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Original Article

Contact toxicity of Essential Oils from Tithonia diversifolia against Aphis gosypii, Thrips tabaci and Bemisia tabaci.

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Tithonia Diversifolia, 3-Careen, Biopesticide, ATR-FTIR, LD50, Contact Toxicity, Aphis Gosypii, Thrips Tabaci, Bemisia Tabaci.

ABSTRACT

Plants and microbes provide naturally occurring chemicals that are often used for the control of pests all around the world. Biopesticides have been used as a comparatively safer replacement for synthetic pesticides for the past five decades. This study determined the contact toxicity of essential oils from the Tithonia diversifolia plant against Thrips tabaci, Bemisia tabaci, and Aphis gosypii. The essential oils (Eos) were extracted from dry leaves by hydrodistillation using the Clavenger apparatus for 8 hours. GC-MS was utilised to analyse the qualitative and quantitative composition of essential oils, whereas ATR-FTIR was employed to determine the functional groups. 3-Carene was the most abundant compound ion in the Eos. T. tabaci, B. tabaci, and A. gosypii were used in the bioassay of the crude extracts. T. tabaci, B. tabaci, and A. gosypii were tested for contact toxicity against mixed-sex adult pests. Five distinct concentrations were made, each of which was repeated five times. Permethrin, a commercial chemical pesticide was utilised as a positive control and acetone were used as a negative control. The essential oils' LD50 was calculated using SPSS version 26.0 Probit analysis. After 24 hours, the pests' response to the treatments was assessed using a blunt instrument probe, and those that did not respond were counted as dead. Essential oils from T. diversifolia had the lowest LD50 against T. tabaci, with a value of 0.085 µL. This suggests that T. diversifolia could be employed as a T. tabaci contact toxicant. ($P < 0.05$, $\alpha = 0.05$) The outcomes were statistically significant. As a result, essential oils from T. diversifolia can be employed as a biopesticide against T. tabaci as a contact toxicant.

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INTRODUCTION

The application of synthetic pesticides is by far the most widely used approach of agricultural pests, and it entails the use of various pesticides (Wei et al., 2018). Pesticides are categorised based on a variety of factors, including chemical components, target pests, formulation, mechanism of action, and source (Pandya, 2018; Gogi et al., 2017). They are categorised as synthetic or biopesticides depending on their source (Kavit et al., 2013). Biopesticides are compounds that naturally exist and are used to protect crops in non-toxic and environmentally acceptable ways (Kumar, 2015). As a result, the word “biopesticide” is used to refer to pest management tactics that employ bioactive microorganisms from animals, bacteria, viruses, and plants in order to create sustainable agriculture (Ivase et al., 2017). They include plant integrated protectants (PIP), microbial, and biochemical pesticides (Kareru et al., 2013).

Biochemical insecticides are made from plant secondary metabolites (Archaya, 2014). Terpenoids, quinines, flavonoids, and alkaloids are secondary plant metabolites that help plants defend themselves against herbivores and phytophagous insects (Belete, 2018). Plants and plant materials with harmful secondary metabolites are suitable for use as biochemical insecticides (War et al., 2018). Different biopesticides as listed differ in the mode of action. The biopesticide deters pests in three main ways, by either acting as repellents, antifeedants or oviposition deterrents (Kareru et al., 2013). This research sought to investigate the contact toxicity of essential oils from *Tithonia diversifolia.*

The leaves of *T. diversifolia* contain sesquiterpenes lactones, diterpenes, and other polyphenolic compounds, the extracts of *T. diversifolia* have significant pesticidal properties and have been widely used for whiteflies, thrips, and aphid management in East and South Africa (Kandungu et al., 2013).

Bemisia tabaci, *Aphis gosypii*, and *Thrips tabaci* cause huge losses in agriculture. In Kenya, *B. tabaci* are major vectors of viral diseases, they reduce cassava (*Manihot esculentum*) yield by up to 40% and at times up to 100% (Njoroge et al., 2017); *T. tabaci* are found in all onion growing areas in Kenya and cause up to 59% loss in yield of onions (*Allium cepa*) (Waiganjo et al., 2006). Aphid can cause more than 90% of losses in wheat (*Triticum aestivum* L) produce (Njuguna et al., 2016). These pests are mainly controlled using chemical insecticides such as pyrethroids, organophosphates, nicotinic receptors agonists, insect growth regulators, carbamates, inorganic pesticides, and neonicotinoids (Wakil et al., 2017).

However, from the documented negative effects of most synthetic pesticides, biopesticides have been sought after as an alternative globally (Auamcharoen & Chandrapatya, 2010). Most biopesticides have a high potential for use in sustainable agriculture because most are reported to be environmentally benign, less toxic to human beings and non-target organisms, pest-specific, among others (Mazid et al., 2011). Plant-based biopesticides are among the most utilised alternatives to chemical pesticides globally (Mazid et al., 2011). Research shows that *T. diversifolia* contains essential oils that show some activities against phytophagous plant pests (Green et al., 2017). This research is therefore directed towards testing the contact toxicity of essential oils from *T. diversifolia* against *T. tabaci*, *B. tabaci*, and *A. gosypii.*

MATERIALS AND METHODS

5.0 kg of fresh leaves were collected during the rainy seasons when the plants were almost flowering as identified by Mr Patrick Mutiso, a

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taxonomist from the University of Nairobi. Flowers were deposited in the University of Nairobi herbarium under voucher specimen number as follows *T. diversifolia* (JNM003/2021). *T. diversifolia* plant leaves were collected from Kandara in Murang'a County, Kenya. The fresh leaves were weighed using a spring balance. They were rinsed with tap water to remove any physical or chemical impurity. The leaves were dried on the benches at the Mount Kenya Pharmacognosy Laboratories for seven days to a constant mass by losing 90% of the total moisture content. The total dry mass was weighed using an analytical grade balance Shimadzu ATY224 uniblock at the Mount Kenya University Pharmacy laboratory and the total dry mass of 0.50 kg was recorded. The percentage moisture content lost was computed by the following formula

$$Percenatge\ moisture\ lost = \frac{mass\ of\ fresh\ leaves - mass\ of\ dry\ leaves}{mass\ of\ fresh\ leaves} x100\%$$

The dry plant leaves from *T. diversifolia* were pulverised using a kitchen blender at the Pharmacognosy laboratory in Mount Kenya University. The dry powdered plant materials were hydro distilled using the Clevenger apparatus for eight hours at 100℃ and ambient atmospheric pressure. The vial was weighed had a mass of using Shimadzu ATY224 uniblock 17.964 g. The vials and the oils were re-weighed and had a mass of 18.972 g. The mass of essential oils was calculated by subtracting the mass of the vials from the mass of oils and the vials. The rate at which the oils were produced were computed as follows:

$$\frac{0.0001\ Kgof\ essential\ oils}{0.2\ Kg\ of\ dry\ leaves}$$

Analysis of Essential Oil Extracts

The analysis was done using Gas chromatograph-mass spectrometry analyses (GC-MS) and Attenuated Total Reflectance Fourier transform infrared spectroscopy (ATR-FTIR).

ATR-FTIR Analysis

The samples were analysed using a Brucker alpha 11 ATR-FTIR spectrophotometers at the Government Chemist Laboratories in Nairobi County, Kenya producing an ATR-FTIR spectrum from the computer libraries. The peaks in the spectrum were analysed and corresponding functional groups were assigned and the probable molecules were identified (Kumar, 2016)

GC-MS Analysis

Essential oils from *T. diversifolia* were diluted with a 1:4 mixture of pure n-hexane (non-polar solvent) and Dichloromethane (DCM-polar solvent) 2% v⁄v for GC-MS analysis sample solution (Wei et al., 2018) and a sample solution of 1 µL was injected into GC-MS equipment using an auto-injector. Calibration of the equipment was done prior to analysis and time and temperature were determined and programmed at the time of analysis. GC-MS was used for the determination of both the qualitative and quantitative composition of the essential oils. Qualitatively the components were identified based on the retention index and by comparison with NIST-11 database (Wei et al., 2018). Quantitatively, the relative amounts of the constituents of the EOs were expressed as a percentage by peak area approximated peak height approximation (Wang et al., 1996).

20 µL of the crude essential oils of *T. diversifolia* samples were diluted using 1 mL of a mixture of n-hexane and dichloromethane in the ratio of 1:4. The component identification was achieved by Agilent 5977A MSD and 7890B GC system, Chemetrix; Agilent Technologies, DE (Germany) at the Government Chemist Department Laboratories in Nairobi County, Kenya. Helium was used as carrier gas at a constant flow of 1 mL/min and splitless mode. An injector volume of 1 µl was employed with the mass spectra scanned from 40 to 560 m/z at an injector temperature of 250 °C. Ionisation was by electron impact (70 eV, source temperature 250 ℃). The oven temperature was programmed from 50 °C (isothermal for 1 min.), with an increase of 10 °C/min, to 300 °C and held for 3 min isothermal at 300 °C. Total GC running time was 32 min. Chromatographic separation was done using DP5-MS-UI.

Contact Toxicity Analysis *T. diversifolia*

Twenty pests from each species of *A. gosypii*, *B. tabaci*, and *T. tabaci* were released on filter in

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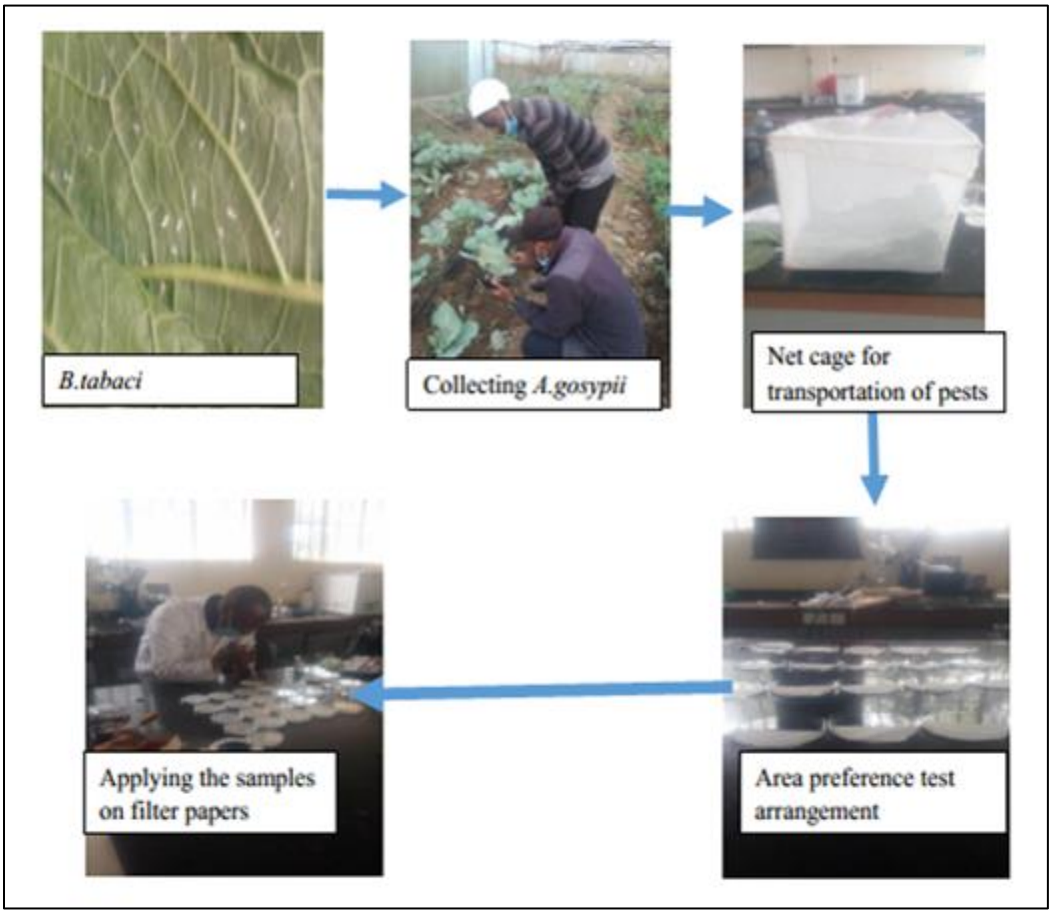
papers treated with acetone as the blank and replicated five times. The same step was repeated with filter papers different treated with different concentrations of essential oils 0.00, 0.01, 0.25, 0.75 and 1.0 µL of *T. diversifolia* essential oils and permethrin as the positive control in covered Petri dishes. The responses to the treatments were observed after a 24 hours period. Probing was done to the pests using a blunt object. The pests that did not respond to probing were counted as dead. The data was then computed and transformed using SPSS version 26.0, analysed by Probit analysis to determine LD_{50}. The figures below show the collection and the bioassay procedure.

Figure 1: Pest collection and the bioassay procedure



RESULTS AND DISCUSSIONS

ATR-FTIR Analysis of *T. diversifolia* Essential Oils

From the ATR- FTIR profiles of essential oils of *T. diversifolia*, a total of 3 major compounds were identified, these were (+)-2-Pinene, Pinene and (±)-α-Pinene which had a hit quality of above 600. *Table 1* below shows chemical constituents as identified by comparison with ATR-LIB-PHARMA-2-472-2S01 libraries

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Figure 2: A spectrum of (+/-)-A-Pinene

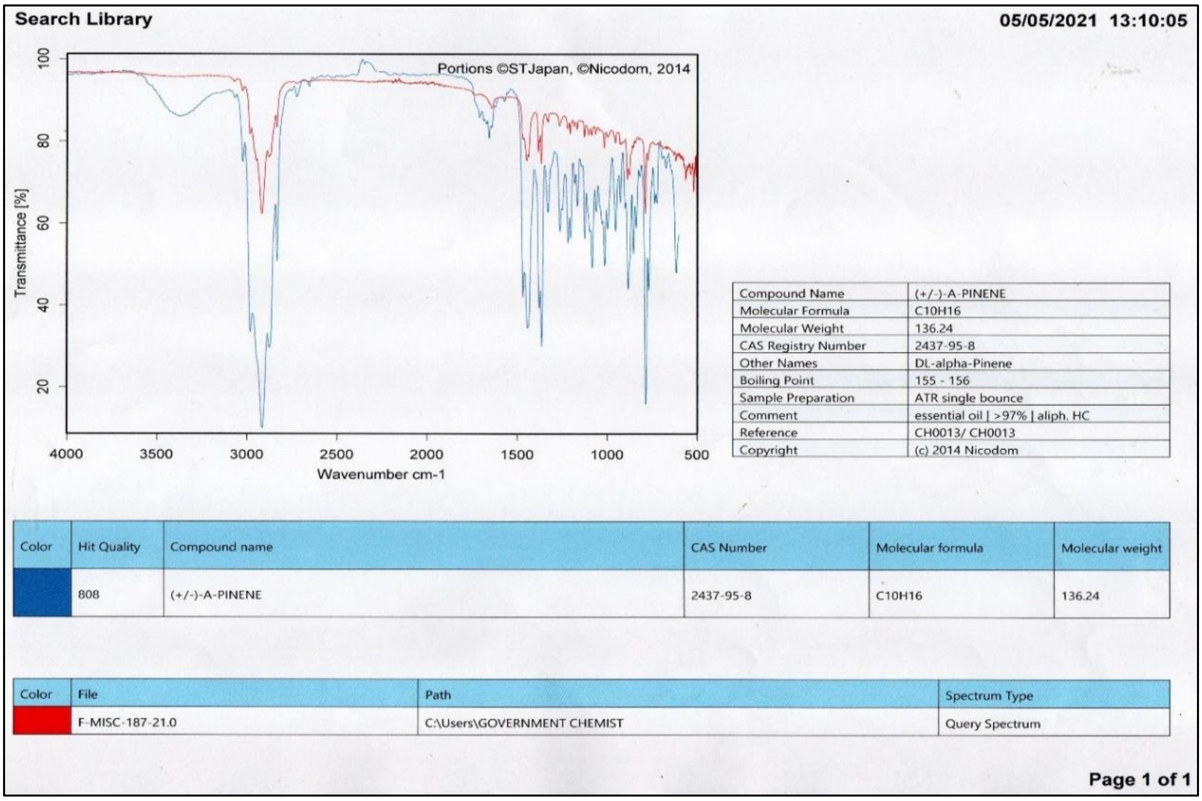

Figure 3: A spectrum of Pinene

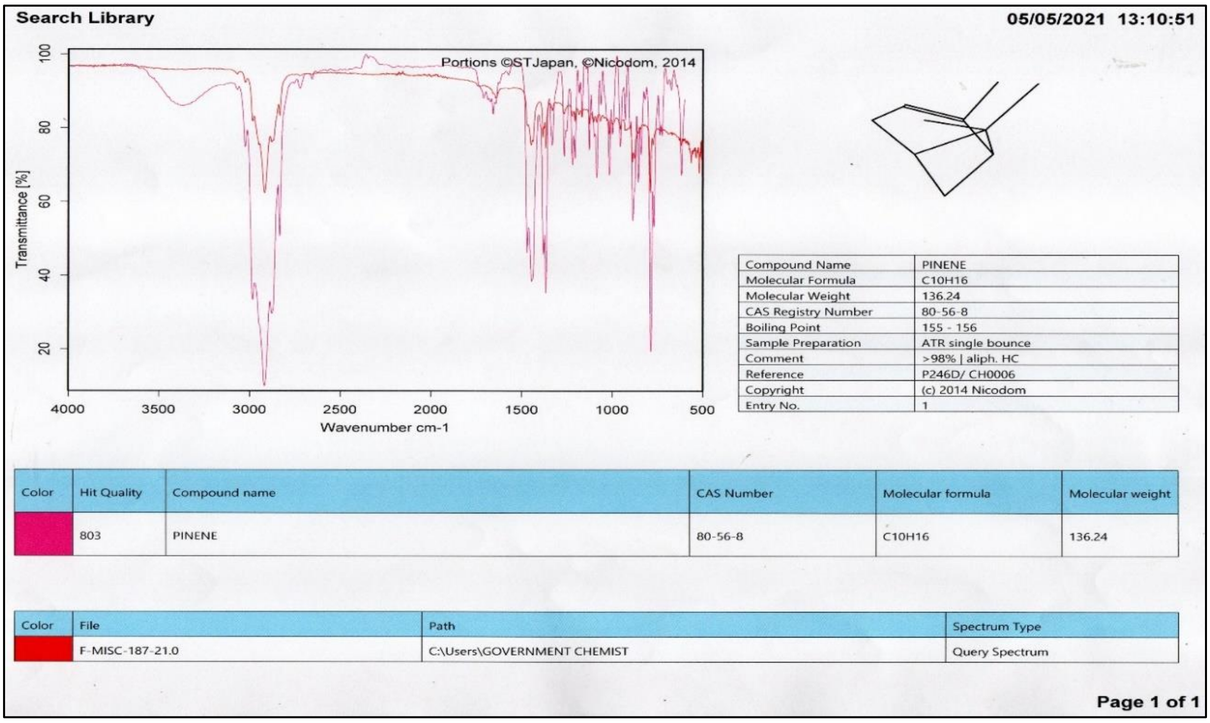

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Figure 4: A spectrum of (+)-Pinene

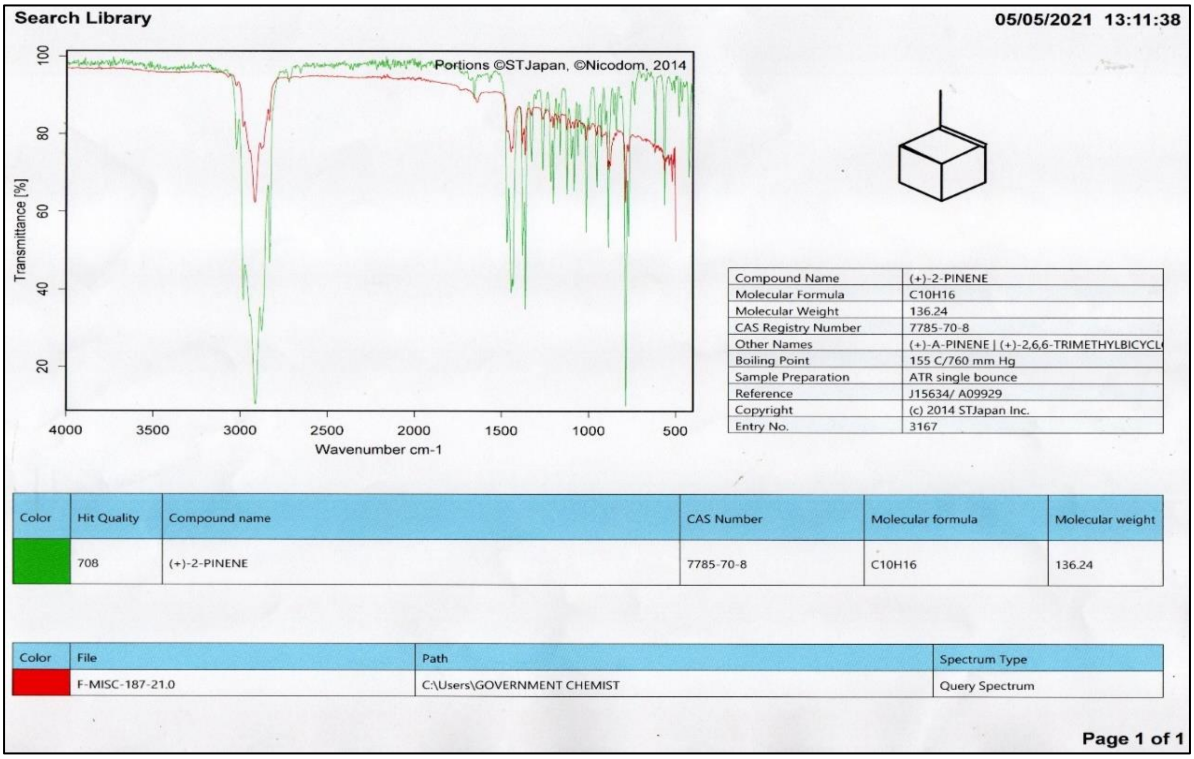

Table 1: Chemical constituents of the essential oil derived from *T. diversifolia* by ATR-FTIR analysis

Compound name	Hit quality	Major peaks Wave numbers	Molecular formula	Classification
(+)-2-Pinene	708	2800cm^{-1}	$C_{10}H_{16}$	Bicyclic monoterpene
Pinene	803	2800cm^{-1}	$C_{10}H_{16}$	Bicyclic monoterpene
(±)-α-Pinene	808	2800cm^{-1}	$C_{10}H_{16}$	Bicyclic monoterpene

The major peaks at 2800 cm^{-1} correspond to –C-H stretching in alkanes. These findings from the research agree with the findings of Sousa et al. (2019) that found that the analysis of essential oils from *T. diversifolia* constituted 88.2% monoterpene (Sousa et al., 2019).

GC-MS Analysis of *T. diversifolia* Plant Essential Oils

The essential oils from *T. diversifolia* leaves revealed several peaks representing different compounds as shown in the total chromatogram by Gas Chromatography-Mass Spectrometry analysis. The peaks in the chromatogram were integrated and were compared with the database of the spectrum of known components stored in the Gas Chromatography-Mass Spectrometry library and the peak area determined using the cut and weigh method. Gas Chromatography-Mass Spectrometry Analysis of the essential oil leaves revealed the presence of different bicyclic monoterpenes, sesquiterpenoids, cyclic monoterpenes and fatty alcohol. The results of GC-MS are described in *Table 2* below. The constituent compounds of *T. diversifolia* identified by GC-MS were drawn using chemsketch software.

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Figure 5: A GC-MS spectrum of the essential oils from *T.diversifolia*

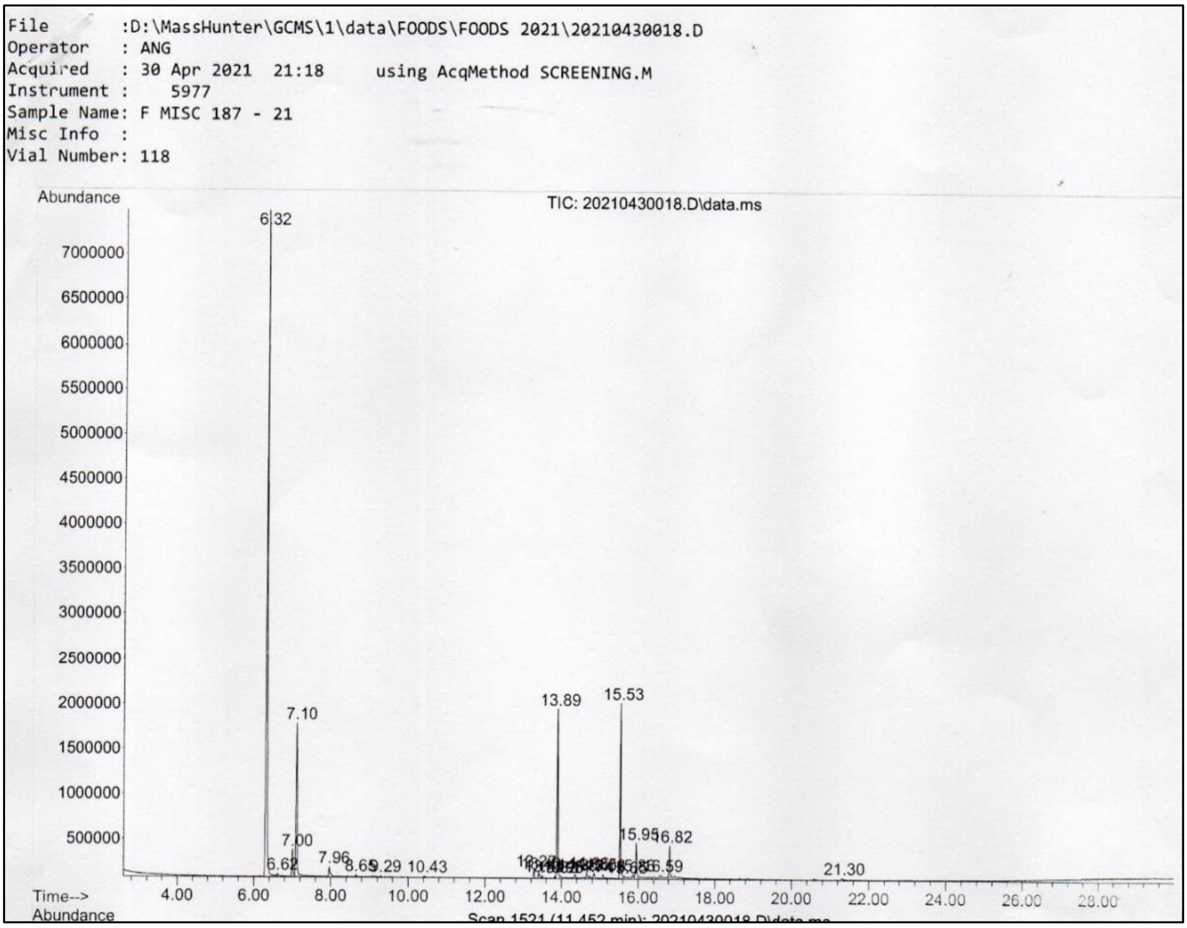
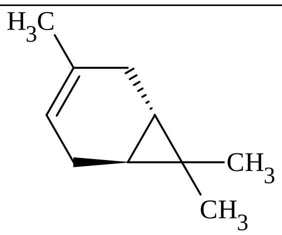
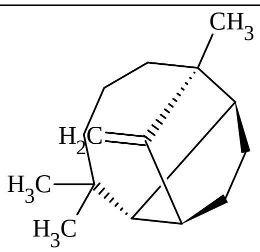
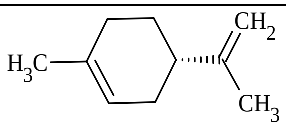


Table 2: Chemical constituents of the essential oil derived from T.diversifolia by GC-MS analysis

Compound name	Retention time	%	Molecular formula	Molecular structure	Classification
3-carene	6.3189	64.8%	$C_{10}H_{16}$		Bicyclic monoterpene
1,4-methanoazulene (junipene)	13.8892	16.7%	$C_{10}H_{16}$		Sesquiterpenoid
Limonene	7.9626	15.4%	$C_{10}H_{16}$		Cyclic monoterpenes

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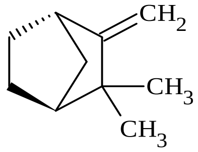
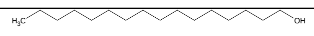
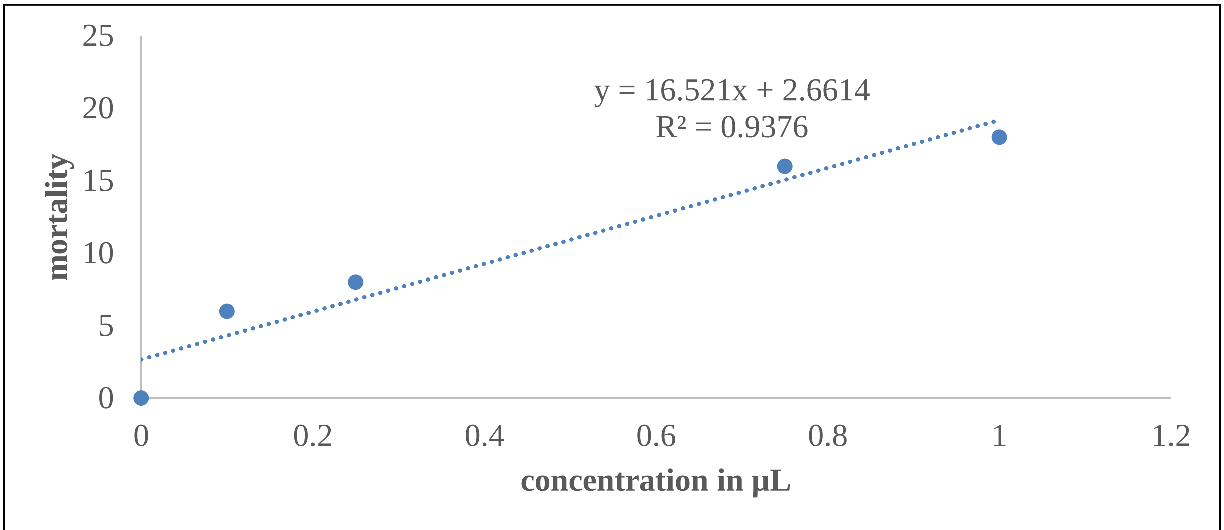
Compound name	Retention time	%	Molecular formula	Molecular structure	Classification
Camphene	6.6144	2.5%	$C_{10}H_{16}$		Bicyclic terpene
1-hexadecanol	14.6522	0.1%	$C_{16}H_{34}O$		Fatty alcohol

In this research conducted in western Kenya, GC-MS analysis of essential oils from *T. diversifolia* showed that they constituted 54% monoterpenes and 46% sesquiterpenoids. Miranda et al. (2016) confirm that limonene is one of the major constituents of essential oils. The findings of this research are in agreement with previous research findings.

LD_{50} of T. diversifolia against A. gosypii

LD_{50} for A. *gosypii* was calculated as 0.089 µL. 0.089 µL led to the death of 50% of *A. gosypii*. A correlation of $R^2 = 0.9376$ showed there is a strong relationship between the increase in the concentration of *T. diversifolia* essential oils and the percentage mortality of *A. gosypii*. *Figure 6* represents a curve that was obtained from the Probit analysis.

Figure 6: Probit analysis between *T. diversifolia* and the mortality of *A. gosypii*



LD_{50} of T. diversifolia against T. Tabaci

LD_{50} for *T. tabaci* was calculated as 0.085 µL. 0.085 µL of *T. diversifolia* essential oils led to the death of 50% of *T. tabaci*. $R^2 = 0.8457$ showed there is a strong relationship between the increase in the concentration of *T. diversifolia* essential oils and the percentage mortality of *T. tabaci*. *Figure 7* below represents a curve that was obtained from the Probit analysis.

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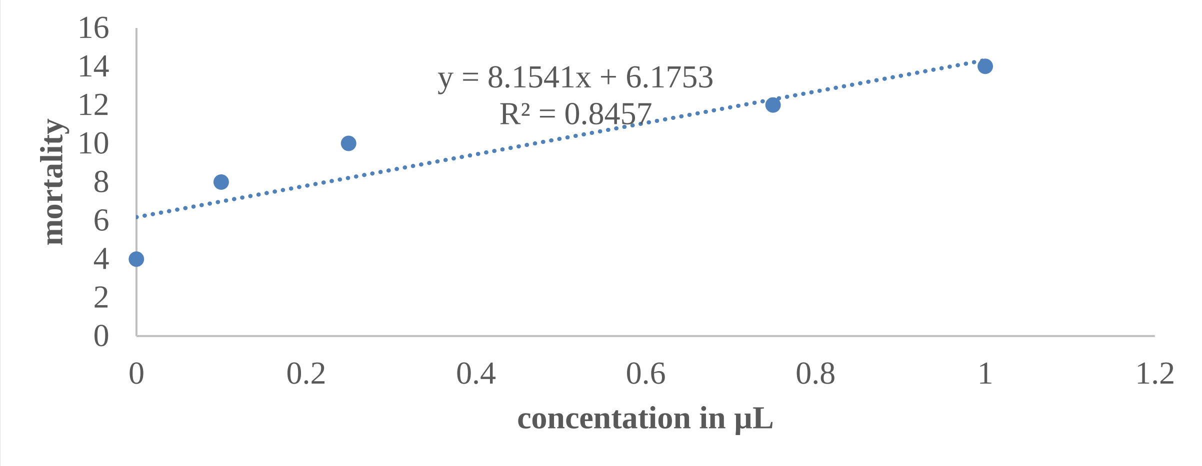
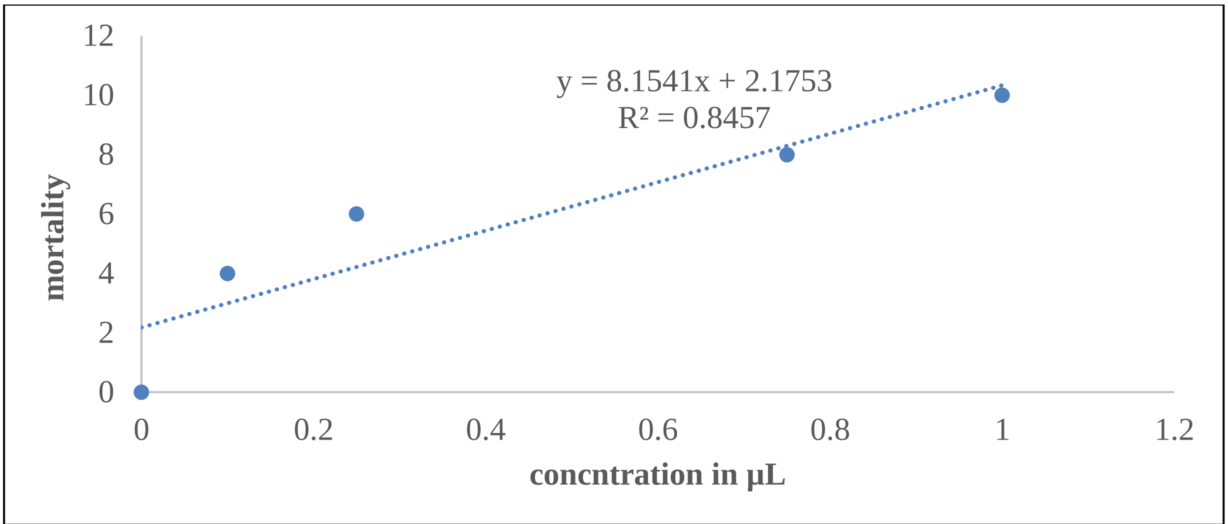
Figure 7: Probit analysis between *T. diversifolia* essential oils and mortality of *T. tabaci*



LD_{50} of T. diversifolia against B. tabaci

LD_{50} for *B. tabaci* was calculated as 2.490 µL. 2.490 µL of *T. diversifolia* essential oils led to the death of 50% of *B. tabaci*. R^2= 0.8457 showed there is a strong relationship between the increase in the concentration of T. diversifolia essential oils and the percentage mortality of *B. tabaci***.** Figure 8 below represents a curve that was from the Probit analysis

Figure 8: Probit analysis between *T. diversifolia* essential oils and the mortality of *B. tabaci*



The results indicated that *T. diversifolia* essential oils had the highest mortality against *T. tabaci* at LD_{50} of 0.085 µL and *A. gosypii* at LD_{50} 0.089 µL. The essential oils from *T. diversifolia* have the potential to be used to effectively manage *T. tabaci* and *A. gosypii*.

CONCLUSIONS

The *T. diversifolia* essential oils were found to be more effective against *T. tabaci* than the *A. gosypii* and *B. tabaci* at an LD_{50} was 0.085 µL. This is because the essential oils were effective at lower concentrations. The oils could therefore be used in the effective management of *T. tabaci* than *A. gosypii* or *B. tabaci* as a

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contact toxicant. The activity of the oils could be attributed to the presence of 3-carene, which is a leaf toxin with activity against predation by herbivores3-Carene is a bicyclic monoterpene. The essential oils from *T. diversifolia* have a high potential for use as a contact toxicant biopesticide against *T. tabaci* than *A. gosypii* and *B. tabaci*.

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DECLARATION OF COMPETING INTEREST

The authors declare that there are no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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