

# Potential of four tropical plant powders as grain protectants against *Callosobruchus maculatus* (Coleoptera: Chrysomelidae)

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## ABSTRACT

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Effects of four tropical plant (*Aframomum melegueta*, *Zanthoxylum zanthoxyloides*, *Piper guineense* and *Eugenia aromatica*) were investigated under tropical laboratory storage conditions for the protection of cowpea seeds against insect infestation. The plant materials were pulverised into fine powder after air drying and admixed with 20 g of cowpea seeds at the rates of 0.2, 0.3, 0.4 and 0.5 g in 125 ml plastic containers for contact toxicity experiment and 0.5 g of each plant powder to 50 g cowpea seeds for the fumigant toxicity experiment. Ten unsexed *Callosobruchus maculatus* were used for contact toxicity experiment, while two copulating pairs of *C. maculatus* were used for fumigant toxicity experiment. *Callosobruchus maculatus* response to the plant powders was recorded at 24 and 48 hrs post treatment for contact toxicity bioassay, while observation for fumigant activity was recorded at 24, 48, 72 and 96 hrs after treatment. All plant powders significantly ( $P < 0.05$ ) exerted adult mortality in relations to dosage and exposure time. Cowpea seeds treated with 0.5g of *E. aromatica* had highest mortality of 90% and 100% at 24 and 48 hrs after exposure respectively for contact toxicity, while *A. melegueta* recorded the highest adult mortality for all exposure periods for the fumigant toxicity. The insects' reactions to the plant powders admixed with cowpea seeds were restlessness, loss of coordination, knock-down and eventual death. The study indicated that the plant powders could be used as suitable alternative to synthetic insecticides to suppress *C. maculatus* infestation in stored cowpea seeds among the resource-poor farmers.

**Keywords:** Contact toxicity; Copulating pairs; Fumigant toxicity; Pulverised; Unsexed

## Introduction

Protection of stored cowpea grains against *Callosobruchus maculatus* (Fabricius, 1775) (Coleoptera: Chrysomelidae: Bruchinae) infestation is of utmost importance to secure continuous, safe legume supply and enhance food security all over the world. Damages caused by *C. maculatus* include severe powdering and seed weight loss; poor seed quality, contamination of produce, predisposition of grains to pathogen attack, reduction in market value and germination of the infested seeds (1-2).

Cowpea (*Vigna unguiculata* (L.) Walp.) plays a prominent role to cater for the quantitative and qualitative dietary protein requirement of large population of human in developing countries because it serves as cheap alternative source of protein as a result of the high cost of animal protein (3-4). Therefore, to meet the dietary protein demand of the ever-growing population, man depends solely on the use of synthetic insecticide as major control measure to curb the damaging effect of this notorious insect. However, the shortcomings associated with the use of synthetic insecticides in stored product protection, which include their effects on nontarget organisms, development of resistance, environmental pollution, risk to human health, their persistency and residue in stored grains, have made farmers and researchers to seek for alternative control measures that are safe for the environment and human health, readily available, effective and biodegradable (5-6).

Use of locally available plants with medicinal and insecticidal properties to control insect pests, is a common traditional method employed by farmers in developing countries in their storage facilities before the advent of synthetic insecticides. This method appears to be a safe and suitable alternative to controlling insect pests of stored food grains (7-9), in view of the increasing efforts to develop safe alternatives to synthetic insecticides and fumigants especially from plant sources for the protection of food grain products against insect infestations and damages. This study was conceived to provide information on grains protectant potentials of *Aframomum melegueta*, *Zanthoxylum zanthoxyloides*, *Piper guineense* and *Eugenia aromatica*) powders against adult stage of *C. maculatus*

infesting stored cowpea seeds.

## Materials and methods

### *Insect culture*

The initial stock of *C. maculatus* used for starter culture was obtained from infested cowpea purchased from Southgate Market, Federal University of Technology, and Akure, Nigeria. Untreated and uninfested 'drum' local cowpea cultivar obtained from the market and was disinfested in a deep freezer at  $-20^{\circ}\text{C}$  for 96 hrs and allowed to air dried for an hour to prevent mouldiness. *Callosobruchus maculatus* adults were then introduced into the clean (disinfested) cowpea seeds and allowed to oviposit for 14 days, after which the adults were sieved out. The emerged adults were transferred into another jar of disinfested cowpea covered with muslin cloth. This was to make sure that the emerged adults used as the culturing stock for the experiment were of uniform size and age. The culture was maintained at ambient temperature of  $30 \pm 2^{\circ}\text{C}$ ,  $75 \pm 5\%$  relative humidity and 12L:12D photoperiodic regime in Entomology Laboratory, Biology Department, Federal University of Technology, and Akure, Nigeria.

### *Preparation of cowpea seeds*

Seeds of drum cowpea cultivar were properly sorted to remove broken or damaged seeds. Remained plants were kept in a freezer for 72 hrs to kill any immature stage (if any). The cowpea seeds were then removed and allowed to air dried under ambient laboratory conditions before use.

### *Preparation of plant powders*

The plant materials used was purchased from the herbal stall at Erekesan, Akure, Nigeria and authenticated at the Department of Forestry and Wood Technology, Federal University of Technology, Akure, Nigeria. The plant materials were washed in clean water and air dried and was then pulverized with an electric blender to obtain fine powder.

Disinfested wholesome cowpea grain was weighed (20 g each) and put into separate plastic dish (125 ml) and admixed with plant powders in the following proportions 0.2, 0.3, 0.4 and 0.5 g, while the control had no plant powders admixed to it. Thereafter, 10

unsexed newly emerged teneral adult *C. maculatus* were introduced into the treatments. Each treatment was replicated three times and the setup kept inside a wire mesh cage in the laboratory under ambient tropical conditions. Beetle mortality was recorded daily for 4 days starting at 24 hrs interval after treatment (10). This was done by putting the set up in the refrigerator at 4°C for 20-30 mins before sieving out the insect from the set ups for counting. This was to reduce the flight activity of the insect (11). An insect was considered dead if it does not respond to probing after pricking it with sharp needle in the abdomen.

**Table 1. Plant materials used for the experiment and plant parts used for powder preparation.**

| Scientific name                   | common name               | family        | part used |
|-----------------------------------|---------------------------|---------------|-----------|
| <i>Zanthoxylum zanthoxyloides</i> | candlewood                | Rubiaceae     | root      |
| <i>Aframomum melegueta</i>        | Alligator pepper          | Zingiberaceae | seeds     |
| <i>Myrcianthes fragrans</i>       | clove                     | Myrtaceae     | fruits    |
| <i>Piper guineense</i>            | West African Black pepper | Piperaceae    | fruits    |

#### *Fumigant toxicity of plant powders to adult C. maculatus and their effect on oviposition*

To determine the fumigant toxicity effects, 0.5 g or 2.5% v/w of each plant powder was weighed and put in a 500 ml Kliner jar. The vials were then sited at the middle of the jar with 50 g of disinfected cowpea seeds. The lids were not cut open at any spot so that the containers can be airtight to disallow the escape of some of the plant powder fumes. Two copulating adult pairs of *C. maculatus* were introduced and replicated 4 times in each case. The entire experiment was arranged in Complete Randomised Design (CRD) and left undisturbed in a wire mesh cage in the laboratory. Adult mortality and oviposition were observed for 4 days starting at 24 hourly intervals after infestation (10).

#### *Data collection and statistical analysis*

Data were corrected for mortality in the control using Abbott's formula (12) and data from the replicates of the experiment were pooled together and was subjected to Analysis of Variances (ANOVA), number of egg laid were subjected to square root transformation and

percentages were arcsine transformed before analysis to normalize the data (13). Significant treatment means were separated using Tukey test at 5% level of probability.

## Results

### *Fumigant toxicity of plant powders on adult C. maculatus*

Average mortalities of fumigant toxicity of the various plant powders against adult *C. maculatus* are presented in Table 2. The results indicated that adult mortality increases as exposure time increases. The percentage adult mortalities obtained ranged from 0.00–17.17%, with maximum adult mortality recorded from cowpea seeds treated with *A. melegueta* which exhibited significant difference compared to other plant powders and *Z. zanthoxyloides* recorded minimum adult mortality over period of exposure and recorded same or similar mean value in untreated (control) dishes.

### *Contact toxicity of plant powders on adult C. maculatus*

Considerable difference in adult mortality of insects to plant powders was observed with different concentrations and exposure times; the mortality of the insect pest increased with increase in plant powder concentration and exposure time (Table 3). From the result, it can be seen that, *E. aromatica* powder was toxic to adult *C. maculatus* survival as total mortality (100%) of this insect pest was achieved after 48 hrs of exposure, at 0.3, 0.4 and 0.5% (w/w) concentrations.

For cowpea seeds treated with *A. melegueta*, *P. guineense* and *Z. zanthoxyloides* adult mortality ranges from 3.33–60.00%, at 24 and 48 hrs exposure.

Maximum mortality was recorded on cowpea admixed with 0.2% w/w *A. melegueta*, *P. guineense* and *Z. zanthoxyloides* powder except for *P. guineense* that recorded maximum adult mortality at 48 hrs exposure (43.33%). Minimum adult mortality was observed 0.5% (w/w) in all plant powder treatments except for *P. guineense* at 48 hrs exposure. The untreated cowpea seeds recorded the lowest adult mortality in all exposure periods.

### *Mean oviposition by adult C. maculatus*

Mean oviposition by adult *C. maculatus* as presented in

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Table 4 revealed that at 24 hrs exposure time, mean oviposition recorded, ranged between 39.25–60.50 and were not significantly ( $P < 0.05$ ) different from each other. At 72 hrs, mean oviposition observed on cowpea seed treated with *A. melegueta* was significantly suppressed, suggesting the presence of some toxic

substances. *Eugenia aromatica* was able to achieve 90% adult mortality at 2.5% (w/w) dosage rate 24 hrs post treatment and evoked compared to other treatments that were not differ significantly and mean number of eggs deposited on treated cowpea seeds ranges from 49.75–63.00.

**Table 2. Fumigant Toxicity of plant powders on adult *C. maculatus*. Each value is the mean of three replicates. Mean followed by the same letter within each column are not significantly different ( $P < 0.05$ ) from each other using Tukey's Test.**

| Treatments               | Concentration % (w/w) | Mean fumigant activity |                        |                        |                        |
|--------------------------|-----------------------|------------------------|------------------------|------------------------|------------------------|
|                          |                       | 24 h                   | 48 h                   | 72 h                   | 96 h                   |
| <i>E. aromatica</i>      | 2.5                   | 0.00±5.81 <sup>a</sup> | 0.25±5.81 <sup>a</sup> | 8.58±5.81 <sup>a</sup> | 8.83±5.81 <sup>a</sup> |
| <i>A. melegueta</i>      | 2.5                   | 0.50±9.21 <sup>a</sup> | 0.75±9.21 <sup>a</sup> | 8.83±9.21 <sup>a</sup> | 7.17±3.21 <sup>a</sup> |
| <i>P. guineense</i>      | 2.5                   | 0.00±0.18 <sup>a</sup> | 0.75±0.18 <sup>b</sup> | 0.75±0.18 <sup>b</sup> | 1.00±0.18 <sup>b</sup> |
| <i>Z. zanthoxyloides</i> | 2.5                   | 0.00±0.19 <sup>a</sup> | 0.00±0.19 <sup>b</sup> | 0.25±0.19 <sup>a</sup> | 0.50±0.19 <sup>a</sup> |
| Untreated                | 0.0                   | 0.00±0.24 <sup>a</sup> | 0.25±0.24 <sup>a</sup> | 0.50±0.24 <sup>a</sup> | 0.50±0.24 <sup>a</sup> |

**Table 3. Contact toxicity of plant powders on adult *C. maculatus*. Each value is the mean of three replicates. Mean followed by the same letter within each column are not significantly different ( $P < 0.05$ ) from each other using Tukey's Test.**

| Concentration % (w/w) | % mortality         |                |                     |                |                     |               |                           |                           |
|-----------------------|---------------------|----------------|---------------------|----------------|---------------------|---------------|---------------------------|---------------------------|
|                       | <i>E. aromatica</i> |                | <i>A. melegueta</i> |                | <i>P. guineense</i> |               | <i>Z. zanthoxyloides</i>  |                           |
|                       | 24 hrs              | 48 hrs         | 24 hrs              | 48 hrs         | 24 hrs              | 48 hrs        | 24 hrs                    | 48 hrs                    |
| 0.2                   | 80.00 ± 6.83a       | 83.33 ± 5.58a  | 16.67 ± 6.49a       | 30.00 ± 3.65b  | 23.33 ± 2.98b       | 36.67 ± 3.65b | 53.33 ± 4.47 <sup>b</sup> | 60.00 ± 8.69 <sup>b</sup> |
| 0.3                   | 86.67 ± 6.83b       | 100.00 ± 5.58b | 13.33 ± 6.49a       | 33.33 ± 3.65b  | 16.67 ± 2.98ab      | 30.00 ± 3.65b | 20.22 ± 4.47a             | 46.67 ± 8.69b             |
| 0.4                   | 93.33 ± 6.83b       | 100.00 ± 5.58b | 6.67 ± 6.49a        | 16.67 ± 3.65ab | 16.67 ± 2.98ab      | 30.00 ± 3.65b | 20.00 ± 4.47a             | 36.67 ± 8.69ab            |
| 0.5                   | 90.00 ± 6.83b       | 100.00 ± 5.58b | 3.33 ± 6.49a        | 16.67 ± 3.65ab | 20.00 ± 2.98b       | 43.33 ± 3.65b | 13.33 ± 4.47a             | 46.67 ± 8.69b             |
| Untreated             | 3.33 ± 6.83a        | 3.33 ± 5.58a   | 0.0 ± 6.49a         | 0.00 ± 3.65a   | 3.33 ± 2.98a        | 3.33 ± 3.65a  | 3.33 ± 4.47a              | 3.33 ± 8.69a              |

**Table 4. Mean oviposition by adult *C. maculatus*. Each value is the mean of three replicates. Mean followed by the same letter within each column are not significantly different ( $P < 0.05$ ) from each other using Tukey's Test.**

| Treatments               | Concentration % (w/w) | Mean oviposition        |                          |                          |                         |
|--------------------------|-----------------------|-------------------------|--------------------------|--------------------------|-------------------------|
|                          |                       | 24 h                    | 48 h                     | 72 h                     | 96 h                    |
| <i>E. aromatica</i>      | 2.5                   | 54.25±1.56 <sup>a</sup> | 56.00±1.56 <sup>ab</sup> | 60.50±1.56 <sup>b</sup>  | 68.50±1.56 <sup>c</sup> |
| <i>A. melegueta</i>      | 2.5                   | 48.50±1.25 <sup>a</sup> | 54.00±1.25 <sup>b</sup>  | 57.00±1.25 <sup>bc</sup> | 62.00±1.25 <sup>c</sup> |
| <i>P. guineense</i>      | 2.5                   | 43.50±1.82 <sup>a</sup> | 49.75±1.12 <sup>ab</sup> | 53.75±1.82 <sup>b</sup>  | 62.50±1.82 <sup>c</sup> |
| <i>Z. zanthoxyloides</i> | 2.5                   | 39.25±2.76 <sup>a</sup> | 50.75±2.76 <sup>ab</sup> | 55.50±2.76 <sup>b</sup>  | 61.25±2.76 <sup>b</sup> |
| Untreated                | 0.0                   | 60.50±1.83 <sup>a</sup> | 63.00±1.83 <sup>ab</sup> | 68.75±1.83 <sup>a</sup>  | 73.50±1.83 <sup>c</sup> |

Number of eggs lay by adult *C. maculatus* at 72 hrs after exposure, indicated significant treatment effects with the exception of cowpea seeds admixed with *P. guineense* and *Z. zanthoxyloides* that exhibited non-significant difference. While at 96 hrs, *Z. zanthoxyloides* plant powder exerted significant difference on oviposition compared to other treatments including control. In all the treatments hours after exposure, dishes treated with *P. guineense* powder recorded the minimum oviposition with exception of 96 hrs after exposure where *Z. zanthoxyloides* powder recorded minimum oviposition (61.25±2.76), while untreated (control) dishes recorded the maximum oviposition throughout the exposure time.

## Discussion

Plant materials with medicinal/insecticidal properties have been used traditionally by peasant farmers mixing with stored grains to prevent insect damage for generations throughout the world (15). Various researchers have reported the use of *E. aromatica*, *A. melegueta*, *P. guineense*, *Z. zanthoxyloides*, as potential biopesticides for the protection of food grains against stored products insect pests in Tropical and Subtropical countries of the world (15-24). The results of this study further provide additional information to support these findings. The various powders exhibited different levels of toxicity against adult *C. maculatus* upto 100% adult mortality 48 hrs post treatment. This

result is similar to previous findings (25) where 1.7% *E. aromatica* powder evoked 100% mortality on yam moth (*Euzopherodes vapidella*). Generally, the other plant powder had low direct toxicity ranges from 3.33–46.67% on *C. maculatus* with exception of *Z. zanthoxyloides* that caused between 53.33–60% adult mortality within 24 and 48 hrs post treatment. For fumigant toxicity, the different plant powders exhibited varying degrees of mortalities with *A. melegueta* having the highest percentage mortality. In this study adult mortality increased as the concentration of the plant powders and exposure time increased. Adult mortality recorded from this study might be as a result of the insecticidal properties of the various plants evaluated; this is evident in the physical discomfort to the *C. maculatus* when in direct contact with the plant materials as evidenced by staggering or loss of coordination and restlessness. Also, the characteristic pungent and peppery odour of *E. aromatica*, *A. melegueta* and *P. guineense* could have caused serious injury to the cells controlling opening and closing of the spiracles, thereby interfere with normal respiratory activity by blockage of spiracles of the insects, resulting in asphyxiation (suffocation) and subsequent death. The lower percentage survival recorded against *C. maculatus* could also be due to the abrasive nature of plant powders or acted as a physical poison which usually affects the cuticle of the insects and causes death of the insect through desiccation (26).

Plant powders interfered with the developmental process of *C. maculatus* by reducing the number of eggs laid on treated cowpea seeds compared to untreated cowpea seeds. The significant oviposition deterrent activity of the plant materials could be due to the following: (i) The brief survival time of the adult weevils on grains treated with the various plant powders at all dosages did not allow enough time for oviposition since most female *C. maculatus* deposit their eggs within 3 days of adult life (27, 28) and any reduction in adult life span is expected to contribute to reduced oviposition (24), (ii) the pungent and peppery smell of the plant powders (24, 30) and the toxicity of the plant bioactive compounds which alter the behaviour and physiology of *C. maculatus* adversely and thus preventing them from oviposition, and (iii) *C. maculatus* laid their eggs on seed coat and plant powder which are abrasive, when admixed with food grains

adhere to grains or seed coat depending on particle size will reduce insect movement, disrupting mating and sexual communication, thus deterred female insects from laying eggs (30). Oviposition inhibition by *C. maculatus* was reported (16, 24, 30-32) therefore support the present findings suggesting that certain plant powders play a significant role in deterring the insect to oviposit.

Plants are rich sources of bioactive compound which might act deadly on the insect physiological system as well as behavioural patterns (33). The high significant toxicity effect of the various plant powders which manifest through contact and fumigant action and oviposition suppression could be linked to phytochemical constituent of each plant material. *Aframomum melegueta* was reported to contain the following bioactive molecules:  $\alpha$ -caryophyllene,  $\beta$ -caryophyllene, E-nerolidol, linalool, gingerdione, gingerol, 2-heptanol, 2-heptyl acetate, paradol, shagaol and humulene (30, 34, 35) and *E. aromatica* contains eugenol, eugenyl acetate, cariofilen,  $\beta$ -caryophyllene, 1,8-Cineole, oleanolic and Cadinene (36-38). Piperine, chavicine,  $\beta$ -Phellandrene, eugenol, limonene, linalool,  $\alpha$ -pinene, and  $\beta$ -pinene are bioactive compounds that have been established to be insecticidal in *P. guineense* (39). The root bark of *Z. zanthoxyloides* contains zanthoxylol, N-isobutyldeca-2, 4-dienamide, N-isobutyllocta-2, 4-dienamide, 8-methoxydictamine, benzophenanthridines fagaronine, dihydroavicine, chelerythrine, oxychelerythrine and furoquinolines (40). These chemical constituents of the various plants are toxic and inhibit the growth of insect developmental stages as it evoked varying degrees of adult mortality. Since the beetle's mortality increases in relation with exposure time, the toxic constituents of *P. guineense*, *E. aromatica* and *A. melegueta* show some level of persistence (10, 41-43).

## Conclusion

The study shows that the plant powders evaluated recorded a remarkable contact, fumigant and anti-oviposition properties against *C. maculatus*; thus serve as promising alternatives to synthetic insecticides and fumigants for the protection of stored cowpea grains, thereby save resource poor farmers who are usually constrained to sell their production immediately after harvest or have their stored cowpea prone to infestation from severe post-harvest damages and loss.

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#### Editorial Note

Volume 7, issue 2 of Progress in Biological Sciences was initially scheduled to be published in December 31, 2017. However, some administrative changes led to a major delay in processing of the manuscripts. This issue is actually published in May 1, 2020. Editor-in-chief apologizes deeply for any inconvenience caused especially to the authors.



