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Original Research Article

Insecticidal Effects of *Paullinia pinnata* (L.) Extracts on *Callopistria sp.* (Noctuidae: Fern Caterpillar)

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Abstract: The increasing herbivory of *Nephrolepis* species by *Callopistria floridensis* larvae (fern caterpillars) threatens the ecological integrity of wetland habitats in Bayelsa State, Nigeria. This study evaluated the insecticidal potential of *Paullinia pinnata* (L.) foliage and fruit extracts against *C. floridensis*, alongside an assessment of the plant's phytochemical composition. Foliage and fruit samples of *P. pinnata* were collected, air-dried, and extracted using aqueous and ethanol solvents following standard protocols. Quantitative phytochemical screening revealed that ethanol-based foliage extract contained the highest concentrations of alkaloids (1.091 mg/L), tannins (2.242 mg/L), glycosides (1.970 mg/L), saponins (2.260 mg/L), and phenols (0.810 mg/L), while the aqueous foliage extract showed the highest flavonoid content (1.846 mg/L). Laboratory bioassays were conducted using 720 larvae of *C. floridensis* in a randomized complete block design (RCBD). Extracts were applied at five concentrations (2.5 - 20 mg/L), and larval mortality was observed at 2.5, 5, 7.5, and 10 hours post-treatment. Results showed a dose and time dependent increase in larval mortality ($p \leq 0.05$), with ethanol foliage extracts achieving 100 % mortality by 10 hours at concentrations ≥5 mg/L. The aqueous extracts also showed significant activity, though delayed and less potent at lower concentrations. Ethanol extracts of both foliage and fruits consistently outperformed aqueous counterparts across all time intervals, while the fruit extracts were comparatively less effective, especially the aqueous type. The control treatments consistently recorded <3% mortality throughout the time frame. These findings confirm that *P. pinnata* contains potent bioactive compounds and offers strong potential as a botanical pesticide for managing *Callopistria sp*. infestations, contributing to sustainable pest control in tropical agroecosystems.

Keywords: *Paullinia pinnata,* insecticidal activity, *Callopistria floridensis* larvae, phytochemical composition, larval mortality.

INTRODUCTION

Ferns, particularly those belonging to the genus *Nephrolepis*, are ecologically significant components of tropical wetland environments, contributing to soil stability, biodiversity, and nutrient cycling (Della, 2022; Siallagan et al. 2025). These plants are vital to erosion prevention through their extensive root systems and enhance soil fertility by serving as hosts for nitrogen-fixing bacteria (Chioma Linda *et al.,* 2021; Lopez, 2022; Idul and Cayme, 2023; Azevedo-Schmidt *et al.,* 2024). Among these species, *Nephrolepis biserrata* is a dominant component in secondary vegetation of disturbed habitats in wetlands and swamps such as in Bayelsa State, Nigeria, and often utilized as a bio-indicator due to its sensitivity to environmental perturbations (Akinsoji *et al.,* 2016; Sharp *et al.,* 2010). These ferns are widely distributed in Bayelsa state, where they form dense vegetation, and contribute significantly to ecological balance and aesthetic value. Nephrolepis species hold cultural and economic importance, its frond decoction treats lower abdominal pains (Jiofack *et al.,* 2008). Anyi-Ndenye Women (Eastern Cote d'Ivoire) utilized *N. biserrata*, epiphytes of the oil palm for foetus development and facilitating childbirth (Malan and Neuba 2011), the dried fronds have historically been used in mattress stuffing and metal polishing (Idul and Cayme, 2023). Significant amounts

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of bioactive compounds were reported in *Nephrolepis cordifolia,* among which were antifungal, antibacterial, anioxidant, and anticancer (Adebiyi *et al.,* 2019). Despite these benefits, the species are increasingly threatened by herbivory from *Callopistria* sp. (Noctuidae), commonly referred to as the fern-caterpillar. This lepidopteran pest exhibits a voracious feeding behaviour, particularly during the dry season causing severe defoliation of fern fronds (Jesudasan *et al.,* 2024; Jaffe *et al.,* 2019). The extensive damage inflicted by the larvae results in a characteristic scorched or burnt appearance across large expanses of Nephrolepis vegetation, compromising not only the aesthetic appeal, but also disrupting microhabitats and reducing overall biodiversity

Historically, synthetic insecticides have been the primary means of controlling insect pests; however, their application poses significant environmental and human health risks. The widespread use of synthetic pesticides has led to concerns regarding bioaccumulation, toxicity to non-target organisms, and the emergence of pesticide-resistant insect populations (Niroumand *et al.,* 2016; Souto *et al.,* 2021). As a result, research efforts have increasingly focused on the development of botanical insecticides, which are considered more environmentally friendly due to their biodegradability, selective toxicity, and lower likelihood of inducing resistance (Kayode *et al.,* 2016; Ngegba *et al.,* 2022). Several studies have demonstrated the efficacy of plant-derived insecticidal compounds in managing plant pests. Ugwu *et al.,* (2021) reported that aqueous extracts from five aromatic plant species native to Nigeria significantly reduced leaf damage caused by *Leucinodes orbonalis* by 39.64%. Similarly, neem (*Azadirachta indica*) extracts have shown broad-spectrum insecticidal activity against aphids, mites, and other pests affecting crops (Singh and Kataria, 1991; Zaki, 2008; Sharma *et al.,* 2012). Some plant species in Nigeria have been evaluated for their insecticidal properties. Ofuya *et al.,* (2023) assessed the effectiveness of plant-derived insecticides in managing vegetable crop pests, Ileke *et al.,* (2020) revealed the significant insecticidal activity of botanical powders and extracts against maize weevils. A number of studies: Okunlola *et al.,* 2008; Ileke and Oni, 2011; Benson *et al.,* 2014; Degri and Sharah, 2014; Obembe and Kayode, 2016) had evaluated the potential of plant-based pesticides to reduce insect infestations and enhance plant yield.

Among the promising plants for insect pest control is *Paullinia pinnata* (L.), a climbing shrub from the Sapindaceae family, widely distributed across tropical Africa. Traditionally, *P. pinnata* has been used in ethnomedicine for treating various ailments due to its bioactive compounds, which exhibit antimicrobial, antifungal, and insecticidal properties (Adeyemo-Salami and Choudhary, 2021; Ikhane *et al.,* 2015). Recent studies have demonstrated its significant bioactivity against insect pests. Ogunwande *et al.,* (2017) reported that *P. pinnata* extracts achieved 100% fumigant toxicity against *Sitophilus zeamais* (maize weevil) at a concentration of 150 mg/mL. Yagi *et al.,* (2013) studies reported 100% insecticidal activity of *Senna italica* pods against *Callosbruchus analis*. In Fred-Jaiyesimi and Anthony (2011), significant larvicidal activity of *P. pinata* against *Anopheles gambiae*, the primary vector of malaria in Africa was reported. Despite these documented bioactivities against insect pests, no studies have specifically evaluated its efficacy against *Callopistria sp*., the fern-caterpillar responsible for damaging Nephrolepis species. Given the broad-spectrum insecticidal properties of *P. pinnata*, it is hypothesized that the plant extracts could serve as effective biopesticide against *Callopistria* sp., and proffers eco-friendly alternative to synthetic pesticides. This study aims to assess the phytochemical composition of *P. pinnata* extracts, while evaluating their insecticidal activity against *Callopistria* sp. By providing insight into these neglected aspects, this study contributes to the ongoing search for sustainable, plant-based pest control solutions that minimize environmental and ecological risks associated with conventional insecticides.

MATERIALS AND METHODS

Study Area- Collection and Identification of the Plant Materials and the Insect Larvae

The study was carried out in biochemistry laboratory, Federal University Otuoke. Plant materials (foliage and fruits) of *Paullinia pinnata* used for the study were obtained from a fallow land in Otuoke community, also the *Callopistria floridensis* larvae were harvested from infested Nephrolepis vegetation on coordinate Lat. $N4^{0}47^{1}9.5$ and Long. $E6^{0}17^{1}57.5$. Seven-hundred and twenty (720) specimens of *C. floridensis* larvae were collected from infested Nephrolepis vegetation together with the fronds they were herbivory, using transparent container and a glove while avoiding contact with the insects. Identification of the species were done in the Department of Biology, Federal University Otuoke.

Processing of the Plant Materials

Fresh samples (foliage and fruits) of *P. pinnata* collected were processed and air-dried. The dried plant materials were subsequently pulverized with clean automatic electrical blender (Model MS-223, China). The resultant powders were stored in airtight containers until required for analyses.

Extraction Preparation

Thirty grams (30 g) of each powdered sample were sequentially extracted using a Soxhlet apparatus with 300 mL each of ethanol (at 56–60°C), acetone (at 50–56°C), and deionized water (at 90–100°C) until the solvents ran clear. The collected extracts were subsequently concentrated to dryness using a rotary evaporator. The semi-solid residues obtained were transferred into sterile plastic containers and stored for further phytochemical investigations.

Aqueous Extraction

Twenty grams (20 g) of pulverized *Pongamia pinnata* foliage and fruit were each soaked in 200 mL of distilled water at ambient temperature in separate conical flasks. The flasks were sealed with sterile cotton wool and subjected to gentle heating. The resulting mixtures were filtered successively through sterile cotton wool and Whatman No. 1 filter paper into clean beakers, and the filtrates were reserved for subsequent analysis.

Experimental Design

Callopistria floridensis larvae were maintained at room temperature in the laboratory. A total of 36 tamper-evident 2-liter containers were arranged on a laboratory workbench using a randomized complete block design (RCBD). The experiment consisted of four treatments applied in two separate groups (labeled A and B), each with three replicates. Controls were similarly replicated. Each container was populated with 20 larvae along with the fronds on which they fed. Aqueous and ethanolic extracts of both *P. pinnata* foliage and fruits were applied at concentrations of 2.5 mg/L, 5 mg/L, 10 mg/L, 15 mg/L, and 20 mg/L to groups A and B, respectively. The treatments were administered by sprinkling while ensuring that the extracts did not directly contact the larvae. The experimental setup was monitored over a 10-hour period to assess larval mortality and determine the efficacy of each extract concentration.

Quantitative Phytochemical Analysis
Saponin Determination

The test extract was dissolved in 80% ethanol. Two milliliters (2 mL) of vanillin (in ethanol) were added, followed by thorough mixing. Subsequently, 2 mL of 77% sulfuric acid were introduced, and the solution was incubated in a water bath at 60°C for 25 minutes. The absorbance of the resulting complex was measured at 540 nm using a spectrophotometer, with a blank lacking the extract serving as control. Saponin content was calculated by comparing absorbance values against a diosgenin standard curve and expressed as diosgenin equivalents.

Alkaloid Determination (Bromocresol Green Method)

A 1 mL aliquot of the extract, pre-dissolved in 2 N HCl and filtered, was mixed with 5 mL of phosphate buffer (pH 4.7) and 5 mL of bromocresol green (BCG) solution. The mixture was extracted with 4 mL of chloroform through vigorous shaking to isolate the alkaloid–BCG complex. This extraction was repeated three times, and the organic layers were combined and diluted to a final volume of 10 mL. Absorbance was measured at 470 nm using a blank solution for calibration. Recovery analysis, conducted by spiking with 10 µg/mL atropine, showed a recovery rate of 98%. Alkaloid concentration was quantified using an atropine calibration curve and expressed in atropine equivalents.

Total Phenolic Content

One hundred milligrams (100 mg) of the extract were dissolved in 100 mL of triple-distilled water. From this stock, 1 mL was mixed with 0.5 mL of 2 N Folin–Ciocalteu reagent and 1.5 mL of 20% sodium carbonate. The mixture was brought to a final volume of 8 mL with distilled water and shaken thoroughly. After 2 hours of incubation at room temperature, absorbance was recorded at 765 nm. Phenolic content was determined using a gallic acid standard curve and reported as gallic acid equivalents (GAE).

Total Flavonoid Content

A 100 µL aliquot of methanolic extract (10 mg/mL) was reacted with 100 µL of 20% aluminum chloride solution and a drop of acetic acid. The total volume was adjusted to 5 mL with methanol, and the mixture was left at room temperature for 35 minutes to allow for flavonoid–aluminum complex formation. Absorbance was read at 415 nm using a blank lacking aluminum chloride. Recovery tests were conducted with 100% spiking to validate accuracy. The flavonoid concentration was calculated using a rutin standard curve and expressed as rutin equivalents.

Tannin Content Determination

A 500 mg sample of *P. pinnata* powder was extracted with 50 mL of distilled water by shaking for 1 hour at room temperature. The mixture was filtered, and the filtrate was adjusted to a final volume of 50 mL. From this, 5 mL was mixed with 2 mL of 0.1 M ferric chloride (prepared in 0.1 N HCl) and 2 mL of 0.008 M potassium ferrocyanide. The resulting blue-green solution was analyzed within 10 minutes by measuring absorbance at 720 nm. Tannin content was quantified via comparison with a standard curve and reported accordingly.

Glycoside Determination

Following a modified method from El-Olemy et al. (1994), 2 g of the powdered extract were soaked in 15 mL of 70% ethanol for 2 hours at room temperature. The solution was filtered and purified using lead acetate and sodium hydrogen phosphate. Baljet's reagent was added to the filtrate, and the mixture was allowed to react for 1 hour at room temperature. The absorbance of the resulting complex was measured at 495 nm. Glycoside concentration was quantified using a digoxin standard curve and expressed as digoxin equivalents. Spike recovery tests confirmed approximately 100% accuracy.

Terpenoid Determination

The method described by Indumathi *et al.,* (2014) was employed with minor adjustments. Five grams (5 g) of powdered sample were macerated in 50 mL of absolute ethanol and left to stand for 24 hours at room temperature. The ethanol extract was concentrated using a rotary evaporator at 40°C. The residue was reconstituted in 20 mL of distilled water and subjected to liquid-liquid extraction with 10 mL of petroleum ether in a separating funnel. After vigorous shaking and settling,

the upper petroleum ether layer containing terpenoids was collected. This procedure was repeated twice more, and the combined ether extracts were evaporated to dryness. The weight of the dried residue was used to calculate terpenoid content, expressed as a percentage of the original sample weight using the formula: Terpenoid (%) = (Weight of residue / Weight of sample) × 100

Data Analysis

The data were subjected to descriptive statistical analysis, and analysis of variance (ANOVA) was performed to determined statistical significance at $p \leq 0.05$. Mean mortality percentages were plotted against concentrations and time intervals to assess efficacy trends. The mean mortality percentage was determined using the formula:

$$\text{Mortality Percentage} = \frac{\text{Number of Deaths x 100}}{\text{Total Number of Individuals at the start}}$$

Where: Number of Deaths = the count of individuals that died during the observation period. Total Number of Individuals at the Start = the initial number of individuals before any deaths occurred.

RESULTS AND DISCUSSION

Table 1 shows the results of the quantitative phytochemical analysis of *Paullinia pinnata* (L.) extracts derived from both foliage and fruit parts using both aqueous and ethanol solvents. The ethanol extract of the foliage exhibited higher concentration of alkaloids (1.091 mg/l), compared to the aqueous extract (0.478 mg/l). Other phytochemicals such as glycosides (1.970 mg/l), tannins (2.242 mg/l), terpenoids (1.024 mg/l), saponins (2.260 mg/l), and phenols (0.810 mg/l) were also more abundant in the ethanol extract of the foliage than in its aqueous counterpart.

Table 1: Results of the quantitative analysis of *P. pinnata* (L.)

Sample	Alkaloids (mg/l)	Flavonoids (mg/l)	Glycosides (mg/l)	Tannins (mg/l)	Terpenoids (mg/l)	Saponins (mg/l)	Phenols (mg/l)
Foliage (aqueous)	0.478	1.846	1.786	2.052	0.832	1.940	0.403
Foliage(ethanol)	1.091	0.927	1.970	2.242	1.024	2.260	0.810
Fruits (aqueous)	0.169	0.024	1.052	1.013	0.221	0.722	0.021
Fruits (ethanol)	0.286	0.127	1.663	1.865	0.397	0.890	0.093

However, the flavonoid content was higher in the aqueous foliage extract (1.846 mg/l), than in the ethanol extract (0.927 mg/l), suggesting that some flavonoids may be more soluble or stable in polar solvents such as water. A similar trend was observed in the fruit extracts. The ethanol-based extractions yielded higher values of alkaloids (0.286 mg/l), glycosides (1.663 mg/l), tannins (1.865 mg/l), terpenoids (0.397 mg/l), saponins (0.890 mg/l), and phenols (0.093 mg/l) compared to the aqueous extract. Yet, flavonoid concentrations in fruit extracts were significantly low in both solvents, with aqueous and ethanol values recorded as 0.024 mg/l and 0.127 mg/l respectively ($p<0.05$). This low flavonoid content in fruit suggests that the major insecticidal phytocompounds may reside primarily in the foliage, aligning with its traditional use and previous phytochemical studies (Begum *et al.,* 2010; Ghada *et al.,* 2025).

Figures 1-4 shows the insecticidal effects of *P. pinnata* extracts against *Callopistria* sp. larvae using both ethanol and aqueous extracts from foliage and fruits across various concentrations (2.5mg/l, 5mg/l, 10mg/l, 10mg/l and 20 mg/l) and at post-application intervals (2.5, 5, 7.5, and 10 hours after treatment application, HATA). At 2.5hours post-treatment (Fig. 2), a clear dose-dependent mortality effect was observed. Higher concentrations of extracts resulted in increased larval mortality. Notably, all extract types reached ≥95% mortality at the highest concentration (20 mg/ml). The rapid mortality suggests a fast-acting mode of action attributable to the bioactive constituents of *P. pinnata*. These findings align with previous work of Oigiangbe *et al.,* (2007), who reported similar dose-dependent insecticidal effects using *Alstonia boonei* extracts on *Sesamia calamistis* larvae.

The ethanol foliage extract exhibited the highest potency, achieving over 50% mortality even at moderate concentrations (10 and 5 mg/ml), followed closely by the aqueous foliage extract. In contrast, fruit aqueous extracts showed a steep decline in efficacy at concentrations below 10 mg/ml, indicating lower potency and possible limited extraction efficiency of insecticidal compounds in water. The fact that the ethanol extracts, particularly from foliage, consistently outperformed aqueous ones, may likely be due to better solubilization of bioactive compounds such as alkaloids, saponins, and terpenoids in organic solvents (Jeyaraj *et al.,* 2021; Kabubii *et al.,* 2023). Whereas the negligible mortality recorded in the control groups (aqueous and ethanol solvents without plant extract), affirm the bioactivity of *P. pinnata* extracts. The results presented in Figure 2, shows mortality percentages 5hours post-application. The mortality percentages increased further across most treatments. The ethanol foliage extracts exhibited the highest and most consistent mortality rates across all tested concentrations, achieving 100% mortality at 20 mg/ml, 15mg/ml and 5 mg/ml. Similarly, the aqueous foliage extract maintained high activity across concentrations, with near-total mortality at higher concentrations and over 70% mortality at the lowest concentration.

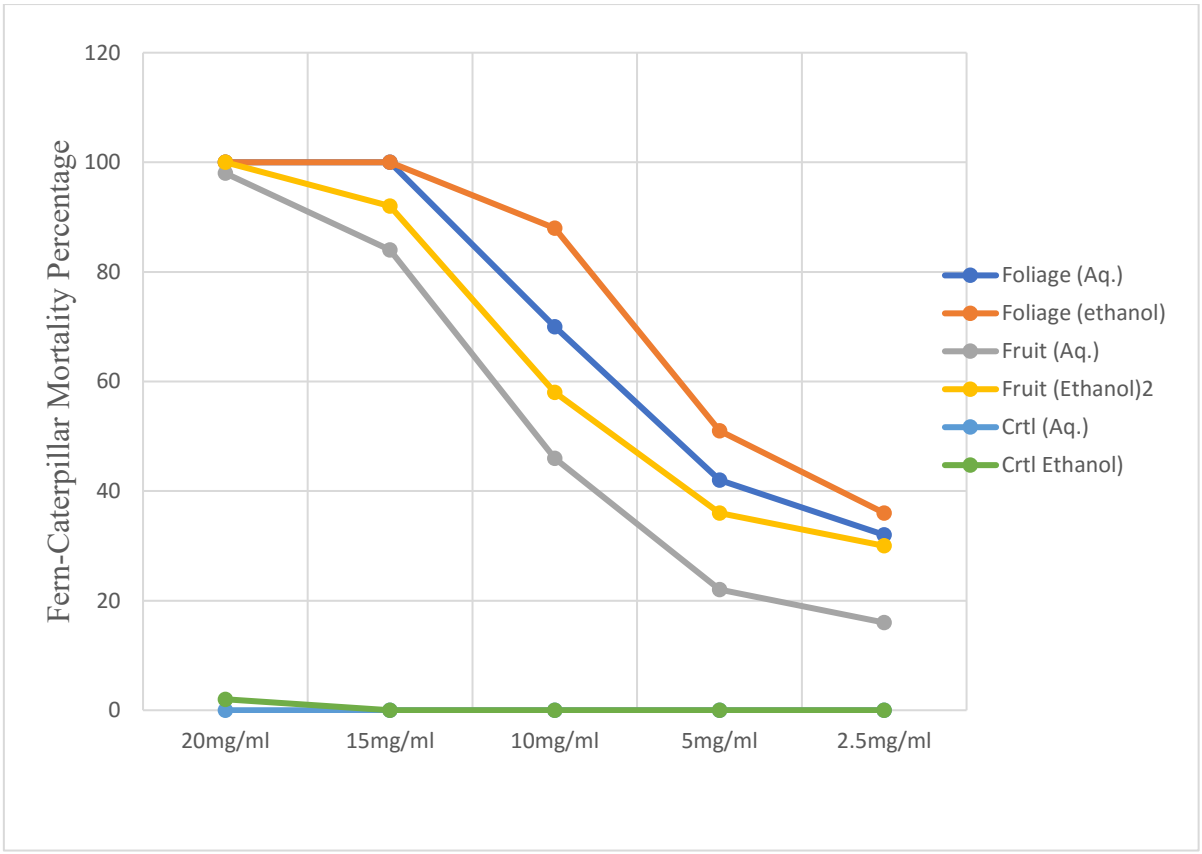

Fig. 1: Fern-Caterpillar Mortality Percentage over Concentrations 2.5 hours Post Application

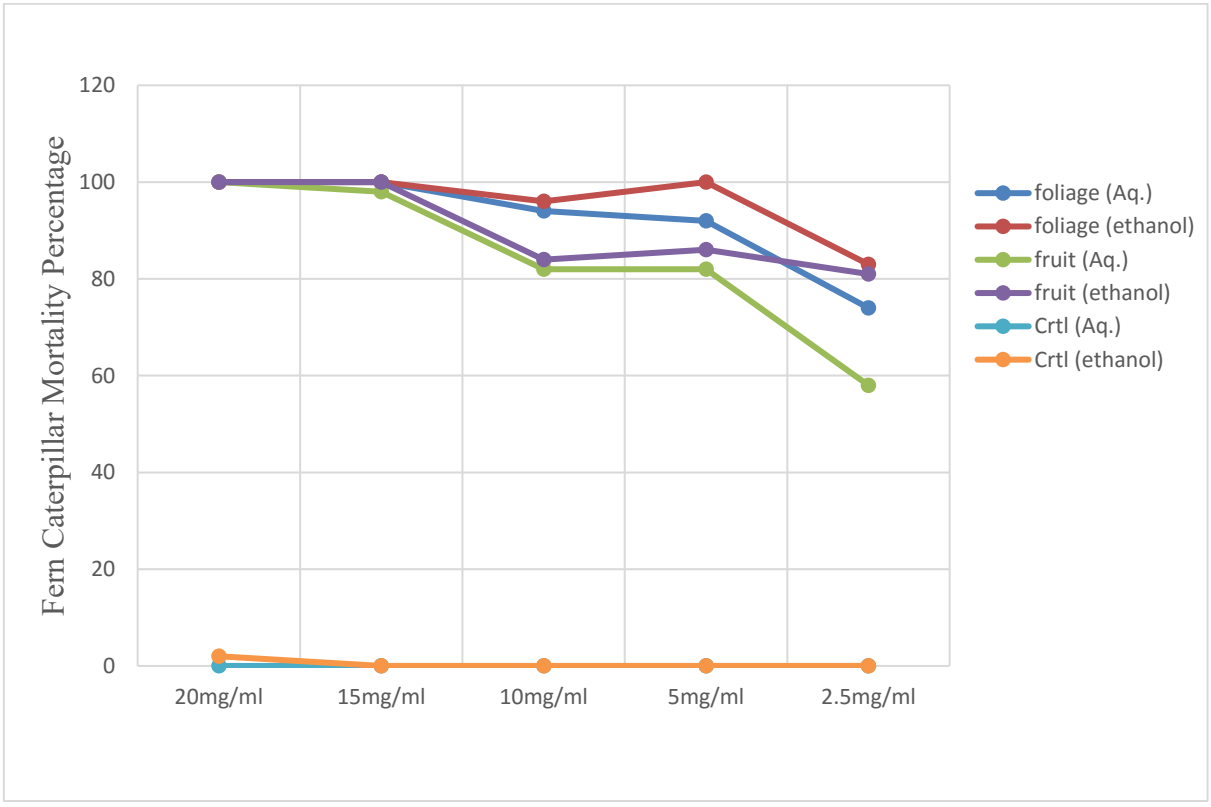

Fig. 2: Fern-Caterpillar Mortality Percentage Over Concentrations 5hours Post Application

However, the aqueous extracts of both foliage and fruit showed substantial insecticidal effects, especially at higher concentrations (20 - 10 mg/ml), their effect dropped noticeably at the lowest concentration

(2.5 mg/ml). The fruit aqueous extract recorded only about 58% mortality at this concentration, indicating reduced stability or solubility of active constituents in water. The observed time-dependent increase in insecticidal activity supports the assertion of a progressive physiological disruption in larvae, potentially through systemic or contact-based mechanisms. These findings are corroborated Mouden *et al.,* (2017) and Huang *et al.,* (2019), who reported reduced long-term efficacy of aqueous plant extracts at lower concentrations.

The results of the mortality rate at 7.5hours post-application is presented in Figure 3. The results reveal a conspicuous rise in insecticidal effect of *P. pinnata* extracts across treatments. Foliage ethanol extract maintained 100% mortality from 20 mg/ml down to 5 mg/ml, and also showed >80% mortality at 2.5 mg/ml. The aqueous extracts, while less potent than their ethanol replica, also showed significant improvements in efficacy ($p<0.05$) compared to earlier time points. Besides, the aqueous extracts showed increased performance: foliage aqueous extract achieved near-total mortality at all concentrations, while the fruit aqueous extract induced about 70% mortality at the lowest dose.

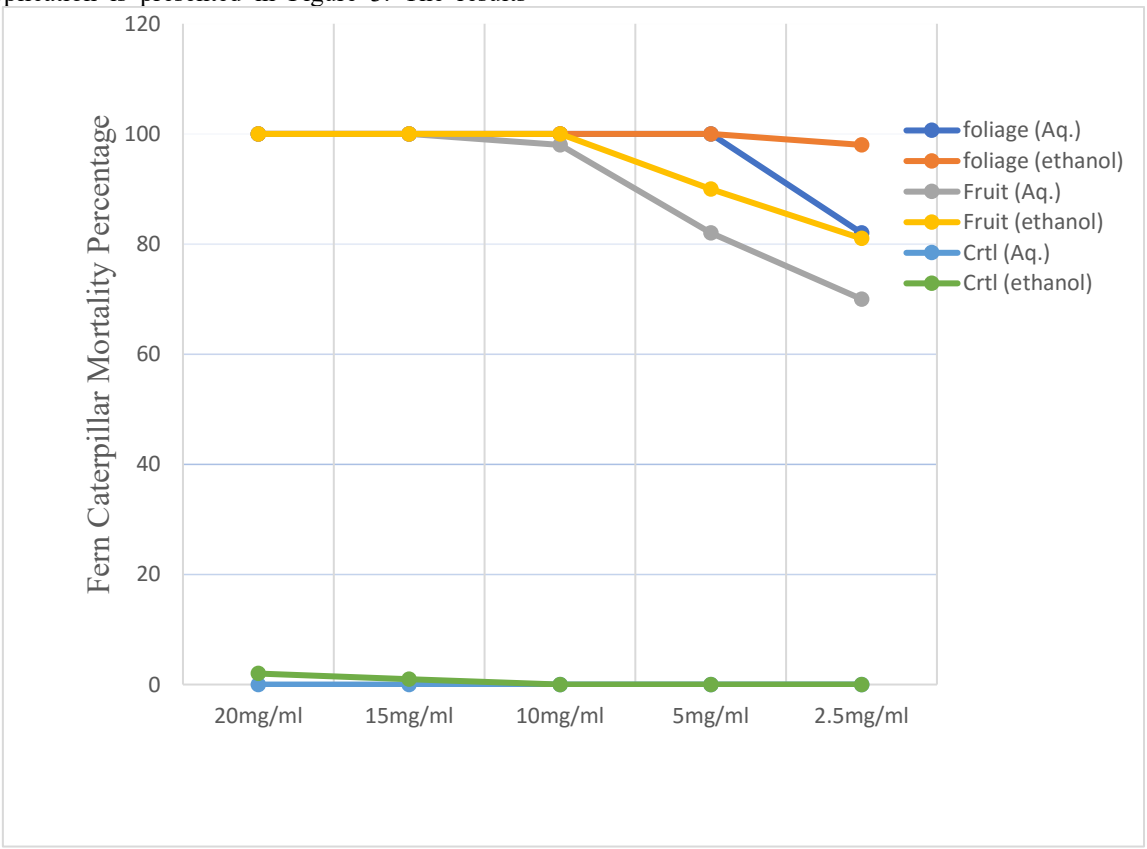

Figure 3: Fern-Caterpillar Mortality Percentage Over Concentration 7.5hours Post Application

These results indicate that the insecticidal compounds in P. pinnata may require time to penetrate insect integuments or act via ingestion, which aligns with reports of delayed action in botanical insecticides (Ateyyat *et al.,* 2009; Alpkent, 2025). Whereas, the controls at this time point continued to show minimal mortality (<5%), affirming the specificity of *P. pinnata*'s insecticidal effects. The results of the ten (10) hours post-treatment (Figure 4), revealed the full insecticidal potential of *P. pinnata* extracts. The ethanol foliage extract maintained 100% mortality across all concentrations, including the lowest (2.5 mg/ml), indicating strong residual toxicity and potential dose-independent effects. The aqueous foliage extract, although slightly less potent at 2.5 mg/ml (83% mortality), performed excellently at higher doses. Ethanol fruit extracts also maintained 100% mortality down to 10 mg/ml and recorded 81% at 2.5 mg/ml, while the aqueous fruit extract ended with 70% mortality at the lowest concentration.

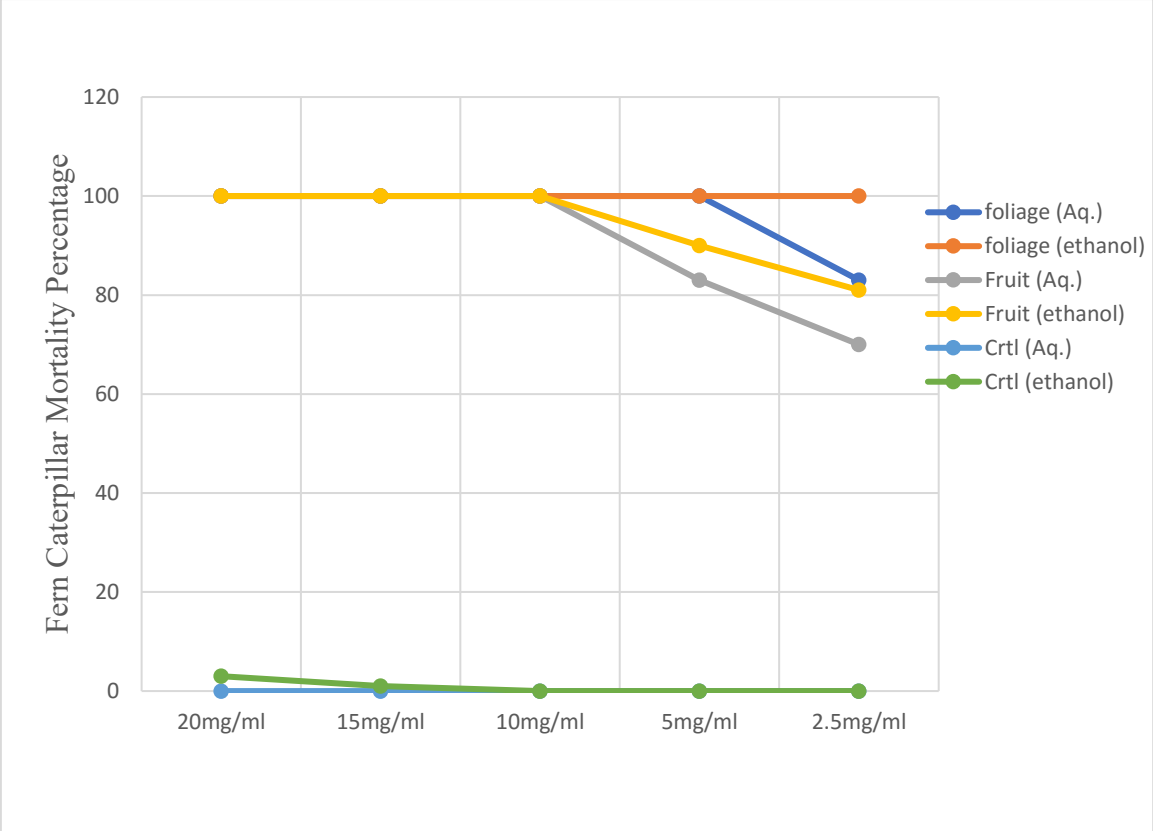

Figure 4: Fern-Caterpillar Mortality Percentage Concentrations 10hours Post Application

These results suggest that ethanol is a more effective solvent for extracting potent insecticidal compounds from *P. pinnata*, this may be due to better solubility of key phytochemicals such as alkaloids, flavonoids, and saponins (Begum *et al.,* 2010; Adeniye *et al.,* 2010). The delayed peak efficacy observed in the aqueous extracts further supports the assertion that water-based formulations may require longer exposure times to achieve optimal activity (Li *et al.,* 2019). Throughout the study, control groups consistently showed minimal mortality (0-3%), confirming that larval deaths were due to the plant extracts rather than solvent effects. Across all observation periods, a time-dependent increase in mortality was evident, with ethanol extracts (especially from foliage) exhibiting the earliest and most sustained activity. These findings substantiate the potential of *P. pinnata* as a source of rapid and effective botanical insecticides for managing *Callopistria sp*. infestations

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