Okunade, 2001). A casual perusal of labels on synthetic pesticides sold in Nigeria will reveal that the active material in most expires in three to five years. The shelf life of *Eugenia* powder (four or probably more years) therefore compares favourably with that of many synthetic insecticides. This is very important if *E. aromatica* powder becomes formulated into insecticidal dusts that will be marketed and sold for use.

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