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Assessment of Plants Alkaloids Isolated from *Calotropis Procera* for Managing Pod Sucking Bug, *Clavigralla tomentosicollis* Stal (Hemiptera: Coreidae) Infestation on Cowpea

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ABSTRACT
Crude plant extracts used to control insect pests are less specific and the active compounds may have antagonistic effects. The trials were thus conducted to assess the effectiveness of alkaloids from *Calotropis procera* in controlling the pod sucking bug *Clavigralla tomentosicollis* on cowpea. Three cowpea varieties, Ife brown, IT07K-318-33, and IT07K-292-10, were grown in beds assigned to four treatment groups: 500µg/ml, 250µg/ml, 125µg/ml, and 0µg/ml of the extracts as a control. Each treatment was replicated three times in a randomized complete block design. The findings revealed a significant decrease ($p<0.05$) in the population of *C. tomentosicollis* in the treated plots compared to the control. The highest level of protection was observed in plots sprayed with 500µg/ml and 250µg/ml, with no significant difference ($p>0.05$) between treatment and spray regimes (1-week after spray (1-WA**S**) and 2-weeks after spray (2-WA**S**). The average percentage of pod damage was significantly lower at higher treatment concentrations (500µg/ml and 250µg/ml) compared to lower concentrations and the control. There was no significant difference in pod damage among the three cowpea varieties. The relative adult mortality of *C. tomentosicollis* followed a similar pattern, with higher concentrations (500µg/ml and 250µg/ml) resulting in a significant decrease in ITOK-292-10 compared to other varieties. In the ITOK7-318-33 variety, there was no significant difference in mortality rates between treatments (500µg/ml, 250µg/ml, and 125µg/ml) and spray regimes. This implied that treatment of cowpea ITOK-292-10 with the higher concentration of the Alkaloid (2-piperidinone, N-(4-bromo-n-butyl) would yield an optimum level control of the notorious bug.

Keywords:
Alkaloid,
Calotropis procera,
C. tomemtosicollis,
Control

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp) is a crucial leguminous crop widely cultivated in tropical climates, particularly in the savanna regions of West Africa and other arid areas around the globe It serves as a significant source of plant protein and fodder for livestock. Additionally, cowpeas play a vital role as a cover crop, aiding in nitrogen fixation (Degri *et al.*, 2013). Nigeria stands out as the leading producer, contributing to 2.1 million tonnes in 2000 out of the global production of 3.3 million tonnes. Sub-Saharan Africa alone accounts for nearly 70% of the total global production, as estimated by IITA in 2007. The yield of cowpea is often hindered by insect pests, with notable post-flowering pests in tropical Africa being the flower bud thrips, pod borer *Maruca vitrata*, and pod-sucking bugs complex dominated by *Clavigralla tomentosicollis* Stal (Opaerake *et al.*, 2005). *C. tomentosicollis* is medium sized, hairy, and grey. Nymphs form large colonies on cowpea pods and peduncle and are not easily disturbed. Adults are not strong fliers and have longevity of 100 – 150 days. Eggs are laid in batches of 10 -70, and, on average, about 200 eggs are laid by each female. Each instar lasts about 2 days, but the last instar is about 6 days. The total nymphal period is about 14 days (Steele *et al.*, 2017).

Complete crop failure may occur where insecticide protection is not introduced, especially the improved high-yielding varieties. The high cost of insecticidal sprays poses a challenge for resource-constrained farmers in Nigeria. The removal of agricultural subsidies further exacerbates this issue, prompting the exploration of alternative insecticide sources for sustainable and eco-friendly crop protection strategies (Agbogidi and Egho, 2012). Limited information exists on the use of herbal landraces in Nigeria for controlling field pests on crops. Previous trials utilizing plant extracts have mainly been conducted on the use of crude plant extracts in controlled environments, such as screen house studies, which may

not accurately reflect field conditions. This trial is aims to explore the potential use of a specific bioactive compound found in plant extracts for managing the pod sucking bug *Clavigralla tomentosicollis* on cowpea. The use of conventional insecticides, which contain toxic ingredients, can have serious and often fatal effects on both humans and the environment. Therefore, there is a growing need for biopesticides, which are effective in small quantities and decompose quickly, resulting in lower persistence and minimal pollution issues compared to conventional pesticides (Singh *et al.,* 2015).

While many studies utilize crude plant extracts to control insect pests, the active ingredients in these extracts may have antagonistic effects, leading to ineffective results. Hence, this study aims to assess the effectiveness of a specific active compound (alkaloids) derived from *Calatropis procera* in controlling the pod sucking bug *C. tomentosicollis* on cowpea. The research aims to evaluate the effect of plant alkaloids from *Callotropis procera* on the control of the pod sucking bug *Clavigralla tomentosicollis* on cowpea.

MATERIALS AND METHODS

Study Environment

Experimental Setup The experiment was conducted at two different locations: the Plantation Section within the Teaching and Research Farm of the Faculty of Agriculture, Bayero University Kano (11” 58.616"N; 8” 25.552"E) at an elevation of 464m above sea level during the 2021 growing season. The laboratory work was carried out at the Entomology laboratories of the Department of Biological Science, Bayero University Kano. Cowpea

Utilized Cowpea Varieties

The cowpea cultivars are IT07K-292-10, IT07K-318-33, and Ife brown were selected for the study due to their visual appeal and popularity among farmers and consumers, despite being susceptible to major cowpea insect pests. These seeds were sourced from the Seed Production Unit of the International Institute for Tropical Agriculture (IITA) in Kano State.

Collection of Test Plant and the Process of Extraction

Fresh leaves of *C. procera* were gathered from different locations in the wild Bordering Bayero University Ungogo LGA of Kano metropolis. The plant material was verified and confirmed at the herbarium of the Department of Plant Science, Bayero University, Kano. The plant's leaves were carefully washed with running tap water to eliminate dirt and other impurities, then chopped and left to air-dry. Subsequently, the dried leaves were ground using a mortar and pestle, and meticulously sieved to obtain a fine powder.

Soxhlet Extraction Method

The plant was extracted using the soxhlet extraction method. A total of 600g of powder was mixed with 80% ethanol v/v (80:20, ethanol: distilled water) in the soxhlet apparatus. Each 60g portion of the dried powder was placed in a whatman filter paper No 6. The filter paper with the powder was then inserted into the thimble of the soxhlet extractor, which was positioned on top of a 500ml flask containing the extraction solvent, ethanol. The soxhlet apparatus was set up with a condenser, and the solvent was heated to reflux. The solvent vapor rose up the distillation arm and condensed into the thimble, where it dissolved some of the compounds in the powder. Once the thimble was nearly full, the solvent was drained back into the distillation flask through the siphon side arm. This process was repeated multiple times over a period of four hours (James *et al.,* 2014)

Chromatographic Fractions) Column Separations

Column Chromatography was performed using silica gel with a mesh size of 60-120. A glass tube with a diameter of 5.0 cm and a length of 87 cm was vertically clamped to serve as the column. The lower end of the column was sealed with a stopper and cotton wool was used as a support. A slurry of silica gel was prepared by mixing 250 g of silica gel with 500 ml of n-hexane, which was the solvent used for separation. The slurry was carefully added to the column while tapping gently to prevent cracks. This process was repeated until a uniform column of the desired length was achieved. To obtain a homogeneous mixture, 27.30g of the crude extract obtained from soxhlet extraction was mixed with 25 g of silica gel. The mixture was then poured into the column and different fractions were eluted using various solvents such as n-hexane, chloroform, and methanol. Each extracted fraction was collected separately in a 100 ml volumetric flask. The solvent of the collected fractions was evaporated at room temperature (Kumar and Sachin, 2013; Bajpai *et al.,* 2016).

Thin layer chromatography (TLC)

A small amount of the fractions was applied onto a Merck Aluminium plate that had been pre-coated with silica gel 60F254 and had a thickness of 0.2mm using a capillary tube. The plate was then developed in a solvent system consisting of n-hexane: chloroform: methanol. Subsequently, the plate was air dried and visualized under UV light at 254 and 366nm to identify spots of different compounds (Gujjeti and Mamidala, 2013). Fourier transforms infrared spectroscopy (FTIR) FTIR analysis was conducted on all the fractions obtained from the TLC process in order to screen for Alkaloid functionality before proceeding to GCMS analysis.

Gas Chromatography Mass Spectrometry GC/MS analysis

The analysis of the samples obtained from the ethanol leaf extract of *Calotropis procera* was conducted using Agilent GC 7890B and MSD 5977A from Agilent Technologies USA. The software MASSHUNTER equipped with AB innowax column (60 × 0.25 mm id, film thickness 0.25 µm) was utilized for the Gas Chromatography Mass Spectrometry (GC/MS) analysis. The identification of compounds was carried out by comparing their retention indices following the method described by Devender and Ramakrishna (2017).

Preparation of Stock and Standard Solution

The stock solution was prepared by adding ten (10) milligrams of the sample to 100ml of Dimethylsulphoxide (DMSO). This solution is expressed in weight per volume (w/v). The standard solution, on the other hand, was created through serial dilutions following the method described by Haydelba D'Armas and Ordaz (2018).

Crops Establishment and experimental design

The field was prepared for planting by ploughing, harrowing, and ridging with a tractor. A total of thirty-six plots were established, organized into three blocks with twelve plots each. The plot size was 3m x 2m, with spacing of 30cm between rows and 23cm between plants. Replications were set at a distance of 90cm. The experiment was conducted using a Randomized Complete Block Design (RCBD) with four treatments, including a control, replicated three times. Three varieties of cowpea - IT07K-318-33, IT07K-292-10, and Ife brown - were sown with three seeds per hole at a spacing of 75cm by 20cm. Interspaces between plots and blocks were maintained at 1m and 2m, respectively. Weeds were manually removed to prevent competition with the crop for space, water, nutrients, and light. Seedlings were thinned to two plants per stand at 2 weeks after sowing (WAS). Each plot consisted of 5 rows of 25 cowpea stands.

Application of Treatments

The treatment was applied by spraying each plot in the early morning using the correct concentration of the compound. The application was done on a weekly basis. The treatment application started 35 days after planting. This method was similar to the one used by Singh *et al.*, 2015). The treatment was applied three times: first at bud initiation (35 days after planting), second at 50% flowering (42 days after planting), and lastly at 60% podding.

Determination of Infestation and damage caused by *C. tomentosicollis*:

Visual sampling was conducted on *C. tomentosicollis*, with the bugs counted on 5 plants randomly selected per plot (Oparaeke *et al.,* 2005). Pod damage by *C. tomentosicollis* was evaluated in the field through visual observation. The assessment took place at 10 weeks after spraying (WAS), when the pods were fully filled and matured yet still green. Infestation by pod-sucking bugs was evaluated on five randomly chosen cowpea stands per plot and marked. Damage to pods, including holes, twisting, shriveling, or poor development, was documented. The mean numbers of damaged and undamaged pods were recorded, and the percentage of pod damage was calculated using the formula outlined by Oparaeke *et al* (2005).

$$\% \text{ pod damage} = \frac{\text{Total No. of Pods Produced per Plant} - \text{No. of undamaged pods}}{\text{Total No. of Pods Produced per Plant}} \text{ x100}$$

Determination of Adult mortality of *C. tomentosicollis*

The mortality of adult bugs was determined following the method outlined by Rauf and Sadar (2011). Mortality rates were assessed by applying the specified concentrations, including a control, in three replications. The treatment was applied to different cowpea varieties in replicated tagged field cages at 60% podding using a hand Knapsack sprayer at different induction time (1-week after spray (1-WA**S)** and 2-weeks after spray(2-WA**S**) before introducing the insects. Ten insects were released onto the treated plants after the initial spray, with the process was repeated at interval for the second spray. The number of dead insects was recorded for each treatment (Perillo *et al.,* 2015).

Statistical Analysis

All data collected by counting were subjected to square root transformation while percentages were arcsine transformed prior to analysis. Transformed data were subjected to Analysis of Variance ANOVA using Statistical Analysis using SPSS software 20 (Version 08.12.14). Post hoc test was carried out using the Tukey test at 0.05 level of significance.

RESULTS AND DISCUSSION

GC-MS Analysis of the Extracts

In the analysis of *C. Procera* using GC-MS, a total of 25 compounds were detected in the chloroform/n-hexane fraction after column chromatography and Fourier transform spectroscopy (FTIR). The identification of these phytochemical compounds is based on their peak area, molecular weight, and molecular formula. Among the compounds identified, the only alkaloid present in the fraction was 2-piperidinone, N-(4-bromo-n-butyl), with a retention time of 59.857 and a peak area of 8.37. The

details of the peaks, compound names, and retention times for the other compounds can be found in Table 1.

Effect of the Alkaloid Extracts of *C. procera* on the Infestation of *C. tomentosicollis*

The results indicate a significant reduction ($p<0.05$) in the insect population in the treated plots compared to the untreated control (1WA**S** and 2WA**S**). The highest level of protection was observed in plots sprayed with 500 (µg/ml) and 250 µg/ml, which did not show a significant difference. However, there was a significant difference in plots sprayed with 125 µg/ml and 0 µg/ml. The untreated control plots with 0 (µg/ml) recorded the highest incidence of *C. tomentosicollis* in all the cowpea varieties.

Effect of Alkaloid 2-piperidinone, N-(4-bromo-n-butyl) on damage by *C. tomentosicollis.*

Table 2 presents the average percentage of cowpea pod damage caused by *Clavigralla tomentosicollis* adult and nymph at 70DAP when treated with Alkaloid 2-piperidinone, N-(4-bromo-n-butyl)-. The results indicate that the damage inflicted by *C. tomentosicollis* was significantly reduced for all concentrations sprayed compared to the untreated control. The highest level of protection was observed in ITOK-292-10 with 500 (µg/ml), which did not show a significant difference from plots sprayed with 250 (µg/ml). The control cages exhibited the highest percentage of pod damage. Furthermore, there was no significant difference in pod damage among the three cowpea varieties.

Effect of the alkaloid 2-piperidinone, N-(4-bromo-n-butyl) on the mortality of *C. tomentosicollis*

The results presented in table 3, show the effect of different concentrations of the alkaloid compound on the adult mortality rate of *C. tomentosicollis* in ITOK7-318-33, ITOK-292-10, and IFE BROWN varieties. Significant differences were observed among the mean values of the concentrations in all the tested varieties at different spray regimes. In the ITO7K-292-10, plants treated with 500, and 250µg/ml showed a significant mortality of *C. tomentosicollis* compared to other treatments group. There was however, no significant ($P>0.05$) difference between the effective treatments (500 µg/ml and 250 µg/ml) in both sprays. Furthermore, in the IFE BROWN variety, the cowpea plants treated with 500 µg/ml, 250 µg/ml, and 125 µg/ml showed no significant difference in terms of the mortality rate, but all concentrations differed significantly from the control group treated with 0 µg/ml.

Table 1: Chemical Constituents Present in the N-Hexane /Chloroform Fraction Using GC-MS Analysis

PK	RT	Area Pct	Names of compounds
1	33.736	0.28	pentadecane
2	35.285	0.27	Trichloroacetic acid, pentadecyl ester
3	37.583	0.99	1-tridecane
4	37.876	1.66	Hexadecane
5	39.854	0.66	Methoxyacetic acid, 2-tetradecyl ester
6	40.038	0.41	Cyclohexane, nonadecyl-
7	41.869	1.94	Heptadecane
8	42.052	1.49	Pentadecane, 2,6,10,14-tetramethyl
9	43.554	0.26	1-Octadecanesulphonyl chloride
10	43.811	0.28	Dodecyl isobutyl ether
11	44.544	0.67	Oxalic acid cyclobutyl hexadecyl ester
12	45.386	3.15	1-Octadecane
13	45.697	3.92	Hexacosane
14	45.972	1.27	Methoxyacetic acid 2-tetradecyl ester
15	46.632	0.32	Triacontyl pentaflouro propionate
16	47.914	0.69	Cyclohexane, (4-methylpentyl)-
17	49.013	0.49	Tetrapentacontane, 1,54-dibromo-
18	49.270	3.50	Nonadecane
19	50.039	0.37	Isobutyl tetratriacontyl ether
20	50.222	0.68	Tetracosyl heptaflourobutyrate
21	51.504	3.07	Dibutyl phthalate
22	52.530	4.07	Cycloeicosane
23	52.713	8.35	Eicosane
24	55.497	18.82	Heneicosane
25	59.857	8.37	2-piperidinone, N-(4-bromo-n-butyl)-

RT=retention time, PK= peak

Table 2: Effects of the Alkaloid 2-piperidinone, N-(4-bromo-n-butyl) on *C. tomentosicollis* Infestation

Treatments (µg/ml)	IT07K 292-10		ITOK7318-33		Ife brown	
	1 week After Spray	2 weeks After Spray	1 week After Spray	2 weeks After Spray	1 week After Spray	2 weeks After Spray
500	20.0^{a}	16.7^{c}	16.7^{c}	16.7^{c}	22.4^{de}	10.0^{e}
250	23.3^{a}	20.0^{b}	16.7^{c}	26.7^{cd}	26.7^{cd}	20.0^{d}
125	36.7^{b}	36.7^{cd}	31.0^{bc}	33.3^{bcd}	36.7^{cd}	33.3^{bcd}
Control	43.3^{a}	53.3^{e}	43.3^{a}	40.00^{b}	36.00^{f}	40.00^{b}

Means followed by the same letters within column are not significantly different at p<0.05 (LSD).

Table 3. Effect of Alkaloid 2-piperidinone, N-(4-bromo-n-butyl) on cowpea pod damage by *C . tomentosicollis*

Treatment No.	Concentration (µg/ml)	Mean pod damage (%) for the three varieties		
		IT07K-292-10	IT07K-318-33	Ife brown
1	500	16.7^{c}	16.7^{c}	16.4^{c}
2	250	23.8^{bc}	23.3^{b}	23.4^{bc}
3	125	74.4^{bc}	86.7^{b}	77.8^{b}
Control	0	43.3^{a}	53.7^{d}	43.3^{a}

Means followed by the same letters within column are not significantly different at p<0.05 (LSD).

Table 4: Effect of Alkaloids 2-piperidinone, N-(4-bromo-n-butyl)- on *C. tomentosicollis* nymph and adult mortality.

Treatments (µg/ml)	IT07K 292-10		ITOK7318-33		Ife brown	
	1 week After Spray	2 weeks After Spray	1 week After Spray	2 weeks After Spray	1 week After Spray	2 weeks After Spray
500	76.7^{a}	76.3^{ba}	96.9^{c}	100.0^{b}	83.3^{e}	86.9^{bf}
250	76.3^{b}	76.4^{b}	80.0^{d}	80.0^{bc}	83.3^{e}	86.7^{b}
125	46.5^{bc}	43.4^{c}	46.5^{bc}	46.3^{bd}	56.33^{f}	53.7^{d}
Control	30.0^{ad}	36.5^{e}	23.3^{a}	33.3^{d}	23.3^{a}	30.0^{ad}

Means followed by the same letters within columns are not significantly different at p<0.05 of fisher LSD test

Discussion

The examination of the alkaloid extract from *C. procera* leaves for its insecticidal effects against *C. tomentosicollis* demonstrated its strong efficacy against the target pest. The extract exhibited higher insecticidal potency at doses of 500 and 250 (µg/ml) compared to lower concentrations and the control group. The compound 2-piperidinone, N-(4-bromo-n-butyl)- was found to inhibit the activity of acetylcholinesterase (Lucie *et al.,* 2013). Acetylcholinesterase is a well-known target of organophosphates, carbamate, and certain natural products (Lucie *et al.,* 2013). It is plausible that the chemical compounds identified in this study have the ability to inhibit the activity of acetylcholinesterase. There is currently no literature available on the insecticidal activity of *C. procera* alkaloids against *C. tomentosicollis*, although (Ge *et al.,* 2015) has reported their insecticidal properties. Previous research has suggested that the insecticidal properties of certain plant extracts may be attributed to anticholinergic alkaloids like scopolamine, hyoscyamine, meteloidine, and atropine (Ge *et al.,* 2015). Since tropane alkaloids have not been isolated from *C. procera*, quinolizidine alkaloids could be considered as another class of alkaloids with insecticidal properties.

The study conducted revealed that the Alkaloid compound 2-piperidinone, N-(4-bromo-n-butyl)- extracted from *C. procera* showed significant effectiveness against *C. tomentosicollis*. When sprayed at 500µg/ml, the compound notably reduced PSB infestation. This reduction in insect infestation is likely attributed to the toxic effects on *C. tomentosicollis* or the repellent properties of the compound. The findings of this research demonstrate that the aqueous leaf extracts of Calotopis procera resulted in varying levels of reduction in pod sucking bugs and provided different levels of protection to cowpea plants compared to the untreated control plot. Moreover, the study highlights the potential of *C. procera* leaf extracts as a biocide for managing *C. tomentosicollis* on cowpea plants. The number of *C. tomentosicollis* and the associated damage significantly decreased in plots treated with the alkaloid extracts. The mode of action is likely through contact, as some bugs were observed on the ground shortly after spraying, displaying symptoms such as hypo-excitability, staggered walking, abdominal extrusion, and eventual death. The

insecticidal properties of alkaloids have been tested against various notorious insect pests, including *Spodoptera litura* Fabricius and *Lipaphis erysimi* (Kaltenbach) (Ge *et al.,* 2015) and *Locusta migratoria* (Doumandji-Mitiche and Bahi, 2013).

The mortality rate percentage increase ed as the extract dosage increased, aligning with the findings of Emmanuel *et al.* (2013) who noted that the difference between the medicinal and toxic effects of alkaloids (or any drug) often lies in the dosage. The application of the compounds had a more significant effect on insect mortality at 500µg/ml compared to 250 µg/ml and 125µg/ml. This effect differed from that of the untreated control. In terms of insect infestation and damage, there were no significant differences observed among the three cowpea varieties used, namely IT07K-318-33, IT07K-292-10, and Ife brown. This suggests that all varieties are susceptible to cowpea pests, including *Clavigralla tomentosicollis*.

The outcomes presented in this research demonstrate that alkaloid extracts from *C. procera* have significant potential as biopesticides, offering viable alternatives for pest management in small-scale, low-input agriculture on field crops commonly found in tropical regions, all while maintaining environmental integrity.

CONCLUSION

The findings revealed a significant decrease in the population of *C. tomentosicollis* in the treated plots with 2-piperidinone, N-(4-bromo-n-butyl) compared to the untreated control. The highest level of protection was observed in plots sprayed with 500µg/ml and 250µg/ml, with no significant difference between them ($p>0.05$). The average percentage of pod damage caused by *Clavigralla tomentosicollis* adults was significantly lower at higher treatment concentrations (500µg/ml and 250µg/ml) compared to lower concentrations and the control. There was no significant difference in pod damage among the three cowpea varieties. The relative adult mortality of *C. tomentosicollis* followed a similar pattern, with higher concentrations (500µg/ml and 250µg/ml) resulting in considerably higher mortality in ITOK-292-10 compared to other varieties. There was however no significant difference in mortality rates in the ITOK7-318-33 variety, between treatments (500µg/ml, 250µg/ml, and 125µg/ml) and spray regimes. This implied that treatment of cowpea ITOK-292-10 with the higher concentration of the Alkaloid (2-piperidinone, N-(4-bromo-n-butyl) could yield an optimum level control of the notorious bug.

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