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Phytochemical Screening and Bio-Insecticidal Efficacy of Alligator Pepper (*Aframomum melegueta*), Pepper Fruit (*Dennettia tripetala*), and Guinea Cubeb (*Piper guineense*) Seed Powder on Cockroaches (*Blattella germanica;* Order: *Blattodea*, Family: *Ectobiidae*)

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Abstract
A laboratory investigation was conducted to assess the efficacy of seed powders derived from Alligator pepper [*Aframomum melegueta*], Pepper fruit [*Dennettia tripetala*] and Guinea cubeb [*Piper guineense*] in managing German cockroach [*Blattella germanica*] nymphs. Different concentrations (0.0, 10, 20, 30, and 40% w/v) of the seed powders were topically applied to the dorsal surface of the thorax of individual nymphs using a microcapillary tube. The nymphs were then placed in 9 cm diameter air-tight Petri dishes and maintained under ambient conditions (25-30°C and 75 ± 5% RH) for 24 hours to assess mortality and for 48 hours for metamorphic tests. The experimental design followed a completely randomized design with duplicate trials. Statistical analysis of the data revealed that the seed extract treatments exhibited significantly higher mean mortality levels of nymphs compared to the control group ($P < 0.05$). Among the treatments, *P. guineense* exhibited the highest toxicity with a mean standard error value of 16.5±2.8, followed by *A. melegueta* (15.0±2.7), and *D. tripetala* (5.5±1.7), compared to the normal control (water). The LD50 values were calculated as 22.0% w/v for *P. guineense* and 10.0% w/v for *A. melegueta*. P hytochemical analysis of the treatments revealed the presence of various secondary metabolites including flavonoids, tannins, saponins, alkaloids, terpenoids, glycosides, and anthraquinones. Among these compounds, saponins are known to disrupt cell membranes, while tannins act as polyglycosides with antifeedant properties, interfering with insect digestion. These findings suggest that the observed mortality of the cockroach nymphs may be attributed to the presence of saponins and tannins in the seed powders.

Keywords: Aframomum Melegueta, Dennettia Tripetala, Piper Guineense, Blattella Sp., Seed Powder.

Introduction

Insects constitute the most diverse group within the phylum Arthropoda, encompassing over a million distinct species and exhibiting unparalleled numerical abundance (Johnson, 2003). Within this taxonomic phylum, insects display characteristic traits while also possessing unique attributes not found in other arthropods (Pedigo & Rice, 2009). Among the myriad species, both beneficial and detrimental to ecosystems and human interests, notable examples include grasshoppers, termites, honeybees, and various others. However, the common cockroach stands out as a particularly pervasive household pest, presenting a significant epidemiological challenge due to its role as a vector for bacterial transmission (Kutrup, 2003). Recent research conducted by Purdue University scientists has underscored the formidable challenge posed by the evolving resistance of cockroaches to synthetic insecticides, rendering chemical extermination strategies increasingly ineffective (AIFS, 2020).

In contrast, natural spices such as Piper guineense, Aframomum melegueta, and Dennettia tripetala seeds have long held cultural, culinary, and medicinal significance in many societies. Piper guineense, belonging to the Piperaceae family, thrives in the humid regions of West Africa, yielding a spice derived from its dried fruits. Referred to by various names including Benin pepper, Ashanti pepper, etinkeni, Edo pepper, guinea pepper, masoro, false cubeb, and kanafuru (Younis & Mohamed, 2019), P. guineense contains notable quantities of the chemical piperine, contributing to its characteristic spiciness, heat, and pungency (Dawodu et al., 2021).

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Aframomum melegueta, commonly utilized in Nigeria and other West African regions as a spice for culinary and entertainment purposes, possesses a rich history of folkloric utilization in traditional medicine. Its therapeutic applications include the treatment of stomach ache, diarrhea, and snakebites (Umukoro & Ashorobi, 2007; Ilic et al., 2010). Furthermore, the seed extract of A. melegueta has been subject to comprehensive evaluation, revealing a spectrum of pharmacological activities such as anti-nociceptive, antiulcer, antimicrobial, anti-inflammatory, antioxidant, and sexual performance enhancing properties (Onoja et al., 2014). Dennettia tripetala, a member of the Anononaceae family predominantly found in Eastern Nigeria and Cameroon, has emerged as a promising agent for pest control. The phytochemical composition of D. tripetala, including pyridine and benzylnitrite among others, has been implicated in its insecticidal activity (Elekwa et al., 2011). Notably, studies have documented the efficacy of D. tripetala seed powder in safeguarding stored products against insect pests (Babarinde et al., 2015; Babarinde et al., 2016). Additionally, investigations have explored the synergistic effects of combining powders derived from P. guineense and D. tripetala in protecting stored commodities against pest infestations (Dawodu and Ofuya, 2000). Despite the existing body of research attesting to the efficacy of such powders in pest management within storage contexts, there remains a distinct necessity to assess the impact of A. melegueta, D. tripetala, and P. guineense seed powders specifically on Blattella Sp., a genus of cockroach species. This evaluation could provide valuable insights into the potential utility of these natural substances as bio-insecticides against a common household pests.

Materials and Methods

Fresh seeds of three indigenous plants, namely Alligator Pepper (*Aframomum melegueta*), Pepper Fruit (*Dennettia tripetala*), and Guinea Cubeb (*Piper guineense*) Seed, were procured from the Igwuruta market situated in the Ikwerre Local Government Area of Rivers State, Nigeria. These plant materials were sourced to facilitate research endeavors aimed at investigating their potential bioactive properties and applications, particularly in the context of pest management and other pertinent areas of study. The selection of these plant species for investigation underscores their cultural significance and traditional uses within the region, as well as their documented pharmacological activities and bioactive constituents. The rigorous procurement process ensured the acquisition of high-quality, fresh seeds essential for conducting reliable and scientifically sound experiments. These botanical specimens hold immense promise for scientific exploration, offering valuable insights into their phytochemical composition, biological activities, and potential applications in various fields. The acquisition of these seeds from a local market in the region not only reflects their accessibility but also underscores the importance of indigenous knowledge and practices in guiding research efforts aimed at harnessing the potential benefits of natural resources for societal advancement and well-being.

Alligator Pepperseeds**Pepper fruit containingGuinea Cubeb seeds**

Upon procurement, the fresh fruits of the selected plant species were subjected to a meticulous drying process within the laboratory setting. The fruits were carefully spread out on laboratory trays, ensuring even distribution, and subsequently placed in an oven for dehydration. Notably, the Alligator Pepper seeds were acquired in a dry state and thus did not require the drying procedure. Following the completion of the drying process, particular attention was directed towards the preparation of the seeds for further experimentation. Thirty grams (30g) of each dried seed sample were carefully milled into a fine powder, employing an electrical grinder to ensure uniformity and consistency. The resulting powdered material was mthoroughly ollected and segregated into distinct labeled containers, facilitating accurate identification and differentiation of the samples. To facilitate the experimental setup, fifteen grams (15g) of the finely powdered seeds from each plant

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sample were precisely weighed out. This careful measurement ensured the uniformity of the experimental conditions and minimized potential variations in the outcomes attributable to differences in sample quantities. German cockroaches (*Blattella germanica*) were reared within controlled laboratory conditions to ensure optimal health and uniformity for subsequent bioassays. The rearing containers were maintained at a constant temperature of 25 ± 3°C and a relative humidity of 75 ± 5%, creating a conducive environment conducive to the growth and development of the cockroach colony. Additionally, a consistent photoperiod of 9:15 hours (Light:Dark) was established to mimic natural lighting conditions and regulate the circadian rhythms of the cockroaches. For the bioassays, fifth instar nymphs were selected as the experimental subjects due to their developmental stage, which is particularly sensitive and relevant for toxicity testing. To facilitate handling and ensure uniformity in experimental conditions, the nymphs were subjected to a brief chilling procedure. Specifically, the experimental nymphs were exposed to a temperature of 4°C for a duration of 10 minutes, effectively inducing a state of temporary immobilization without causing harm or altering their physiological status.

The toxicity test, designed to assess the potential lethal effects of the seed powder solutions derived from Alligator Pepper (*Aframomum melegueta*), Pepper Fruit (*Dennettia tripetala*), and Guinea Cubeb (*Piper guineense*) Seed on German cockroach nymphs (*Blattella germanica*), was meticulously conducted following a standardized experimental protocol. Fifteen grams (15g) of each seed powder sample was dissolved in distilled water to create solutions of varying concentrations (10%, 20%, 30%, and 40% w/v). Notably, the seed powders were combined in a single form at a 100% concentration and in a 50:50 ratio for the creation of the different concentrations. Using a micro capillary tube, 0.1 ml of the test solution corresponding to each concentration (0.0 [control], 10%, 20%, 30%, and 40% w/v) of the plant extract was carefully applied to the dorsal surface of the thorax of each experimental cockroach nymph. This procedure was replicated in duplicates. Twenty cockroaches were included per replicate, and subsequent to the application of the test solutions, the nymphs were transferred to a 9 cm diameter Petri dish containing bread crumbs to serve as a food source. Mortality rates were meticulously observed and recorded at specific intervals, including 15 minutes, 30 minutes, 60 minutes, and 24 hours post-exposure. A randomized experimental design was implemented to minimize bias and ensure the robustness of the results. Control groups were treated with equivalent doses or concentrations (0.0, 10%, 20%, 30%, and 40% w/v) of distilled water to serve as the normal control, thereby facilitating the comparison of mortality rates between the experimental and control groups. By adhering to stringent experimental procedures and employing appropriate controls, the study aimed to elucidate the potential insecticidal properties of the seed powder solutions derived from the selected plant species against German cockroach nymphs, contributing valuable insights into their potential utility as bioinsecticides.

The determination of the lethal concentration (LC50) for the seed powder extracts of Alligator Pepper (*Aframomum melegueta*), Pepper Fruit (*Dennettia tripetala*), and Guinea Cubeb (*Piper guineense*) against cockroach nymphs was conducted employing the topical LD50 arithmetic method as described by Shetty et al. (2007) and Randhawa (2009). Procedure: The percentage mortality at each dose level of the seed powder extracts was recorded. These mortality percentages were then converted to log dose and plotted against the percentage mortality response. The LD50 value, representing the dose at which 50% mortality occurred, was determined from the graph as the point where the probit value equaled 5.0, indicative of 50% mortality. This methodology enabled the estimation of the concentration of each seed powder extract required to induce a 50% mortality rate among the tested cockroach nymphs. By employing statistical and graphical analyses, the LD50 values were derived, providing crucial insights into the relative potency and efficacy of the different plant extracts as potential bioinsecticides against cockroach infestations. The qualitative phytochemical screening of the test plants, including Alligator Pepper (*Aframomum melegueta*), Pepper Fruit (*Dennettia tripetala*), and Guinea Cubeb (*Piper guineense*), was conducted following established procedures outlined by Okwu (2001), Udo (2008), and Trease and Evans (2014). These standardized protocols are widely recognized in the field of phytochemical analysis and have been employed to elucidate the presence of various bioactive compounds within plant materials.

The collected data were summarized utilizing the formula mean + standard error of the mean (SEM), providing a concise representation of the central tendency and variability within the dataset. Subsequently, statistical analyses were conducted employing a two-way analysis of variance (ANOVA) utilizing the latest version of SPSS (Statistical Package for Social Sciences). Statistical significance was inferred if the calculated p-value was less than 0.05, indicating a low probability of obtaining the observed results under the null hypothesis. This threshold is commonly accepted in scientific research as indicative of significant differences or associations within the data, warranting further investigation or interpretation. This research project titled

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"Phytochemical screening and bioinsecticidal efficacy of *Aframomum melegueta*, *Dennettia tripetala* and *Piper guineense* seed powder on cockroach *(Blattellagermanica)*" has undergone thorough ethical scrutiny and approval by the [Research and Development, Ignatius Ajuru University of Education, Port Harcourt] Ethics Committee. The following ethical considerations were carefully evaluated and addressed in accordance with established ethical guidelines: The research strictly adhered to principles of animal welfare. No unnecessary harm or distress was inflicted upon the cockroaches involved in the study. All procedures were conducted with the utmost care to minimize discomfort. As cockroaches are not sentient beings capable of providing informed consent, alternative measures were taken to ensure their welfare and minimize any potential harm during the study. The potential environmental impact of using phytochemical screening and bioinsecticidal efficacy of *Aframomum melegueta*, *Dennettia tripetala* and *Piper guineense* seed powder on cockroach *(Blattella germanica)* was thoroughly considered. Measures were taken to mitigate any adverse effects on the environment, and appropriate disposal methods were employed for any waste generated during the study. Any data collected during the research process were handled with confidentiality and respect for privacy. Data were anonymized and securely stored to protect the identities of any individuals involved in the study. The research complied with all relevant local, national, and international regulations pertaining to the use of animals in scientific research, as well as any regulations regarding the use of experimental substances. Any potential conflicts of interest related to the research, including funding sources or affiliations with organizations, were disclosed and managed appropriately to ensure the integrity and impartiality of the study.

Results

The qualitative phytochemical screening of *P. guineense*, *A. melegueta*, and *D. tripetala* seed extracts revealed the presence of flavonoids, tannins, saponins, alkaloids, terpenoids, glycosides, and anthraquinones. Six out of the seven compounds were significantly present in each of the plant extracts. However, anthraquinones were found to be present in lower quantities in *P. guineense* and *A. melegueta* compared to *D. tripetala* (Table 1).

Table 1: Qualitative phytochemical Screening of seed powder extracts of *P. guineense, A. melegueta* and *D. tripetala*

Phytochemicals	*P. guineense*	*A. melegueta*	*D. tripetala*
Flavonoids	+++	+++	+++
Terpenoids	+++	+++	+++
Alkaloids	+++	++	+++
Saponins	++	++	+++
Tannins	++	+++	++
Glycoside	++	++	++
Anthraquinone	+	+	+

Key: - Absent, + =Present (minimal), ++= Actively present (moderate), +++=highly present

The toxicity assessment of Alligator Pepper, Pepper Fruit, and Guinea Cubeb Seed powder extracts against cockroach nymphs revealed higher levels of toxicity compared to the control group. Different concentrations of the aqueous solutions were prepared from 15g of seed powder extract, ranging from 10% w/v to 40% w/v, with the highest dose concentration at 40% w/v and the lowest at 10% w/v. The results indicate that higher doses of the seed powder extracts resulted in increased mortality rates among the experimented cockroaches. The mean mortality rates ± SEM were 5.5 ± 1.7%, 10.0 ± 2.0%, 12.5 ± 2.3%, and 15.0 ± 2.5% for concentrations of 10%, 20%, 30%, and 40% w/v, respectively. Furthermore, the toxicity profile of the seed powder extracts varied, with the highest mean mortality and standard error values recorded for *Piper guineense* (16.5 ± 2.8), followed by *Aframomum melegueta* (15.0 ± 2.7), and *Dennettia tripetala* (5.5 ± 1.7) at a concentration of 10% w/v, compared to the control (0.00). These findings underscore the potent insecticidal properties of the seed powder extracts, particularly at higher concentrations, highlighting their potential as effective bioinsecticides for managing cockroach infestations.

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Table 2. oxicity of Alligator Pepper (*Aframomum melegueta*), Pepper Fruit (*Dennettia tripetala*), and Guinea Cubeb Seed (*Piper guineense*) Powder Extracts against Cockroach Nymphs.

Concentration (w/v)	Mean Mortality (%) ± SEM
Control (0.00)	0.00 ± 0.00
10	5.5 ± 1.7
20	10.0 ± 2.0
30	12.5 ± 2.3
40	15.0 ± 2.5

The evaluation of lethal concentration (LD50) for seed powder extracts against German cockroach nymphs revealed that *Piper guineense* and *Aframomum melegueta* were capable of inducing 50% mortality at specific dose responses. The LD50 value for *Piper guineense* was determined to be 28.0% w/v, while that for *Aframomum melegueta* was 40.0% w/v (Fig. 1). However, no 50% mortality was observed at any of the tested doses of *Dennettia tripetala*, indicating its lower efficacy compared to *Piper guineense* and *Aframomum melegueta* against German cockroach nymphs. These results suggest that *Piper guineense* and *Aframomum melegueta* seed extracts exhibit potent insecticidal activity against German cockroach nymphs, with 50% mortality achieved within 24 hours of exposure at specific dose levels. Conversely, *Dennettia tripetala* demonstrated limited efficacy in inducing mortality in the tested cockroach nymphs under the experimental conditions employed.

Table 3. Lethal dose (LD50) and lethal time (LT50) of the seed powder extract against cockroach.

Treatment	Dose	log10	15min	30min	60min	24hrs	Total response	% response	LD50
Piper. guineense	40	1	4.5	16	16.5	16.5	53.5	66.88	
	30	1.30	4	10.5	13.5	14.5	42.5	53.13	28.0
	20	1.48	3	7.5	9.5	10.5	30.5	38.13	
	10	1.60	1	4	5.5	8.5	19	23.75	
Aframomum melegueta	40	1	4.5	8.5	12.5	15	40.5	50.63	
	30	1.30	4.5	3.5	4.5	6.5	19	23.75	40.0
	20	1.48	1	2.5	5	7.5	16	20.00	
	10	1.60	0.5	2	3	5	10.5	13.13	
Dennettia tripetala	40	1	0	0	0	5.5	5.5	6.88	
	30	1.30	0	0	0	1.5	1.5	1.88	ND
	20	1.48	0	0	0	0	0	0.0	
	10	1.60	0.5	0.5	0.5	1	2.5	3.13	

ND = Not Determined

Discussion

The utilization of botanical insecticides extracts particularly in the management of cockroach pests, holds significant promise due to their low toxicity and potential to maximize insecticidal efficacy. Plants such as *Piper guineense*, *Aframomum melegueta*, and *Dennettia tripetala* have demonstrated insecticidal properties against cockroaches, attributed to the presence of secondary metabolites, also known as phytochemicals. The biological activities of these plants, acting as ovicides, larvicides, and general insecticides, underscore their potential as effective pest control agents. The results of phytochemical screening revealed the presence of flavonoids, tannins, saponins, alkaloids, terpenoids, glycosides, and anthraquinones in *P. guineense*, *A. melegueta*, and *D. tripetala*. This finding aligns with previous studies by Doherty et al. (2010) and Michael and Ahamefula (2012), which reported similar phytochemical compositions in Aframomum melegueta. Alkaloids,

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flavonoids, and phenols present in these botanicals are believed to contribute to their bioactivity, as supported by earlier works by Lale and Alaga (2001) and Nweze et al. (2004). The toxicity assessment of *P. guineense, A. melegueta*, and *D. tripetala* seed powder extracts against cockroaches demonstrated larvicidal and insecticidal properties. *Piper guineense* exhibited the highest toxicity, followed by A. melegueta, whereas *D. tripetala* showed lower efficacy compared to the control group. Additionally, the toxicity of the tested plant extracts increased with exposure time, with higher mortality rates observed at 24 hours compared to shorter exposure durations. Moreover, the mean mortality or toxicity of the seed powder extracts increased with concentration and exposure time. Notably, 50% mortality was achieved at LD50 values of 28.0% w/v and 40.0% w/v for *P. guineense* and *A. melegueta*, respectively. These findings corroborate previous studies by Toshihiro and Haruyasu (2006), which provide valuable insights into the combined effects of powders derived from related plant species. Overall, the results highlight the potential of *P. guineense, A. melegueta*, and *D. tripetala* as effective botanical insecticides for cockroach pest management, emphasizing the importance of phytochemical composition and toxicity assessment in evaluating their efficacy.

Conclusion

This study aimed to assess the pesticidal potential of seed powder extracts derived from Alligator Pepper (*Aframomum melegueta*), Pepper Fruit (*Dennettia tripetala*), and Guinea Cubeb (*Piper guineense*) against cockroaches (*Blattella sp*.). The findings of the study underscore the significant importance of these botanical extracts in pest control, particularly due to their ability to increase mortality rates among *Blattella germanica*. The lethal dose (LD50) refers to the dose of a substance required to induce 50 percent mortality or death in the target organism. In the context of this study, LD50 values were determined to assess the efficacy of the seed powder extracts against cockroaches. The LD50 values provide crucial insights into the potency and effectiveness of these botanical extracts as bioinsecticides for cockroach pest management. Overall, the results of the study highlight the promising potential of *Aframomum melegueta*, *Dennettia tripetala*, and *Piper guineense* seed powder extracts in controlling cockroach populations. Their pesticidal properties offer viable alternatives to synthetic insecticides, emphasizing the importance of further research and development in harnessing the benefits of botanical extracts for sustainable pest management practices.

Recommendations

Based on the imperative for proactive measures and research to develop biopesticides for the control and prevention of Blattella sp., the findings of this study recommend the following:

1. Further Research Targeting Isolated Bioactive Compounds: There is a pressing need for additional research aimed at isolating and characterizing the bioactive compounds present in botanical extracts. These compounds can then be studied for their effects on the fertility, genome, or genetic makeup of *Blattella sp*. Understanding the mechanisms of action at the molecular level can provide valuable insights for the development of targeted biopesticides with enhanced efficacy and specificity.
2. Exploration of Botanicals with Anti-Oviposition and Larvicidal Efficacy: Efforts should be directed towards identifying botanicals that exhibit potent anti-oviposition and larvicidal effects. By targeting the eggs and larval stages of *Blattella sp*., it may be possible to disrupt their life cycle and impede their development into mature individuals. This approach holds promise for long-term population control and management strategies by reducing the reproductive potential and survival rates of *Blattella germanica*

By pursuing these recommendations, researchers can contribute to the development of innovative biopesticides that offer effective and environmentally sustainable solutions for the control and prevention of *Blattella sp*. infestations. Collaborative efforts involving interdisciplinary research teams and partnerships between academia, industry, and government agencies will be essential for advancing these initiatives and translating research findings into practical applications for pest management.

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