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Repellent Effect of Plant Terpenes Compound from *Eucalyptus camaldulensis* Leaves Extract for the Regulation of Cowpea Flower Damage Caused by *Megalurothrips sjostedti (*Trybom 1908) (*Thysanoptera*: *Thripidae)*

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Abstract

Cowpea flower thrip causes considerable damage to the cowpea plant, particularly at the flowering stage of the plant. This study employed Eucalyptus camaldulensis leaf extract as a regulatory mechanism to lessen the impact of Megalurothrip sjostedi. The Gas chromatography mass spectrometry (GC-MS) method was used to examine the composition of the essential oil. Three cowpea varieties;ITOK7-318-33, ITOK7-292-10, and VITA-7 were evaluated. The experiment was conducted during the 2017 cropping season and laid out in a randomized complete block design with three replications. Four treatments 500, 250, 125, and 0 µg/mL of a terpene compound from Eucalyptus camaldulensis leaf extract were selected. Flower damage was recorded as browning or drying, and flower bud abscission was estimated for each treatment. Results obtained indicate that Plants treated with 500µg/mL recorded no browning or drying of stipules, leaf, or flower buds and no bud abscission, whereas untreated 0µg/mL plants recorded distinct browning or drying of stipules, leaf, or flower buds and some bud abscission after 24 hours of treatment. The results obtained in this study suggest that Eucalyptus camaldulensis leaf extracts can serve as an alternative product for the control of cowpea flower thrip (M. sjostedti). Additional research ought to be done to determine whether of the separated component from the same plantand the crude additiona is more beneficial.

Keywords Cowpea flower thrip, *Eucalyptus camaldulensis,* Terpene, Flower damage

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INTRODUCTION

Long-grown in tropical climates, cowpea (*Vigna unguiculata* L. Walp) is most likely native to Africa, where wild species are abundant (Jada *et al*., 2015). The wild cowpea's widespread distribution is one of the most compelling arguments in favor of Africa. In addition to Australia, South America, and the West Indies, cowpeas are grown in West Africa Jada *et al*. (2015) . According to the same research by Jada *et al*. (2015), Nigeria is one of the top producing countries in the world for cowpeas, which are widely produced there in a variety of ecological zones. According to estimates from the Food and Agricultural Organization FAO.,(2012), cowpea dry grains are produced globally in amounts of 3.3 million tons. Integrated Pest Management (2001) reported that the crop represents a significant supply of feed for cattle, particularly in the dry savannah of West Africa and the semi-arid tropics. Cowpea, like other legume crops, is an effective crop for fixing nitrogen, providing up to 240 kg/ha of nutrients annually (Jada *et al*., 2015). It also replenishes the nutrients that other non-leguminous crops utilize (Jada *et al*., 2015). Because cowpeas is a resilient crop that grows well in moderately dry conditions and is an affordable source of vegetable protein, it is also an important grain legume in the tropics FAO.,(2012) .According to reports FAO , the crop may fix up to 70 kg of nitrogen (N) per hectare annually, making it significant for improving soil fertility (Adipala *et al*., 2000). The ability to fix N is especially crucial in subsistence-oriented farming systems where the utilization of inorganic fertilizers is almost nonexistent. Cowpea, being highly palatable and nutritious, is a cost-effective protein source in many diets within tropical and sub-tropical regions (Chintkuntla, 2009). With a protein content ranging from 20–30%, cowpea grains serve as an economical plant protein source for those with limited access to animal protein from sources like meat, fish, milk, and eggs (Egho, 2011).

Africa has reported the lowest yields per unit area, with insect pests identified as the primary obstacles to cowpea production (Alabi *et al*., 2003). In particular, flower thrips, such as *Megalurothrips sjostedti*, poses a significant threat to cowpea crops in tropical Africa, attacking reproductive structures during plant development (Mfuti *et al*., 2017). Cowpea flower thrips (CFT) in Africa cause damage to buds, racemes, and flowers, leading to premature abortion of these reproductive organs and resulting in yield losses ranging from 20 to 100% (Alabi *et al*., 2003; Mfuti *et al*., 2017).

The adverse impact of CFT on cowpea development, especially in Tanzania, Ghana, Cameroon, and Nigeria, has prompted the exploration of safe, effective, and natural measures to combat these flower thrips (Science technology., 2011). Plant-based compounds are recommended as a viable method for pest control due to their low toxicity to non-target organisms, minimal environmental impact, and safety for mammals (Wei *et al*., 2011). Currently, Sub-Saharan African countries heavily rely on synthetic insecticides to control *M. sjostedti*. However, the indiscriminate use of these chemicals has led to issues such as insecticide resistance, the accumulation of toxic residues in food, health risks to consumers and livestock, and environmental contamination (Abtew *et al*., 2015). Therefore, there is an urgent need to develop alternatives that are safe, effective, biodegradable, and highly selective. Plant-based pesticides are proposed as a superior alternative to synthetic insecticides in addressing these concerns. This research was therefore aimed at determining the major terpene compound in *Eucalyptus camaldulensis* leaf extract and assessing its effect on flower damage caused by the legumes flower thrips.

MATERIALS AND METHODS

Experimental site

The experiment was conducted at the Teaching and Research Farm of the Faculty of Agriculture, Bayero University, Kano, during the 2017 cropping season. Kano lies between longitude 9^0 30, and 12^0 north, longitude 9^0 30, and 8^0 42, east in the sudan savannah zone of Nigeria. Laboratory experiments were conducted at the Postgraduate Laboratory of the Department of Biological Sciences, Bayero University, Kano.

Collection Of Experimental Seeds

Seeds of two resistant cowpea varieties, namely ITO7K-318-33 and ITO7K-292-10, and one susceptible variety (Vita-7) were collected from the International Institute of Tropical Agriculture (IITA), Kano station.

Preparation of the Extract

Eucalyptus camaldulensis leaf powder weighing 150 g was dispensed in 300 mL of ethanol in a dark bottle container, which was kept at laboratory temperature for two weeks with shaking at regular intervals, after which the content was filtered using Whatman filter paper Number1. The crude extract obtained was kept in a sterile beaker to allow for evaporation, as described by Herborn (1998).

Column Chromatography of the extract

Silica gel with a 60–120 mesh size (a product of Lobachemie laboratory reagents and fine chemicals) was used as a stationary phase in the column. A glass measuring 5.0cm in diameter and 87cm in length was clamped vertically to prepared th column.The lower end of the column was fitted with a stopper and plugged with cotton wool as support. The slurry of silica gel was prepared in the solvent used for separation (250 g of silica gel in 500 mL of n-hexane). The slurry was added to the column gradually and with gentle tapping to avoid cracks. This process was continued until a uniform column of the desired length was obtained. 24.42 g of the crude extract obtained from cold extraction was mixed with 25 g of silica gel to obtain a homogenous mixture. This mixture was poured into the column, and different fractions were eluted with different solvents, viz., n-hexane, chloroform, and methanol. The extracted fractions were collected separately in 100 mL of a volumetric flask. The solvent of the collected fractions was evaporated at room temperature.

Thin-layer chromatography

A tiny amount of the fractions was put on a merck plate had previously been silica gel-coated (60 ˚C 254) of 0.2 mm thickness using a capillary tube. The plate was developed in a solvent system of n-hexane, chloroform, and methanol. The plate was then air-dried and visualized in UV 254 and 366 nm to detect spots of different compounds (Dwivedi *et al*., 2014).

Fourier Transform Infrared Spectroscopy

Fourier transform infrared spectroscopy (FTIR) was performed for all the fractions pulled after thin-layer chromatography (TLC) to screen the fractions for terpene functionality before GCMS analysis. This was carried out at the Department of Biochemistry Laboratory, Bayero University, Kano

Gas Chromatography Mass Spectrometry Analysis

The GCMS analysis of the sample derived from the ethanol leaf extract of *Eucalyptus* leaves was performed using Agilent GC 7890B and MSD 5977A, made by Agilent Technologies USA. Library: NIST14. L SOFTWARE MASSHUNTER equipped with an AB inno wax column (60

× 0.25 mm id, film thickness 0.25 µm) was used to read and interpret the outcome. For GCMS detection, an electron ionization system with an ionization energy of 70.007 eV was used. Helium gas was used as a carrier gas at a total flow rate of 7.4206 mL/min. Injector and mass transfer line temperatures were set at 270 and 280 °C, respectively. The oven temperature was programmed from 50 to 180 ˚C at 3 ˚C min^{-1} with a hold time of min^{-1} and from 180 to 250 ˚C at 6 ˚C min^{-1} with a hold time of 20 min, respectively. Diluted samples (prepared with methanol) of 0.2µL were manually injected in the splitless mode. Identification of compounds in the samples was based on the GC retention time on AB in the no-wax column; the total GC running time was 30 min (Verma *et al.*, 2013, Dwivedi *et al.*, 2014).

Preparation of Stock and Standard Solution

Ten (10) milligrams of the sample were added to 100 ML of dimethylsulfoxide (DMSO) to make the stock solution. It is expressed in weight per volume (w/v). The standard solution was made by serial dilutions. The concentrations are shown in the table 1below. Three (3) replicates of each dose are made. To make the first dose, which was in a ratio of 1:1, 25 mL of stock was added to 25 mL of distilled water in a 50 mL container. For the second dose (a ratio of 1:2), 12.5 mL of stock was added to 37.5 ml of distilled water in a 50 mL container. In the third dose (ratio of 1:3), 6.25 mL of the extract was added to 43.75 mL of water (Imam and Tajuddeen, 2015). The 0 µg/mL served as a control (Dauda and Ali, 2004).

Table 1: Preparation of stock and standard solutions according to dilution ratios

Dilution(v/v)	Concentration (µg/mL)	Requiredamount (mL)
1:1	500	25
1:2	250	12.5
1:3	125	6.25
Control	0	0

Source: Imam and Tajuddeen (2015).

Sampling and Analysis

Soil samples were randomly collected at a depth of 0–10 cm and 10–20 cm using a soil auger. The physical and chemical properties of the soil, including texture, pH, organic matter, cation exchange capacity, total nitrogen, available phosphorus, and exchangeable bases, using soil kid analysis, were determined in the soil laboratory of the Department of Soil Science at Bayero University Kano.

Layout and Design

Planting was done on the field during the rainy season after the land was plowed and harrowed with a tractor. The experimental plot size of 18 x 20 m was demarcated, and the beds were made manually using a hoe. Their size was 1 x 1 m with an inter-bed space of 0.5 m, and the distance between the replications was 1m at both locations. The experiment was laid out in a randomized complete block design (RCBD) with three replications.

Sowing of Experimental Plant

Sowing of cowpea varieties on the prepared land was done by direct seeding at a rate of 2–3 seeds per hole. The cowpea seedlings were later thinned to two plants per stand two weeks after germination. Gap filling was done three weeks after germination to replace failed seedlings.

Caging
Caging of cowpea was done when the pest was fully established using mosquito nets. The cages were designed in such a way that they did not interfere with the ventilation and aeration of the growing plants within the mesh. The bottom edges of the cages were inserted into the soil on all sides to check for the escape or entry of other insects, as described by Musa (2016).

Data Collection

Damage to flowers by *Megalurothrips sjostedti* (Trybom)
Flower damage by *Megalurothrip sjostedti* was assessed in the field by a visual rating on a scale of 1–9 as depicted in Table 2 (Jackai and Singh, 1988). The assessment was done 40 days after planting (DAP), when flowers were filled and matured. The visual rating of damage (flower drop, stipules, or raceme damage) was done on the plant in one of the border rows in each plot. Changing colours and drops of the flower were used as damage index by *Megalurothrip sjostedti*.

Table 2: Scale for rating *Megalurothrips sjostedti* damage to flower

Rating	Flower damage/appearance
1	No browning/drying (i.e. scale) of stipules, leaf or flower bud; No bud abscission.
3	Initiation of browning of stipules and leaf or flower buds; no abscission.
5	Distinct browning/drying of stipules and leaf or flower buds, some bud abscission.
7	Serious bud abscission accompanied by browning/drying of stipules and buds; no elongation of peduncles
9	Very severe bud abscission, heavy browning, drying of stipules and buds; distinct no elongation of (most of all peduncle).

Soure: Jakai and Singh (1988).

Data Analyses
Data were subjected to analysis of variance (ANOVA) and significant means were separated by Fisher's Least Significant Difference Test (LSD), at a 5% level of significance using Microsoft Excels 2007 statistical software. Probit analysis was used to determine lethal concentration 50 (LC_{50}), µg/mL.

RESULTS

Effect of Terpene Compound on Flower Damage Using Rating Scale on ITOK7318-33
Table 3 illustrates the impact of varying concentrations of terpene compound extracted from *Eucalyptus camaldulensis* leaves on cowpea flower damage, assessed using a rating scale, caused by cowpea thrips at 40 DAP across three different cowpea varieties. Following the initial spray, a significant difference ($p < 0.05$) among the treatment means was evident. Specifically, cowpea seeds of variety ITO7K-318-33 treated with 500 and 250 µg/mL displayed no browning/drying (i.e., scale) of stipules, leaves, or flower buds, and no bud abscission. In contrast, seeds treated with 125 µg/mL exhibited the initiation of browning in stipules and

flower buds but no abscission. Contro seeds (0µg/mL) displayed distinct browning/drying of stipules and flower buds with some bud abscission.
A similar pattern emerged after the second spray, with seeds treated with 500 , 250 and 125 µg/mL showing no browning/drying (i.e., scale) of stipules and flower buds, and no bud abscission. In contrast, control plants (0µg/mL) experienced significant bud abscission accompanied by browning/drying of stipules and buds, and no elongation of peduncles.

Table 3: Effect of Terpene Compound on Cowpea thrip in Flower Damage on ITOK7318-33

Treatments (µg/mL)	24 hrs After First Spray	24 hrs After second spray
	Mean ±SE	Mean ±SE
500	1.67±0.33^{a}	1.33±0.33^{a}
250	2.00±0.67^{a}	1.67±0.33^{a}
125	3.67±0.33^{b}	2.33±0.66^{a}
0	6.33±0.66^{c}	7.00±1.15^{d}

Means along column with different superscripts are significantly different at P<0.05 using Pitcher's LSD

Terpene Compound on Flower Damage using Rating Scale on ITO7K-292-10
Results on the cowpea flower damage using a rating scale caused by cowpea thrip at 40 DAP as affected by different concentrations of terpene compounds extracted from *Eucalyptus camaldulensis* leaves in three different cowpea varieties are presented in Table 4. A significant difference ($p \leq 0.05$) was observed among the treatment means after the first spray, cowpea seeds (ITO7K-292-10, Variety) showed significant differences ($p \leq 0.05$) among the treatments, where mean cowpea seeds treated with 500, 250, and 125 µg/mL recorded no browning or drying (i.e., scale) of stipules, flower buds, or bud abscission, whereas plants treated with 0µg/mL treatment recorded serious bud abscission accompanied by browning or drying of stipules and buds; no elongation of peduncles. A similar trend was observed after the second spray, whereas seeds treated with 500, 250, and 125 µg/mL recorded no browning or drying (i.e., scale) of stipules, flower buds, or bud abscission. The seeds left as control with 0µg/mL recorded serious bud abscission accompanied by browning or drying of stipules and buds, with no elongation of peduncles.

Table 4: Terpene Compound in Cowpea Thrip on Flower Damage on IT07K 292-10

Treatments (µg/mL)	24 hrs After First Spray	24 hrs After second spray
	Mean ±SE	Mean ±SE
500	1.33±0.33^{a}	1.00±0.33^{a}
250	1.66±0.33^{a}	1.33±0.33^{a}
125	2.33±0.33^{a}	1.33±0.33^{a}
0	7.00±1.57^{d}	7.67±0.67^{d}

Means along column with different superscripts are significantly different at P<0.05 using Pitcher's LSD

Terpene Compound on Cowpea Thrip on Flower Damage on VITA-7
Table 5 presents the outcomes of cowpea flower damage, assessed using a rating scale, caused by cowpea thrips at 40 DAP, influenced by varying concentrations of terpene compounds extracted from Eucalyptus camaldulensis leaves across three distinct cowpea varieties. Following the initial spray, a significant difference ($p < 0.05$) was evident among the treatment means. Notably, cowpea seeds (VITA-7) were significantly impacted by the treatment at $p < 0.05$, where the application of 500 µg/mL terpene compound from Eucalyptus leaf extract resulted in no browning or drying (i.e., scale) of stipules or flower buds and no bud abscission.

Conversely, plants treated with 250 and 125 µg/mL exhibited the initiation of browning in stipules and flower buds but no abscission. In contrast, plants treated with 0µg/mL experienced serious bud abscission accompanied by browning or drying of stipules and flower buds, with no elongation of peduncles. A similar pattern persisted after the second spray, with plants treated with 500, 250, and 125 µg/mL displaying no browning or drying (i.e., scale) of stipules, flower buds, or bud abscission. Control seed on the other hand, demonstrated serious bud abscission accompanied by browning or drying of stipules and buds, with no elongation of peduncles.

Table 5:Terpene Compound in Cowpea Thrip on Flower Damage on VITA-7

Treatments (µg/mL)	24 hrs After First Spray	24 hrs After second spray
	Mean ±SE	Mean ±SE
500	2.33±0.66^{a}	1.67±0.33^{b}
250	3.67±0.67^{b}	1.67±0.88^{b}
125	4.33±0.67^{b}	2.67±0.00^{c}
0	7.00±1.33^{d}	7.67±1.15^{a}

Means along column with different superscripts are significantly different at P<0.05 using Pitcher's LSD

DISCUSSION

Various formulations of terpenes extracted from *Eucalyptus camaldulensis* leaves extract resulted in different degrees of reduction in flower damage caused by cowpea thrips. These formulations also provided varying levels of protection to flowers against thrip-induced damage. Since cowpea flower thrip infestation poses a significant obstacle to improving crop yield, effective management is crucial.

Essential oils, as mixtures of volatile organic compounds, are secondary plant metabolites (Khater, 2012; Grdiša *et al*., 2013). Many essential oils, primarily from families such as Lamiaceae, Myrtaceae, Asteraceae, Rutaceae, Apiaceae, and Laureaceae, exhibit significant insecticidal potential (Khater, 2012; Grdiša *et al*., 2013). These essential oils demonstrate diverse bioactivities against both medically important insect species and agricultural pests (Arshad *et al*., 2014) . Their effects range from toxicity, with ovicidal, larvicidal, pupicidal, and adulticidal activities, to sub-lethal effects like oviposition deterrent, antifeedant activity, repellent action, and influence on biological factors such as life span, growth rate, and reproduction (Arshad *et al*., 2014). Certain essential oils, including rosemary, eucalyptus, clove, thyme, and lemongrass, are recognized for their insect pest control properties (Arshad *et al*., 2014).

Results of this study on flower damage in the ITOK7-318-33 variety revealed significant differences among plants. Those treated with 500 and 250 µg/mL recorded no browning/drying of stipules, leaves, or flower buds, and no bud abscission after both sprays. In contrast,seeds treated with 125 µg/mL showed the initiation of browning in stipules and flower buds but no abscission. Untreated seeds (0 µg/mL) exhibited distinct browning/drying of stipules and flower buds, with some bud abscission after the first spray and serious bud abscission with browning/drying after the second spray. This aligns with previous research on the attractiveness of volatile plant compounds to western flower thrips (Koschier *et al*., 2000).

Similar trends were observed in the study of the ITO7K-292-10 variety, where seeds treated with 500, 250, and 125 µg/mL showed no browning/drying or bud abscission, while control plants (0µg/ml) experienced serious bud abscission with browning/drying. Results for the

VITA-7 variety also indicated that plants treated with 500 and 250 µg/mL displayed no browning/drying or bud abscission, whereas control seeds (0 µg/mL) exhibited browning/drying and some bud abscission. These findings are consistent with research on the insecticidal activity of essential oils from different plants against stored-product pests (Abdurrahman *et al*., 2010).

While the essential oils obtained from plants like *Eucalyptus camaldulensis* show promise for integrated pest management, addressing cost-effective commercial challenges is essential. Essential oil content in aromatic plants is typically around 1-3%, necessitating the processing of large quantities of plant material for commercial-scale applications.

CONCLUSION

The outcomes of this study provided evidence that the terpene compound extracted from *Eucalyptus camaldulensis* leaves, particularly at concentrations of 500 and 250 µg/mL, effectively reduced the incidence of flower damage. This suggests the insecticidal potential of the terpene compound against *M. sjostedti*, highlighting its promising application for the development of botanical insecticides to manage this pest on cowpea plants. Importantly, the compound exhibited no phytotoxic effects on the treated plants. The GC MS analysis identified a total of thirty two (32) compounds, with notable terpenes such as 1,8 cineole falling under the category of monoterpenes.

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