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Comparative efficacy of *Piper guineense* and *Eugenia aromatica* on *Callosobruchus maculatus* and *Sitophilus zeamais* under hermetic and non-hermetic storage

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Abstract
Laboratory experiments were carried out in order to evaluate the insecticidal effects of *Eugenia aromatica* flower buds and *Piper guineense* fruit powders on *Callosobruchus maculatus* (F) and *Sitophilus zeamais* (M) under hermetic and non-hermetic storage conditions. The effects of the powders were observed at different dosage rates: 0.0g (control), 0.2g, 0.4g, 0.6g, 0.8g and 1.0g per 20g of cowpea seeds and maize grains. Percentage beetle and weevil mortality were observed at 12, 24, 36, 48 hours and at 24, 48 and 72 hours respectively. The results revealed 100% mortality of adult *C. maculatus* on cowpea seeds treated with *E. aromatica* powder and *P. guineense* at 36 hours and 48 hours respectively. *P. guineense* powder on *S. zeamais* evoked the highest adult mortality of 56.7% at 72 hours post treatment under hermetic storage condition. Low bruchid and weevil mortality were observed on cowpea seeds and maize grains treated with the plant powders when stored in non-hermetic storage condition regardless of the period of exposure of the insects to the plant powders. In a further evaluation of the plant powders on the survival and development of *C. maculatus* and *S. zeamais*, adult emergence were drastically reduced in all the treated seeds stored under hermetic condition than those stored under non-hermetic condition. The effectiveness of the plant powders in reducing damage due to the control of *C. maculatus* and *S. zeamais* infestation during storage was proved in this work and the plant materials exhibited promising potentials as a substitute to the use of synthetic insecticides in hermetic storage of seeds.

Keywords: *P. guineense*, *E. aromatica*, *C. maculatus*, *S. zeamais*, hermetic, non-hermetic

Introduction
Staple crops such as legumes and cereals (cowpea and maize), have served as major sources of dependence and sustainability for the livelihood of people in Africa [13]. Cowpea, *Vigna unguiculata* (L.) Walp, is among the major sources of plant protein [19]. The high protein content of the seed makes it extremely valuable in Tropical Africa, especially where meat and fish is expensive for the teeming population [14]. Maize (*Zea mays L. ssp. mays*) or corn is high yielding, easy to process, readily digested, cheaper than other cereals and grows across a wide range of agro ecological zones. Maize is a significant source of starch, and a feedstock for the production of corn oil, gluten, high fructose corn syrup, grain alcohol, and biofuels [21]. 55-60% loss in seed weight and 45.50-66.33% loss in protein content in cowpea has been reported due to damage by *Callosobruchus maculatus* [17]. Of all pest infestations, the interaction of *Zea mays* with the maize weevil (*Sitophilus zeamais*) has also been reported to be the most destructive in post-harvest storage resulting to 10-50% maize grain loss, in the tropics and even complete destruction in some cases [6].
Several control measures have been employed by farmers to combat many destructive pests in staple crops' production and storage [18]. At present, much of the pest control measures in storage rely on the use of synthetic chemical insecticides and fumigants which are the quickest and surest method of pest control, but the methods are of high risk [17]. The uncontrolled use of this method has led to a number of problems including insect resistance, food poisoning and environmental pollution in addition to the increasing cost of application [5], and an effect on non-target organisms [7]. In a reaction to this problem, researchers all over the world have resorted to using more available, less costly and eco-friendly botanicals to protect crops from insect damage [11].

Several botanicals which include among others; *Piper guineense*, *Eugenia aromatica*, *Zanthozylum zanthoxyloides*, *Nicotiana tabacum*, *Azadirachta indica and Dennetia tripetela* have been screened for insecticidal activities [1].

In addition, storage problems do not only occur as a result of pest infestations but also often occur in high relative humidity and temperature, and in the presence of adequate oxygen. These problems are eliminated by creating a hermetic storage condition which include the use of air-tight storage containers or structures that reduces the O_2 concentration and increases the CO_2 concentration through respiration processes of biological agents [20]. The present research was carried out to evaluate the insecticidal activities of *E. aromatica* (flower buds) and *P. guineense* (fruits) powders against *C. maculatus* and *S. zeamais* under hermetic and non-hermetic storage conditions.

2. Materials and Methods

The experiments were carried out in the Research Laboratory of the Crop, Soil and Pest Management Department of the Federal University of Technology, Akure, Nigeria, under ambient conditions (28±2ºC and 65±10% RH).

2.1 Preparation of plant products and stored grains

The dry fruits of West African black pepper, *Piper guineense* (Schum and Thonn) and dry flower buds of Clove, *Eugenia aromatica* (Baill) used in this investigation were bought from a local market (Oba market) in Akure, Ondo State, Nigeria. They were selected based on the assumption of absence of mammalian toxicity owing to its use as a popular spice in several diets. They were air-dried in a well-ventilated shaded area and then ground to a fine powder using an electric blender. The spices were stored in separate containers before use. Cowpea seeds (Oloyin variety) and maize grains (T-Suwan) were disinfested by keeping them in a freezer for two weeks and subsequently in room temperature before being used for experimental purposes.

2.2 Rearing of experimental insects

Insects (*S. zemais*) were released at the rate of 60 pairs of sexed adults in plastic jars containing 600g of maize grains while sixty pairs of sexed adults of *C. maculatus* were introduced into plastic jars containing 1.2kg of cowpea seeds. The jars were covered with fine mesh cloths tied with rubber bands and the cultures were maintained under prevailing conditions in the laboratory. Freshly emerged adults of *S. zeamais* and *C. maculatus* from the cultures in the laboratory were removed and used accordingly for the experiments.

2.3 Mortality and progeny assessment assays

The powders of dry *Piper guineense* fruits and dry *Eugenia aromatica* flower buds were added separately to 20g of clean uninfested cowpea and maize seeds in separate small hermetic storage glass bottles and non- hermetic storage containers covered with meshed lids at five dosage rates of 0.2g, 0.4g, 0.6g, 0.8g and 1.0g, while the control treatments had no plant powders. All treatments were replicated three times. The powders were admixed thoroughly with the grains to ensure proper coating of seeds.

Five pairs of freshly emerged adults of 0-1 day old *Callosobruchus maculatus* and *Sitophilus zeamais* were introduced into each treatments. Adult mortality was monitored and counted at 12hrs, 24hrs, 36hrs and 48hrs after infestation for cowpea beetles, while adult mortality of maize weevils was monitored and counted at 24hrs, 48hrs and 72hrs.

Adult weevils were removed 7 days after infestation and the numbers of emerged adult weevils were counted for 15 days while cowpea bruchids were allowed to oviposit for 7 days and dead insects were removed. The various treatments were left on the laboratory workbench and examined daily for the emergence of adults. The emerged adults were removed and counted daily for 10 days.

2.4 Statistical analysis

The experimental design adopted in this experiment was Completely Randomized Design (CRD). Data on % mortality were arcsine transformed and the number of emerged adults were square root transformed before being subjected to analysis of variance (ANOVA). Means separations were conducted using Duncan New Multiple Range Test at 5% level of significance using the statistical package, SPSS version 21.

3. Results

The results in Table 1 revealed that cowpea seeds stored under hermetic storage condition, 100% bruchid mortality were achieved at 36 hours of application by 0.4g-1.0g rates of *E. aromatica* while none of the rates of *P. guineense* was able to cause 100% mortality throughout the time of exposure. Under non- hermetic storage condition, Low insect mortality were achieved with the various rates of the plant powders and their effects differ significantly at $p<0.05$ (Table 2). The percentage of emerged adults of *C. maculatus* from seeds treated with the plant powders under the two storage conditions was significantly reduced compared to the control (Table 5 and 6). Adult emergence decreased with the increase in rates of application.

At 48 hours post treatment, there was a significant difference ($p<0.05$) between 1.0g rate of the plant powders applied and the other rates of the plant powders. In Table 3, *P. guineense* powder applied at 1.0g on maize grains stored under hermetic storage condition, recorded the highest weevil mortality of 56.67% at 72 hours after treatment. Weevil mortality of 16.67% was recorded over 72 hours of exposure with 1.0g of *E. aromatica* powder under non-hermetic storage condition (Table4). There were significant difference between the rates of *P. guineense* powder at $p<0.05$ for all the period of exposure. The powder of *P. guineense* significantly reduced the emergence of adult weevils from treated grains stored under both storage conditions (Table 5 and 6).

Table 1: Percentage mortality of *C. maculatus* on cowpea seeds treated with different rates of *E. aromatica* and *P. guineense* powders under hermetic storage condition.

		% mortality in hours			
P. materials	**Rates(g)**	**12**	**24**	**36**	**48**
E. aromatica	0.0	0.0 ± 0.00^{c}	$0.0 \pm 0..00^{d}$	0.0 ± 0.00^{c}	23.3 ± 3.33^{c}
	0.2	13.3 ± 3.33^{d}	56.7 ± 3.33^{c}	76.7 ± 3.33^{b}	86.7 ± 3.33^{b}
	0.4	23.3 ± 3.33^{c}	86.7 ± 3.33^{b}	100.0 ± 0.00^{a}	100.0 ± 0.00^{a}
	0.6	26.7 ± 3.33^{b}	100.0 ± 0.00^{a}	100.0 ± 0.00^{a}	100.0 ± 0.00^{a}

	0.8	33.3 ± 3.33^{ab}	100.0 ± 0.00^{a}	100.0 ± 0.00^{a}	100.0 ± 0.00^{a}
	1.0	40.0 ± 0.00^{a}	100.0 ± 0.00^{a}	100.0 ± 0.00^{a}	100.0 ± 0.00^{a}
P. guineense	0.0	0.0 ± 0.00^{b}	0.0 ± 0.00^{b}	0.0 ± 0.00^{c}	23.3 ± 3.33^{b}
	0.2	3.3 ± 3.33^{ab}	16.7 ± 3.33^{b}	53.3 ± 6.67^{b}	80.0 ± 0.00^{a}
	0.4	6.7 ± 3.33^{ab}	23.3 ± 3.33^{ab}	60.0 ± 0.00^{ab}	83.3 ± 3.33^{a}
	0.6	6.7 ± 3.33^{ab}	33.3 ± 3.33^{ab}	63.3 ± 3.33^{ab}	83.3 ± 3.33^{a}
	0.8	6.7 ± 3.33^{ab}	46.7 ± 3.33^{a}	66.7 ± 3.33^{ab}	86.7 ± 3.33^{a}
	1.0	13.3 ± 3.33^{a}	56.7 ± 6.67^{a}	70.0 ± 0.00^{a}	86.7 ± 3.33^{a}

Each value is the mean ± standard error of the three replicates. Values in the same column with the same letter(s) do not differ significantly at $p>0.05$ using Duncan's New Multiple Range Test.

Table 2: Percentage mortality of *C. maculatus* on cowpea seeds treated with different rates of *E. aromatica* and *P. guineense* powders under non-hermetic storage condition.

		% mortality in hours			
P. materials	**Rates(g)**	**12**	**24**	**36**	**48**
	0.0	0.00 ± 0.00^{d}	0.0 ± 0..00^{c}	0.0 ± 0.00^{d}	3.3 ± 3.33^{d}
	0.2	3.3 ± 3.33^{cd}	10.0 ± 5.77^{d}	26.7 ± 3.33^{c}	36.7 ± 3.33^{d}
E. aromatica	0.4	6.7 ± 3.33^{bcd}	16.7 ± 3.33^{cd}	30.0 ± 3.33^{c}	46.7± 3.33^{cd}
	0.6	10.0 ± 0.00^{abc}	20.0 ± 0.00^{bc}	40.0 ± 3.33^{b}	56.7 ± 3.33^{bc}
	0.8	13.3 ± 3.33^{ab}	26.7 ± 3.33^{ab}	50.0 ± 3.33^{a}	63.3 ± 3.33^{ab}
	1.0	16.7 ± 3.33^{a}	30.0 ± 0.00^{a}	53.3 ± 3.33^{a}	73.3 ± 3.33^{a}
	0.0	0.00 ± 0.00^{c}	0.0 ± 0.00^{d}	0.0 ± 0.00^{e}	3.3 ± 3.33^{d}
	0.2	10.3 ± 3.33^{b}	13.3 ± 0.00^{c}	26.7 ± 0.00^{d}	33.3 ± 3.33^{c}
P. guineense	0.4	13.3 ± 3.33^{ab}	16.7 ± 3.33^{bc}	33.3 ± 3.33^{cd}	43.3 ± 3.33^{bc}
	0.6	13.3 ± 3.33^{ab}	23.3 ± 3.33^{ab}	36.7 ± 3.33^{ab}	46.7 ± 3.33^{b}
	0.8	16.7 ± 3.33^{ab}	26.7 ± 3.33^{a}	43.3 ± 0.00^{ab}	53.3 ± 3.33^{b}
	1.0	20.0 ± 0.00^{a}	30.0 ± 0.00^{a}	46.7 ± 3.33^{a}	66.7 ± 3.33^{a}

Each value is the mean ± standard error of the three replicates. Values in the same column with the same letter(s) do not differ significantly at $p>0.05$ using Duncan's New Multiple Range Test.

Table 3: Percentage mortality of *S. zeamais* on cowpea seeds treated with different rates of *E. aromatica* and *P. guineense* powders under hermetic storage condition.

		% mortality in hours		
P. materials	**Rates(g)**	**24**	**48**	**72**
	0.0	0.00 ± 0.00^{c}	0.0 ± 0..00^{b}	0.0 ± 0.00^{c}
	0.2	0.0 ± 0.00^{c}	0.0 ± 0.00^{b}	0.0 ± 0.00^{c}
E. aromatica	0.4	0.0 ± 0.00^{c}	0.0 ± 0.00^{b}	3.3 ± 3.33^{c}
	0.6	0.0 ± 0.00^{c}	3.3 ± 3.33^{b}	16.7 ± 3.33^{bc}
	0.8	6.7 ± 3.33^{b}	6.7 ± 3.33^{b}	30.0 ± 5.77^{b}
	1.0	13.3 ± 3.33^{a}	26.7 ± 6.67^{a}	50.0 ± 11.55^{a}
	0.0	0.0 ± 0.00^{b}	0.0 ± 0.00^{c}	0.0 ± 0.00^{d}
	0.2	0.0 ± 0.00^{b}	0.0 ± 0.00^{c}	16.7 ± 3.33^{c}
P. guineense	0.4	0.0 ± 0.00^{b}	0.0 ± 0.00^{c}	20.0 ± 5.77^{c}
	0.6	3.3 ± 3.33^{b}	6.7 ± 3.33^{c}	23.3 ± 3.33^{c}
	0.8	6.7 ± 3.33^{b}	16.7 ± 3.33^{b}	36.7 ± 3.33^{b}
	1.0	16.7 ± 3.33^{a}	26.7 ± 3.33^{a}	56.7 ± 8.82^{a}

Each value is the mean ± standard error of the three replicates. Values in the same column with the same letter(s) do not differ significantly at $p>0.05$ using Duncan's New Multiple Range Test.

Table 4: Percentage mortality of *S. zeamais* on cowpea seeds treated with different rates of *E. aromatica* and *P. guineense* powders under non-hermetic storage condition.

		% mortality in hours		
P. materials	**Rates(g)**	**24**	**48**	**72**
	0.0	0.00 ± 0.00^{b}	0.0 ± 0..00^{b}	0.0 ± 0.00^{c}
E. aromatica	0.2	0.0 ± 0.00^{b}	0.0 ± 0.00^{b}	0.0 ± 0.00^{c}
	0.4	3.3 ± 3.33^{ab}	3.3 ± 3.33^{ab}	3.3 ± 3.33^{c}
	0.6	3.3 ± 3.33^{ab}	3.3 ± 3.33^{ab}	6.7 ± 3.33^{c}
	0.8	6.7 ± 3.33^{ab}	6.7 ± 3.33^{ab}	13.3 ± 3.33^{ab}
	1.0	10.0 ± 0.00^{a}	10.0 ± 3.33^{a}	16.7 ± 3.33^{a}
	0.0	0.0 ± 0.00^{b}	0.0 ± 0.00^{c}	0.0 ± 0.00^{b}
P. guineense	0.2	0.0 ± 0.00^{b}	0.0 ± 0.00^{c}	0.0 ± 0.00^{b}
	0.4	0.0 ± 0.00^{b}	3.3 ± 3.33^{c}	6.7 ± 3.33^{b}

	0.6	3.3 ± 3.33[b]	13.3 ± 3.33[b]	16.7 ± 3.33[a]
	0.8	6.7 ± 3.33[ab]	20.0 ± 0.00[ab]	20.0 ± 0.00[a]
	1.0	13.3 ± 3.33[a]	23.3 ± 3.33[a]	23.3 ± 3.33[a]

Each value is the mean ± standard error of the three replicates. Values in the same column with the same letter(s) do not differ significantly at $p>0.05$ using Duncan's New Multiple Range Test.

Table 5: Number of adult emerged (*S. zeamais*) and % adult emergence (*C. maculatus*) under hermetic storage condition.

Plant materials	Rates (g)	Number of adult emerged (*S. zeamais*)	% Adult emergence (*C. maculatus*)
E. aromatica	0.0	2.0 ± 0.00[a]	28.8 ± 0.58[a]
	0.2	1.3 ± 0.33[b]	5.8 ± 0.29[b]
	0.4	1.0 ± 0.00[bc]	0.0 ± 0.00[c]
	0.6	1.0 ± 0.00[bc]	0.0 ± 0.00[c]
	0.8	0.7 ± 0.33[bc]	0.0 ± 0.00[c]
	1.0	0.3 ± 0.33[c]	0.0 ± 0.00[c]
P. guineense	0.0	1.7 ± 0.33[a]	28.8 ± 0.58[a]
	0.2	1.0 ± 0.58[ab]	17.0 ± 1.66[b]
	0.4	0.3 ± 0.33[b]	15.1 ± 0.79[bc]
	0.6	0.0 ± 0.00[b]	9.3 ± 3.01[c]
	0.8	0.0 ± 0.00[b]	0.0 ± 0.00[d]
	1.0	0.0 ± 0.00[b]	0.0 ± 0.00[d]

Each value is the mean ± standard error of the three replicates. Values in the same column with the same letter(s) do not differ significantly at $p>0.05$ using Duncan's New Multiple Range Test.

Table 6: Number of adult emerged (*S. zeamais*) and % adult emergence (*C. maculatus*) under non-hermetic storage condition.

Plant materials	Rates (g)	Number of adult emerged (*S. zeamais*)	% Adult emergence (*C. maculatus*)
E. aromatica	0.0	11.3 ± 3.18[a]	78.8 ± 0.58[a]
	0.2	7.3 ± 2.85[a]	42.1 ± 2.18[b]
	0.4	5.7 ± 2.08[a]	36.8 ± 1.15[c]
	0.6	5.3 ± 2.19[a]	32.5 ± 0.53[cd]
	0.8	5.0 ± 0.67[a]	29.7 ± 1.87[d]
	1.0	4.7 ± 1.45[a]	20.6 ± 1.35[e]
P. guineense	0.0	8.3 ± 1.86[a]	78.8 ± 0.59[a]
	0.2	7.0 ± 1.00[abc]	55.4 ± 1.14[b]
	0.4	5.0 ± 0.58[abc]	45.9 ± 1.16[c]
	0.6	4.0 ± 0.58[bc]	34.8 ± 1.37[d]
	0.8	4.0 ± 1.15[bc]	28.3 ± 0.82[e]
	1.0	3.0 ± 0.58[c]	19.1 ± 0.63[f]

Each value is the mean ± standard error of the three replicates. Values in the same column with the same letter(s) do not differ significantly at $p>0.05$ using Duncan's New Multiple Range Test.

4. Discussion

The results obtained from this study showed that the effectiveness of the plant powders were dependent on the rate of application, period of exposure and storage condition. The 100% mortality of the bruchids has been linked to an observation reported by [4] that *E. aromatica* powder was the only plant material that elicited complete mortality of *S. oryzae* when tested on the insects alongside *P. guineense* and *Zingiber officinale*. High mortality and low/non emergence of cowpea beetles and maize weevils caused by the plant powders under hermetic storage may be due to the combined effects of the air-tight storage condition of the seeds and the choking smell of the plant materials which must have disrupted the normal respiratory process of the insects, larval feeding activity, mating and sexual communication, as well as deterring females from laying eggs [9]. The effectiveness of hermetic containers against *Prostephanus truncatuus* has been reported before in Mexico, with 100% mortality of *P. truncatus* after only a few days when storing grain in glass containers [15]. It was also reported by [3] and [8] that under sealed storage (hermetic) conditions in maize, insects and fungi deplete the oxygen supply, creating an unfavorable atmosphere for their own survival.

Low bruchid and weevil mortality recorded under non-hermetic storage of the grains may be due to the free flow of air in and out of the storage containers making sufficient oxygen to be available for respiration by the insects, loss of active ingredients of the plant powders to air and also the presence of thick and large exoskeleton on maize weevils which might have made them to develop some levels of tolerance to the plant powders [2]. This is in agreement with the findings of [10] when they used stem and root powder of *Newbouldia laevis* and oil against *S. zeamais* and *S. oryzae*.

5. Conclusion and Recommendation

On the bases of the findings, it could be concluded that plant powders pose potentials as plant derived insecticides against *C. maculatus* and *S. zeamis* when grains were stored under hermetic and non-hermetic conditions. The combined effects of the hermetic condition and plant materials were effective in suppressing the development of the insects. Considering the side effects of synthetic pesticides, it is therefore recommended that the plant materials used in this work could serve as a good substitute to the use of synthetic insecticides in hermetic storage of seeds and also, longer period of exposure may be needed to increase mortality in adults.

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