Chemical variation and implications on repellecy activity of *Tephrosia vogelii* (Hook f.) Essential oils against *Sitophilus zeamais* Motschulsky

N. Kerebba [a], A.O. Oyedeji [b], R. Byamukama [c], S.K. Kuria [d], O.O. Oyedeji[a],*

[a] *Department of Chemistry, University of Fort Hare, P/BagX1314, Alice 5700, South Africa*

[b] *Department of Chemical and Physical Sciences, Walter Sisulu University, P/BagX1, Mthatha 5117, South Africa*

[c] *Department of Chemistry, Makerere University, P.O. Box 7062, Kampala, Uganda*

[d] *Department of Biological and Environmental Sciences, Walter Sisulu University, P/BagX1, Mthatha 5117, South Africa*

*Corresponding Author email: ooyedeji@ufh.ac.za

Abstract

Chemical variability in the components of *T. vogelii* essential oils from eastern Uganda was identified using principal component analysis (PCA) and Agglomerative hierarchical clustering (AHC). Based on the profiles of the compounds of farnesene family three chemotypes were found: farnesol (chemotype 1), springene (β- Springene and α-Springene) and the β-Farnesene were distinctive in chemotype 2 and a mixed variety of farnesol and the Springene. In the three cases, alkybenzenes; o-xylene, m-xylene and ethylbenzene were significant components in the oil. 1,4-dihydroxy-p-menth-2-ene, 5,9-undecadien-2-one, 6,10-dimethyl, and 3-cyclohexen-1-carboxaldehyde,3,4-dimethyl were other prominent constituents. The yields of the essential oils did not vary significantly however the chemical composition varied with harvesting time during the rainy and dry seasons. In choice repellency tests, chemotype 1 and chemotype 2 were more active against *Sitophilus zeamais* than mixed chemotype. Farnesol was found to be effective only at a higher concentration as a repellent against *S. zeamais*. However, further study that aims to optimize and standardize the varieties and harvesting period needed for recommendation to smallhold farmers.

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Key words: Chemotypes, *Tephrosia vogelii*, pesticidal activity, *Sitophilus zeamais*, essential oils.

1. Introduction

Tephrosia vogelii (Hook f.) is a pesticidal plant, in Africa found mainly in the tropics [1]. It is also called fish poison [2]. It is a soft woody branching herb with dense foliage and can grow up to 0.5-4.0 m tall [3] It occurs in climates with an annual rainfall of 850-2650 mm, annual mean temperature of 12.5-26.2^{o}C and is found up to 2100 m above sea level[3]. Attempts have been made in eastern and southern Africa to promote *T. vogelii* for wider application as a pesticide source through earlier reports [4]-[5]. Previous reports however indicate that some plants like *Tephrosia vogelii* (Hook f.) and *Lippia javanica* (Burm. f.) can exhibit an extreme variation of bioactive principles from the same species [6]-[7]. [6] showed that some crude extracts of the leaves of *T. vogelii* possessed rotenoids and were thus pesticidal materials (chemotype1) while others are non-pesticidal due to lack of rotenoids (chemotype 2). This variation was thus based on the profiles of the flavonoids. Additionally, recent reports have indicated three chemotypes of *T. vogelii* materials from East Africa (Kenya and Tanzania) through phytochemical analysis [8]. These variations in the phytocompounds would affect the pesticidal activities of these plants pausing limitation to their use which would slow their adoption. *T. vogelii* volatile extracts have not been widely exploited for insecticidal activities.

To the best of our knowledge, there had been no reports of chemical variation on the essential oils of *T.vogelii* species that exists. The aim of the study was to at investigate chemical variation in the constituents and composition of the essential oils *T. vogelii* collected from different locations in eastern Uganda and evaluate the implications this would have on pesticidal application against stored pest, *S. zeamais*.

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2 Materials and Method

2.1 Plant collection and sites

Collection of plant materials took place in Butaleja district, eastern Uganda. Two plant collection sites were considered for the study; Mazimasa sub-county, Nampologoma Parish, Muyago village and Kachonga sub-county, Kyadongo parish, and Kyadongo B village. Kachonga sub-county surrounds the Doho Wetland found in Mazimasa sub-county and the area is ten kilometres from Mbale Town (33° 55′ to 34° 05′ E and 0° 50′ to 1° 00′ N). The coordinates of the district are: 00 56’ N, 33 57’ E. The district (Butaleja District) area is approximately 653.1km^2. The altitude of the district ranges from 1050 m to 1100 m above sea level. Several tropical climate conditions with average temperatures between 16 ^{0}C and 29 ^{0}C occur due to different altitudes. The mean annual rainfall varies between 1500 mm to 1,750 mm and received within four months [9]. The bimodal rainfall peaks are; March-May and August-September [10]. The soil is sandy with low organic content although some clay soils transferred from neighboring volcanic mountain in Mbale district form along rivers [9].

2.2 Plant Materials and Botanical Identification

Different plant leaf materials of *T. vogelii* plant species were collected from Muyago and Kyadongo villages, Butaleja District, eastern Uganda. Leafy materials were collected from branches, air dried and stored. To determine the effect of geographical and seasonal variations in the existence of *T. vogelii* chemotypes, collection of plant materials was done within two major seasonal rainfall patterns in the district: rainy season (March-May, and August-September) and dry season (January and June-July) (Table 1) from two different villages.

Table 1: Location of sampling points

Village (sample)	Flower color	Location Latitude North	Longitude East	Sampling date	Altitude (m)
Muyago (TV1 muya)	White	0°84′20″	34°03′15″	14/05/2017	1080
KyadongoB (TV1 kya)	White	0°90′14″	34°08′30″	1/06/2017	1098
Kyadongo B (TV1 kyb)	White	0°90′30″	34°09′45″	1/06/2017	1098
Kyadongo B (TV1 kyc)	White	0°80′10″	34°08′40″	1/06/2017	1098
Muyago (TV2 muya)	white	0°84′14.9″	34°03′10″	15/08/2017	1080
Kyadongo B (TV2 kya)	white	0°70′30″	34°07′35″	15/08/2017	1098
Kyadongo B (TV2 kyb)	white	0°90′14″	34°08′30″	15/08/2017	1098
Kyadongo B (TV2 kyc)	white	0°90′20″	34°09′40″	15/08/2017	1098
Kyadongo B (TV2 kyd)	white	0°90′35″	34°08′45″	15/08/2017	1098
Muyago (TV3 muya)	white	0°84′00″	34°02′45″	01/03/2018	1090
Muyago (TV3 muyb)	white	0°84′14″	34°03′09″	01/03/2018	1090
Muyago (TV3 muyc)	white	0°84′14.9″	34°03′10″	01/03/2018	1090
Kyadongo B (TV3 kya)	white	0°90′14″	34°08′30″	01/03/2018	1098
Kyadongo B (TV4 kya)	white	0°70′30″	34°07′35″	10/01/2019	1098
Kyadongo B (TV4 kyb)	white	0°90′14″	34°08′30″	10/01/2019	1098
Kyadongo B (TV4 kyc)	white	0°90′20″	34°09′40″	10/01/2019	1098
Muyago (TV4 muya)	white	0°84′00″	34°02′45″	10/01/2019	1090
Muyago (TV4 muyb)	white	0°84′14″	34°03′09″	10/01/2019	1090
Muyago (TV4 muyc)	white	0°84′14.9″	34°03′10″	10/01/2019	1090

Rain season sampling was during March, May and August. Dry season sampling was between January and June,

The distance between Muyago and Kyadongo villages is about 7 km. The collected plant species were identified by a senior Botanist Rwaburindori Protase at the Department of Botany, Makerere University. The voucher specimen was deposited at Makerere University Herbarium and the voucher name, number and GPS; Kerebba N. No 1- *Tephrosia vogelii* Hook. f. (Leguminosae) (Access No. MHU 50735), were deposited at the Herbarium.

2.4 Extraction and analysis of essential oils

2.4.1 Extraction

Each sample of plant leaf materials (20g) was hydro-distilled for 4hrs using a Clevenger apparatus set up as prescribed by British pharmacotia for essential oils. The oils were collected using a Pasteur pipette and dried using anhydrous sodium sulphate. The dry oil was then put in a small weighed dark brown bottle (5mL) and refrigerated at 4 ^{0}C for analysis. For pesticidal evaluation, more masses of the sample were hydro-distilled.

2.4.2 Identification and quantification of compounds

2.4.2.1 Synthetic chemicals

Ethylbenzene (> 99.8%), (±)-linalool (> 95.0%), 2-undecanone (95%), o-xylene(≥ 99.0%), p-cymene (> 95.0%) and R-(+)-limonene (> 98.0%), undecanoic acid (> 99.0%) were purchased from SigmaAldrich (Gillingham, Dorset, UK). n-decane (> 99.0%) was bought from BDH chemicals. α-Terpeneol (98.0%) was purchased from Fisher Scientific, (Loughborough, Leicestershire, UK). A mixture of xylene isomers (o-xylene and m-xylene) was purchased from pronalys while α-pinene was purchased from B.C. Treatt &co. Ltd. (E)-Beta-farnesene (≥ 98%) and Trans (2E,6E)- farnesol (98.0%) standards were purchased from career henan chemical co. China.

2.4.2.2 Identification of compounds with Gas Chromatography (GC)

GC analysis was done using Brunker 300 Gas Chromatograph equipped with FID detector and ZB-5 column (30 m in length × 0.25 mm i.d × 0.25 µm film thickness). The carrier gas was hydrogen at a flow rate of 1.0 mL/min and inlet pressure 52.6 Kpa. The column oven temperature was programmed to 50-250 ^{o}C at the rate of 3.0 ^{o}C/min. injector and detector temperature were set at 250 ^{0}C, volume injector 1.0 µL of the oil; split ratio 1:5. Peaks were measured by electronic integration. n-alkanes of C_8 to C_{30} were run under the same condition for Kovats indices determination [11].

2.4.2.3 Identification and quantification of volatile constituents by Gas Chromatography-Mass spectrometry (GC/MS)

The essential oil was analyzed by a Bruker 300-MS along with the 431-GC and CP-8400 Autosampler (quadrupole mass spectrometer) equipped with a ZB-5 capillary column (30 m length × 0.25 mm i.d × 0.25 µm film thickness). The oven temperature was programmed from 50 ^{o}C – 250 ^{o}C at the rate of 3.0 ^{o}C/min, electron ionization was at 70 eV. Helium was

used as the carrier gas at a flow rate of 1.0 mL/min. Injector and detector temperature were set at 280 ^{o}C, split ratio 1:5. 1.0 µL of the diluted oil in hexane was injected into the GC/MS. Compounds in the essential oil were identified by matching their Kovats indices and mass spectra with the ones recorded in WILEY NIST 11 library and by comparing them with literature values [12], where possible authentic compounds were co-injected. To quantify the amount for constituents in the oil, standard solutions of 5, 10, 20, 40 and (70≤ Y≤ 100ppb, Y, is based on the equivalent 5 or 10µL stock of compound used due to different densities) for the linear regression curves were prepared from synthetic reference materials. These were run on the same day of the sample analysis and regression equations obtained by plotting peak areas against the concentration levels. For compounds whose standards were available, quantification was done. For unavailable standards, compounds were grouped into chemical classes (hydrocarbons, alkylbenzenes, aldehydes, alcohols, etc.) and subclasses (monoterpenes, sesquiterpenes, oxygenated monoterpenes etc.) and a semi-quantification approach was carried out using one (or more) reference standard per group. The compound composition was then expressed as percentage peak area i.e.

Constituent percentage peak area= (Xs)*100/(1000 x R)

where Xs is the constituent concentration of with respect to its peak area (ppb/µg mL^{-1}) relative to peak area in the injection volume (1µL =1000ppb), and R is the recovery (R was taken as 100% since average recovery on spiking was 93.1±9.8, n=11).

2.5 Principal component analysis (PCA) and Agglomerative hierarchical clustering (AHC) of major chemical components from oils of T. vogelii species

PCA and AHC were performed on the data to group components and samples into clusters using statistical software SPSS for Windows version 25. PCA is a statistical tool that aims to represent the variation present in the data. It allows similarities and differences between data to be seen easily. During PCA, values in the loadings matrix were obtained through the

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transformation of data from correlated to new uncorrelated variables called principal components [13]. PCA was performed on a combined set of data from the two locations giving 19 samples × 23 variables for PCA. Analysis followed the standardization of data using Varimax rotation. Factor loadings generated indicate the correlations of each chemical constituent with its corresponding component. Loading scores which were greater than 5% of the variance of a given variable were considered however only loadings higher than an absolute value of 0.23 were considered meaningful throughout the analysis.

AHC is an algorithm that brings together related objects into clusters. The clustering makes it easier to see the correlations. The endpoint is having clusters that are distinctive other cluster and the objects with in a cluster are very similar. AHC based on the Euclidean distance was used to analyse seasonal and geographical influence on the yield and composition of the samples of *T. vogelii*. Finally, the classification of samples was done based on the composition and chemical constituents.

2.6 Pesticidal evaluation

The repellency and fumigant toxicity for the different varieties were evaluated for selected samples (Tv1kyb, Tv4kyc and TV4muyc).

2.6.0 Rearing of weevils

Plastic containers were used to bleed colonies of the *S. zeamais*. Initial stocks of *weevils* was obtained from infected maize from a market in Mthatha, Eastern Cape province of South Africa. Culturing occurred at 25-29 °C, 60 ± 5% RH and a photoperiod of 12:12 dark: light.

2.6.1 Repellence bioassay against S. zeamais

Repellence assay was done using the area preference method [14]. Here Petri dishes (9.0 × 1.2) cm and discs of filter paper half (31.8 cm^2) were used. Different levels of the test

solutions; 1, 5 and 10 µL/mL of essential oils corresponding to 0.03, 0.16 and 0.31 µL of oil per cm^3 respectively were used to check for the repellent potential of essential oils. Whatman filter papers were cut into two halves. To one half was applied the oil treatment uniformly using a micropipette. To the other half was a control treatment of 1.0 mL of hexane. Both treated halves were allowed to dry so that the solvent could evaporate completely. The halves were then attached with cellophane tape in a manner that would avoid the seepage of the test samples from one disc to another and placed at the bottom of each petri dish. Thirty mixed sex-adult *S. zeamais* were released at the center of each disc and the petri dish was covered and kept in the dark at 25 to 29.5 ^{0}C. Three replicates were done for each test solution of the essential oil. The numbers of the weevils in both the treated and untreated filter paper disc were counted after 1, 12, 24, 48 and 72 hours.

Percentage repellency (PR) was calculated using the formula in equation 1:

$$PR(\%) = \frac{C-T}{C+T}, \quad (1)$$

C = insect number found on untreated half,

T = insect found on treated half

Preference index (PI) obtained using the formula in equation 2:

$$PI = \frac{A-B}{A+B}, \quad (2)$$

A= Percentage of insects in treated halves,

B = percentage of insects in untreated halves

The experiments were repeated twice with three replicates each time and separate controls were set in all the replicates in a completely randomized design. The data for the treatment

means were compared using analysis of variance (ANOVA) and separated by the Fisher LSD test at $P < 0.05$, after being $\log_{10}$ transformed using statistical software SPSS for Windows version 25 for heterogeneity correction. Data was presented as mean percentage repellency± SEM (SEM is a standard error of the mean)

3. Results and discussion

3.1 Chemical constituents and composition of essential oils

Yellow distillates whose percentage yield ranged between 0.18±0.01% to 0.22±0.01% (w/w) dry weight for samples from Kyadongho B (Table 2) and 0.16±0.00% to 0.22±0.01% (w/w) dry weight for Muyago samples (Table 3) was obtained from the hydro distillation of leaf materials.

Table 2: Mean peak area (%±SEM) composition of major chemical constituents identified in the essential oils of *T. vogelii* samples from Kyadogho B

Compounds (RT, KI)[1]	**TV1 kya**	**TV1 kyb**	**TV1 kyc**	**TV2 kya**	**TV2 kyb**	**TV2 kyc**	**TV2 kyd**	**TV3 kya**	**TV4 kya**	**Tv4 kyb**	**Tv4 kyc**	**Ident meth**
Ethylbenzene (4.753, 878)	0.2 (0.0)	4.0 (0.4)	0.6 (0.3)	0.4 (0.1)	1.4 (0.4)	0.2 (0.0)	0.5 (0.0)	0.7 (0.1)	nd	nd	nd	MS/K CI
o-Xylene (4.962, 867)	1.1 (0.0)	29.4 (1.6)	3.2 (2.1)	2.1 (0.3)	9.0 (1.7)	1.1 (0.1)	3.4 (0.6)	6.7 (2.3)	nd	nd	nd	MS/K CI
p-Xylene (5.448, 887)	0.9 (0.0)	nd	1.6 (0.6)	nd	nd	nd	1.5 (0.1)	nd	nd	nd	nd	MS/K
m-Xylene (5.539, 896)	0.3 (0.0)	25.0 (1.2)	3.6 (1.6)	1.0 (0.0)	5.1 (0.8)	0.3 (0.0)	2.1 (0.1)	3.3 (0.6)	nd	nd	nd	MS/K CI
Ethanol,2-butoxy- (5.776, 895)	0.3 (0.0)	2.7 (0.1)	3.4 (0.9)	0.6 (0.2)	1.0 (0.2)	0.2 (0.0)	2.9 (0.3)	1.1 (0.2)	nd	nd	nd	MS/K
D-(+)-Alpha-pinene (6.811, 931)	0.7 (0.1)	1.7 (0.1)	0.7 (0.1)	0.5 (0.1)	1.0 (0.2)	nd	0.5 (0.1)	nd	0.4 (0.1)	0.9 (0.2)	nd	MS/K CI
D-limonene (10.419, 1031)	nd	0.2 (0.0)	0.2 (0.0)	0.8 (0.4)	0.2 (0.0)	nd	0.1 (0.0)	nd	nd	0.1 (0.0)	nd	MS/K CI
Linalool (13.514, 1102)	nd	1.8 (0.0)	nd	nd	nd	nd	nd	nd	nd	nd	nd	MS/K CI
(E,E)-Cosmene (15.875, 1132)	nd	nd	nd	nd	nd	nd	nd	nd	0.2 (0.1)	0.6 (0.1)	0.5 (0.0)	MS/K
5,9-undecadien-2-one, 6,10-dimethyl (28.883, 1453)	nd	0.8 (0.2)	0.9 (0.2)	1.2 (0.4)	1.3 (0.0)	0.3 (0.0)	nd	nd	0.2 (0.0)	0.5 (0.0)	1.1 (0.1)	MS/K
Isocaryophyllene (30.511, 1409)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.2 (0.0)	MS/K
(E)-Nerolidol (33.432, 1564)	nd	0.2 (0.0)	nd	nd	0.2 (0.0)	0.2 (0.0)	nd	nd		nd		MS/K

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(-)-Spathulenol (33.959, 1566)	nd	0.2 (0.0)	0.2 (0.0)	0.3 (0.0)	0.3 (0.0)	0.2 (0.0)	nd	nd	0.2 (0.0)	nd	0.2 (0.0)	MS/K
3-cyclohexen-1-carboxaldehyde,3,4-dimethyl (34.520, 1492)	nd	nd	nd	nd	2.9 (0.6)	nd	nd	nd	nd	nd	nd	MS/K
Cis-p-metha-1(7)-8-dien-2-ol (35.208, 1233)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.2 (0.0)	MS/K
Isoaromadendrene epoxide (37.465, 1577)	nd	0.2 (0.0)	nd	nd	nd	nd	nd	0.2 (0.0)	0.3 (0.0)	0.3 (0.0)	0.7 (0.3)	MS/K
1,4-dihydroxy-p-menth-2-ene (37.767, 1243)	nd	0.2 (0.0)	0.4 (0.0)	nd	1.4 (0.2)	nd	nd	nd	nd	1.1 (0.2)	0.9 (0.1)	MS/K
β-Farnesene (39.188	0.3 (0.0)	nd	nd	nd	0.3 (0.0)	0.8 (0.3)	nd	nd	nd	nd	nd	MS/K
Farnesol(E)-methylether (39.186, 1682)	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.0 (0.0)	nd	MS/K
Z, Nerolidol (39.204,1558)	nd	nd	nd	nd	nd	nd	nd	nd	0.9 (0.1)	nd	nd	MS/K
Farnesol*	nd	1.8 (0.2)	2.2 (0.7)	nd	nd	nd	0.3 (0.0)	0.4 (0.1)	nd	nd	2.9 (0.2)	MS/K CI
β- Springene (39.410, 1918)	nd	nd	nd	0.6 (0.1)	5.7 (2.9)	nd	nd	0.3 (0.1)	nd	nd		MS/K
α-Springene (40.793, 1731)	nd	0.2 (0.0)	0.2 (0.0)	nd	nd	nd	nd	nd	nd	nd	0.2 (0.0)	MS/K
Hexadecane (42.274, 1818)	0.2 (0.0)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	MS/K
Density (g/ml)	1.014 0.014	1.001 0.024	1.026 0.003	0.986 0.014	0.964 0.036	0.979 (0.043)	0.959 (0.013)	0.987 (0.013)	1.087 (0.016)	0.974 (0.002)	1.076 (0.049)	
Yield(%w/w)	0.19 (0.01)	0.21 (0.00)	0.20 0.01	0.18 0.01	0.20 0.01	0.19 0.04	0.18 (0.00)	0.19 (0.01)	0.22 (0.01)	0.19 (0.02)	0.21 (0.02)	

Data presented as mean (SEM)./desity (SEM). [1] RT= Retention time and KI = Kovats index relative to ZB-5 column KI is compared with [11] (90%KI). [2]M= mass spectrum matching with NIST library [10], KI= kovat index and CI= co-injection with standard

Table 3: Mean peak area (%±SEM) composition of major chemical constituents identified in the essential oils of *T. vogelii* samples from Muyago village

Compounds (RT, KI)[1]	**TV1 muya**	**TV2 muya**	**TV3 muya**	**TV3 muyb**	**TV3 muyc**	**TV4 muya**	**TV4 muyb**	**Tv4 muyc**	**Identification method[2]**
Ethylbenzene (4.753, 878)	2.7 (0.2)	2.4 (0.4)	nd	1.7 (0.3)	2.3 (1.6)	nd	nd	nd	MS/KI/CI
o-Xylene (4.962, 867)	17.6 (0.6)	22.1 (1.2)	0.9 (0.0)	8.6 (0.8)	23.4 (17.3)	nd	nd	nd	MS/KI/CI
p-Xylene (5.448, 887)	nd	nd	0.9 (0.0)	3.1 (2.0)	5.2 (3.4)	nd	nd	nd	MS/KI
m-Xylene (5.539, 896)	14.4 (0.2)	18.1 (2.7)	nd	5.3 (0.6)	6.7 (5.1)	nd	nd	nd	MS/KI/CI
Ethanol,2-butoxy- (5.776, 895)	nd	2.2 (0.7)	0.3 (0.1)	3.7 (0.9)	2.7 (0.1)	nd	nd	nd	MS/KI
D-(+)-Alpha-pinene (6.811, 931)	1.2 (0.0)	1.6 (0.3)	nd	1.5 (0.2)	1.8 (1.2)	0.4 (0.0)	0.6 (0.0)	nd	MS/KI/CI
D-limonene	0.2	0.3	nd	0.2	0.1	nd	nd	nd	MS/KI/CI

(10.419, 1031)	(0.0)	(0.0)		(0.0)	(0.0)				
Linalool (13.514, 1102)	nd	1.5 (0.3)	nd	nd	nd	1.0 (0.2)	nd	nd	MS/KI/CI
(E,E)-Cosmene (15.875, 1132)	nd	nd	nd	nd	nd	0.4 (0.2)	0.6 (0.2)	nd	MS/KI
5,9-undecadien-2-one,6,10-dimethyl (28.883, 1453)	0.7 (0.0)	0.6 (0.4)	Nd	0.7 (0.0)	nd	0.7 (0.4)	1.0 (0.2)	0.6 (0.2)	MS/KI
Isocaryophyllene (30.511, 1409)	nd	nd	Nd	nd	nd	0.3 (0.0)	0.2 (0.0)	0.4 (0.0)	MS/KI
(E)-Nerolidol (33.432, 1564)	nd	0.3 (0.0)	Nd	0.2 (0.0)	nd	nd	nd	0.2 (0.0)	MS/KI
(-)-Spathulenol (33.959, 1566)	0.2 (0.0)	0.3 (0.0)	Nd	0.2 (0.0)	0.3 (0.0)	nd	0.2 (0.0)	nd	MS/KI
3-cyclohexen-1-carboxaldehyde,3,4-dimethyl (34.520, 1492)	nd	nd	Nd	nd	3.4 (1.6)	nd	nd	nd	
Cis-p-metha-1(7)-8-dien-2-ol (35.208, 1233)	nd	nd	Nd	nd	nd	nd	nd	1.1 (0.0)	MS/KI
Isoaromadendrene epoxide (37.465, 1577)	nd	nd	Nd	0.8 (0.2)	nd	0.3 (0.0)	nd	nd	MS/KI
1,4-dihydroxy-p-menth-2-ene (37.767, 1243)	1.1 (0.0)	2.3 (2.1)	Nd	nd	nd	nd	1.3 (0.4)	nd	MS/KI
β-Farnesene (39.188, 1456)	nd	nd	Nd	nd	nd	nd	nd	0.8 (0.5)	MS/KI
Farnesol(E)-methylether (39.186, 1682)	0.8 (0.0)	nd	Nd	nd	nd	nd	nd	0.2 (0.0)	MS/KI
Farnesol*	nd	6.3 (1.3)	Nd	4.5 (2.1)	5.9 (1.7)	nd	1.2 (0.8)	0.2 (0.0)	MS/KI/CI
β- Springene (39.410, 1918)	nd	0.2 (0.0)	0.2 (0.1)	0.2 (0.0)		2.0 (0.3)	0.9 (0.1)	2.0 (0.1)	MS/KI
α-Springene (40.793, 1731)	nd	nd	Nd	nd	0.2 (0.0)	nd	nd	0.2 (0.0)	MS/KI
Hexadecane (42.274	0.2 (0.0)	0.5 (0.1)	Nd	0.3 (0.1)	nd	nd	nd	nd	
Density	0.988 (0.038)	0.988 (0.012)	0.942 (0.001)	0.973 (0.000)	0.973 (0.027)	1.030 (0.030)	1.000 (0.000)	1.017 (0.017)	
Yield(%w/w)	0.20 (0.01)	0.22 (0.01)	0.16 (0.00)	0.18 (0.00)	0.18 (0.00)	0.18 (0.00)	0.17 (0.01)	0.18 (0.01)	

Data presented as mean (SEM)./desity (SEM). [1] RT= Retention time and KI = Kovats index relative to ZB-5 column KI is compared with [11] (90%KI). [2]M= mass spectrum matching with NIST library[10], KI= kovat index and CI= co-injection with standard

The densities of these oils were between 0.959±0.013 and 1.087±0.016 g/mL for oils extracted from Kyadongo B samples and for Muyago samples, it varied between 0.942±0.001g/mL and1.030±0.030g/mL. The compositions were expressed as mean peak areas (%±SEM) of the major compounds quantified from sample sites of the two study areas.

The highest composition representation came from the alkylbenzenes; Ethylbenzene, o-xylene, p-xylene and m-xylene. Among these, the composition of o-xylene was highest and varied between nd - 29.4±1.6% in samples from Kyadong B village and n.d-23.4±17.3% for Muyago village. This was closely followed by m-xylene with nd- 25.0±1.2% (Kyadongho B village) and nd- 18.1±2.7% (Muyago village) range and finally the ethylbenzene; n.d – 4.0±0.4% (Kyadong B) and nd to 2.7±0.2% (Muyago) in this category. There was a significant amount of farnesol, varying between nd to 6.3±1.3% (Muyango) and nd to 2.9± 0.2% (Kyadongho B). β- Springene was another major compound with the composition of between nd to 2.0±0.3% (Muyago samples) and nd to 5.7±2.9% (Kyadongho B samples).

Chemotypes of *T. vogelii*

To determine the correlation of major components between various *T. vogelii* samples, PCA and AHC were performed on the data. Principal component analysis led to a total of 8 factors extracted (i.e. whose eigenvalues were greater than unity) and the loading scores were generated. These components could explain about 88% of total variance. Multiple linear regression (MLR) of the elements in the factor score matrix was carried out against the total composition for the data to estimate the contribution of each major component in the chemotype on total component composition of the samples. Significance of the regression coefficients (R^2 = 0.95, observations; N = 19) was at 95% confidence level ($p < 0.05$). The regression results based on 8 factor scores showed that components: 4 (p = 0.35), 5 (p = 0.63), 6 (p = 0.25), 7 (p = 0.96) and 8 (p = 0.33) did not significantly influence the component composition. Therefore, the components were reduced to 3 factors, which explained 55% of the total variance. PC1 could describe about 30% while PC2 about 14% and finally PC3, 11% of the total variance. The MLR equation (R^2 = 0.95, ANOVA significance f < 23.43, p < 0.05) was as follows: Total composition = 19.39SC1 +

7.70SC2+1.92SC4+19.4, where SC1, SC2 and SC4 are factor scores for samples on component 1, 2 and 3 respectively.

Graphical representation of the principal components (Figure 1) reveal three chemical groupings. The first category shows that some samples were majorly farnesol (Fnso) type (Chemotype 1). Farnesol was detected in the following samples: TV1kyb, TV1kyc, TV1 muya, TV2muya, TV2kyd, TV3muyc, TV4kya, TV4kyb, TV4kyc, where in some cases it was one of the major components. Among other compounds in this grouping were: Ethanol, 2-butoxy-(E2B) and D-(+)-Alpha-pinene (αP). Not all these other compounds could be detected in each of these samples as was the case of farnesol. The second grouping was majorly springene compounds (βspringene (βS) and α-springene (αSp)) and the β-farnesene (βF) which was referred to as Chemotype 2. There is positive correlation between β-springene and β-farnesene (Pearson correlation, r=0.3), α-springene and β-farnesene (Pearson correlation r=0.2). However there is no positive correlation between either β-springene or β-farnesene with farnesol signifying a different chemical grouping. β-springene, α-springene and β-farnesene were detected in the rest of the samples other than those detected in farnesol above (TV3muyb, TV3muya, TV2kyb, TV2kya TV4muya and TV4muyc, Tv2kyc and Tv1kya). The other compounds in this Chemotype 2 were: (*E*)-nerolidol(EN), cis-p-metha-1(7)-8-dien-2-ol(zMD), D-limonene(Dl), 1,4-dihydroxy-p-menth-2-ene (Dm) 5,9-undecadien-2-one,6,10-dimethyl (UD), and farnesol(E)-methylether (FnsoEm). β-springene was the most represented in this category since it was encountered in six samples out of a total of 19 samples (TV3muyb, TV3muya, TV2kyb, TV2kya TV4muya and TV4muyc). β-farnesene was detected in 2 samples (Tv1kya and Tv2kyc). All samples, however, were dominated by the alkylbenzenes; ethylbenzenes and xylene isomers for either in abundance or trace amount (treated as non-detectable). There was minimum composition of the mixture of farnesene compounds (farnesol, β-springene and α-springene and β-farnesene) with huge

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amount of alkylbenzenes which formed the mixed chemotype for example in samples like Tv4muyb and Tv3kya. There is a large correlation between alkylbenzenes and farnesol (Pearson correlation, r >0.4, p<0.05, for all alkylbenzene) and also a correlation between α-springene and farnesol (Pearson correlation, r >0.4, p<0.05).

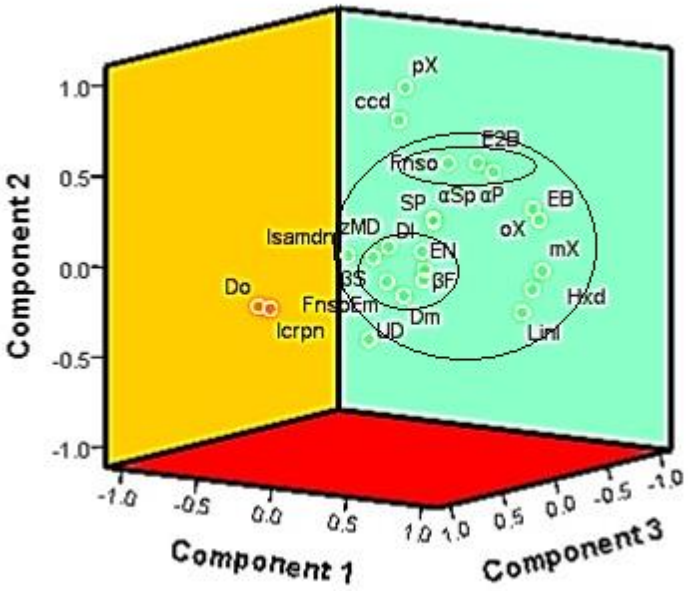

Figure 1: Three dimensional scatter plot of different correlations of chemical components using principal component analysis

Figure 2 shows the major hierarchical clustering classification of major components in the oil. Farnesol, β-springene, α-springene, β-farnesene could form separate clusters affirming the above observations. Acluster of ethylbenzene, o-xylene and m-xylene was formed. The hydrocarbon cluster was the first in the dendrogram. Thus the compounds within this cluster are broadly similar to each other. These arguments are in line with those suggested by [8] who obtained three chemotypes from the *T. vogelii* samples from east Africa; Kenya, Tanzania and Malawi using phytochemical analysis. Chemotype 1 had rotenoids, chemotype 2 lacked rotenoids but had flavanones and flavones while chemotype 3 had a hybrid chemical profile of the chemotype 1 and chemotype 2.

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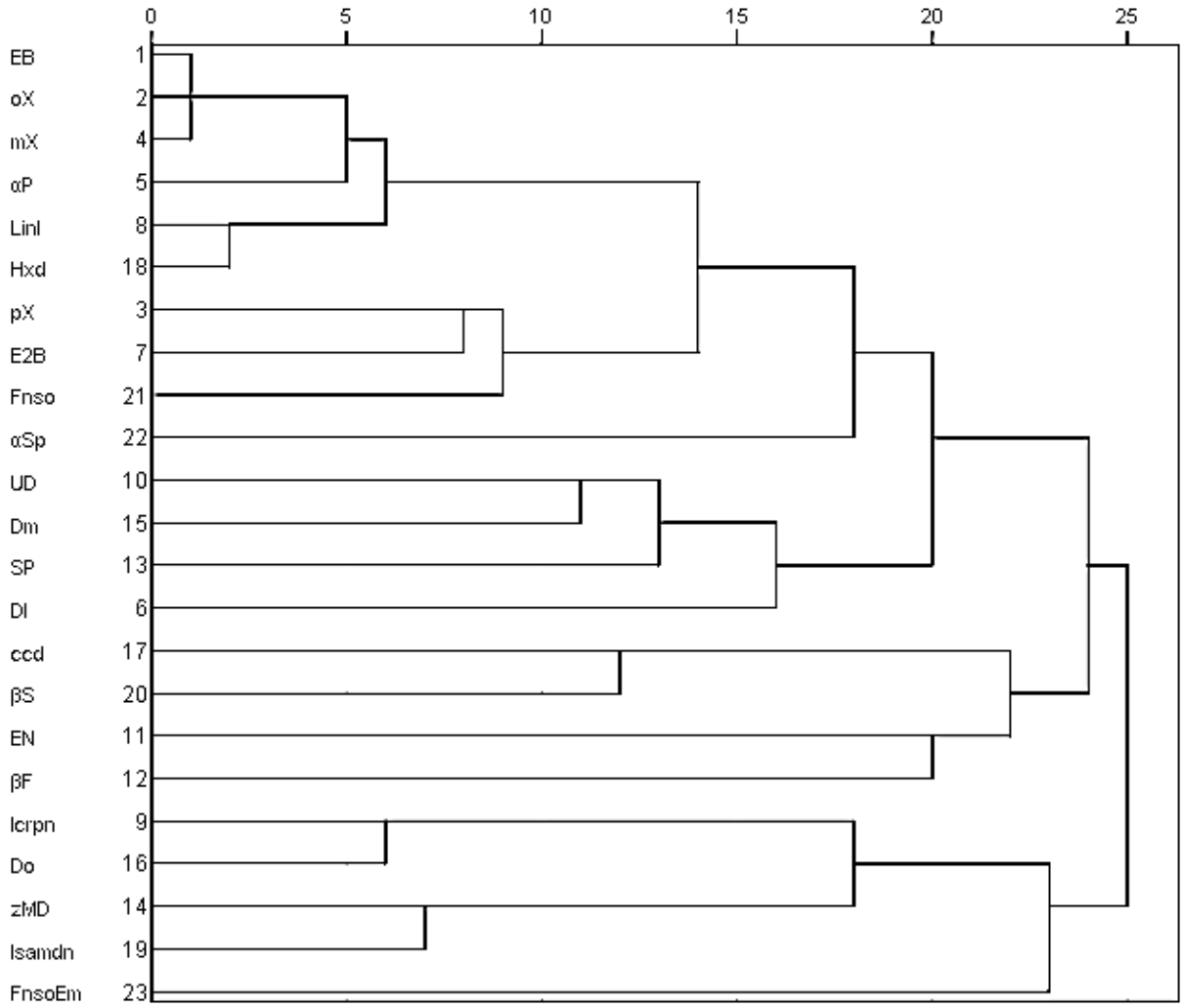

Figure 2: Dendrogram of major components obtained based on the classification of the samples of *T. vogelii* during the two harvest periods

3.2 Effect of season variation on the percentage yield and major composition of the oils

Two seasons variations were considered in the study: rain season and dry season represented with the green pattern and red pattern respectively (Figure 3). The rainfall bimodal peaks in the district occur between March to May and August- September. Samples obtained during this time were: Tv1muya, Tv2muya, Tv3muya, Tv3muyb, Tv3muyc, Tv2kya, Tv2kyb, Tv2kyc, Tv2kyd, and Tv3kya. During the dry season, harvest occurred in January and June and these samples were: Tv1Kya, Tv1kyb, Tv1kyc, Tv4kya, Tv4kyb, Tv4kyc, Tv4muya,

Tv4muyb, and Tv4muyc. Considering this, there was no significant difference between the percentage oil yield between the two seasons and from the two sampled areas.

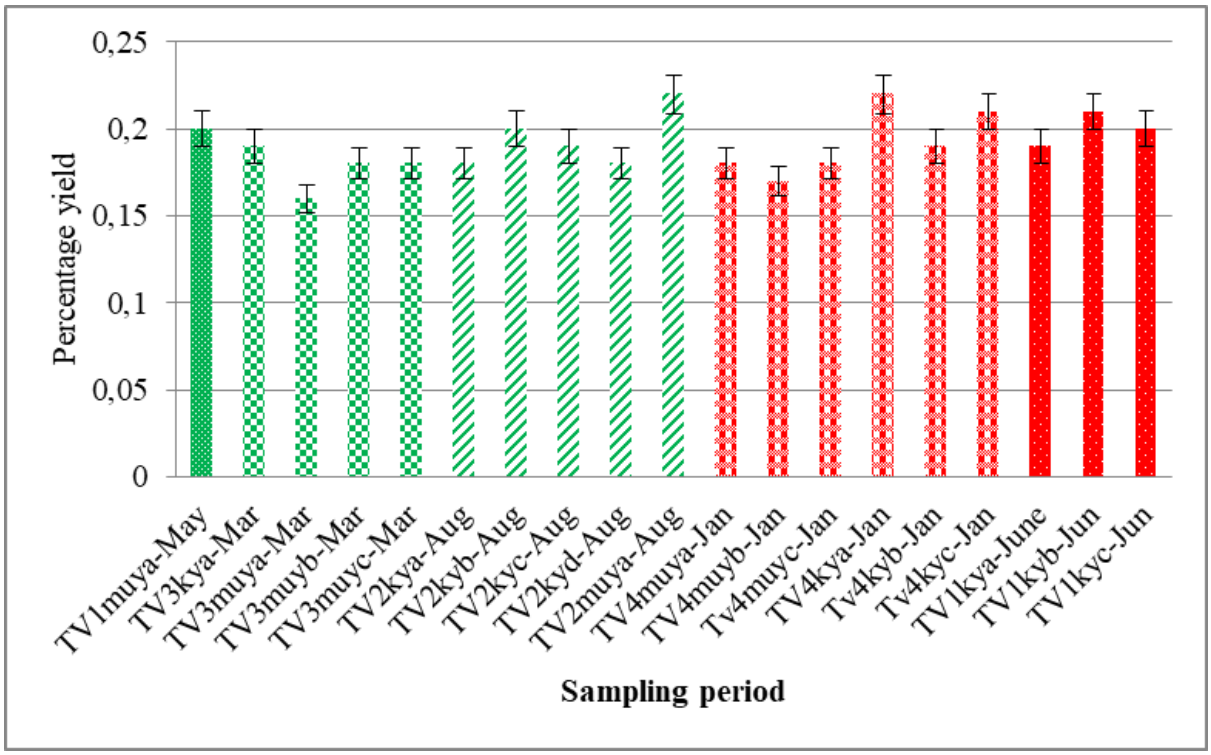

Figure 3: A continuous histogram depicting the percentage yield of the oils from samples of *T. vogelii*

The effect of seasonal variation on the constituents and composition of the samples was done using cluster analysis of the constituent composition in the samples and several clusters were formed (Figure 4). Cluster 1 was majorly for the composition of samples taken during rainy season; March-May and august (Tv1muya, Tv2muya, Tv3kya) and one sample of the dry season, June (Tv1kyb). Cluster 2 and 4 were compositions for the samples during rainy season sampling i.e cluster 2-Tv3muyb and Tv3muyc) were for march sampling, Cluster 4-Tv2kya and Tv2kyb for august sampling. In addition, Tv3muya that formed Cluster 6 was sampled in March thus during rainy season. Cluster 3 and 5 were for major compounds of the samples from dry and rain season (Tv1kyc and Tv2kyd for cluster 3 and Tv2kyc and

Tv1kya for cluster 5). And finally samples of cluster 7 (Tv4muya and Tv4muyc), cluster 8 (Tv4kyc and Tv4muyb), cluster 9 (Tv4kyb) and cluster 10 (Tv4kya) were obtained the dry season (January). These correlations indicate major seasonal effects on the composition of the major constituents. However compounds like the ethylbenzene, o-xylene and m-xylene could not be detected in the samples that were picked in January and but were observed in samples for June. The compounds were however found in trace amount and therefore were not quantified. This clustering certainly could have serious implications on the pesticidal activity of the *T. vogelii* leaf material.

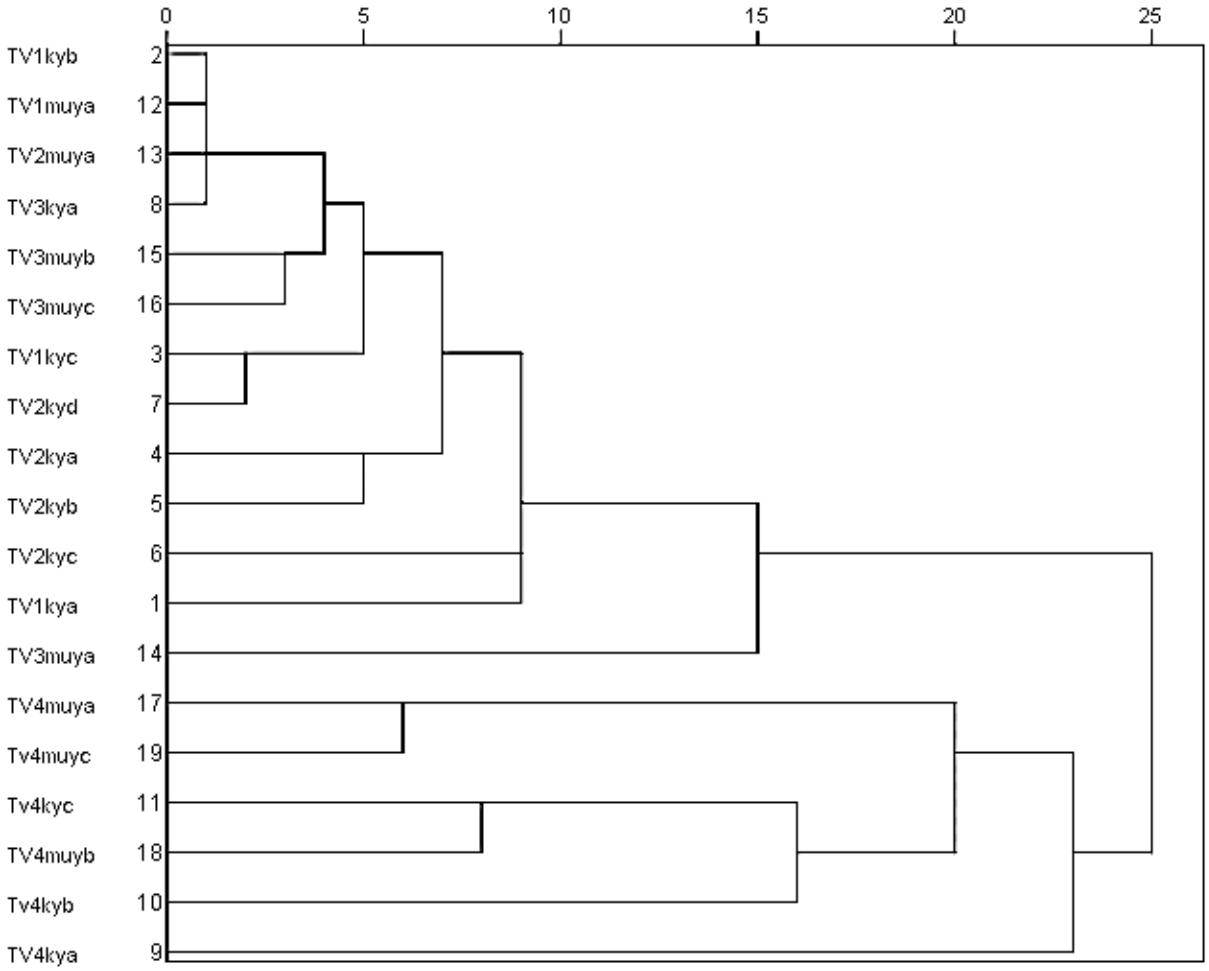

Figure 4: Dendrogram of samples due to classification based on their major composition

3.3 Chemotaxonomic significance of these chemical varieties

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These observations reveal a very significant chemotaxonomic importance where in this part of the world, *T. vogelii* is of three chemical varieties: Farnesol variety and springene type, and the mixed variety thus delimiting the taxonomic status of this plant.

Farnesol is an oxygenated sesquiterpene of various isomers; (*E,Z*)-farnesol, (*Z,E*)-farnesol and (*E,E*)-farnesol. The most common isomer is (*E,E*)-farnesol that could represent 95% of farnesol and was the most identified. Springene is an isoprenoid hydrocarbon of diterpene nature. The two springenes: β-springene (7,11,15-trimethyl-3-methylene-1,6,10,14-hexadecatetraene) and α-springene (E,E,E-3,7,11,15-tetramethyl-1,3,6,10,14-hexadecapentaene) were identified. Both farnesol and the springene, however, belong to the farnesene family. (*E,E*)-β-springene is a diterpene homolog of (*E*)-β-farnesene (4) (which formed part of the second grouping).

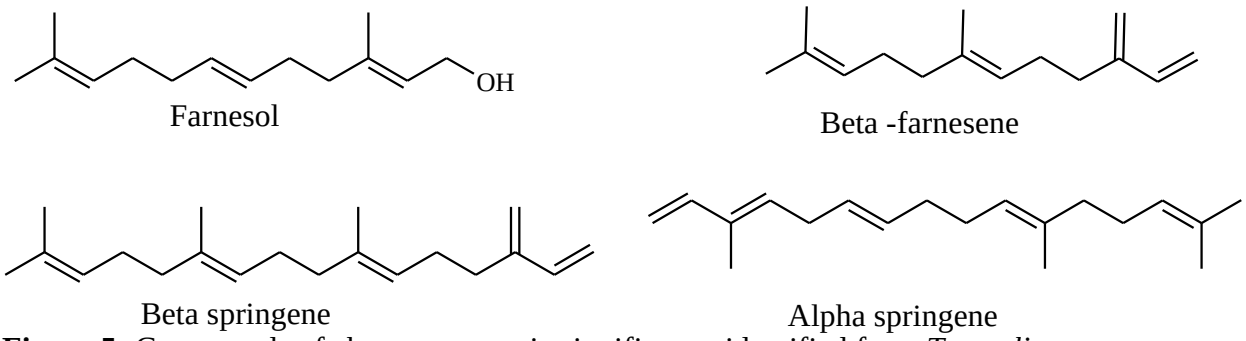

Figure 5: Compounds of chemotaxonomic significancy identified from *T.vogeli*

These are quite uncommon compounds. However β-springene has been previously found in the essential oils of the leaves of the herb *Heracleum persicum* Desf. ex Fischer from Kandavan, northern Tehran in Iran [15] and *Sigesbeckia jorullensis* Kunth (Asteraceae) from North-East of Hamburg, German [16]. It was also detected in *Lagochilus cabulicus* Benth (Lamiaceae) (19.4%); an aromatic plant from Wakhan Corridor in Afghanistan used by the Wakhi and Kyrgyz peoples [17]. Additionally, it was found in very small amounts in *Salvia sclarea* Clary (Lamiaceae) (1.1%) from France [18] and *Salvia reuterana* Boiss (Lamiaceae) (0.3%) from Iran [19]. α-Springene was identified only in the essential oil of *Murraya exotica*

L.(Rutaceae) flowers, collected in India where it was the major constituent (23.8%) [20] and in the essential oil of *Teucrium marum* L. (Lamiaceae) from Corsica where it was detected as one of the main compounds (1.1 – 17.8%) [21]. It is therefore noteworthy to report the presence of α- and β- springene as very important constituents of the Uganda *T. vogelii* essential oils.

3.4 Evaluation of the Repellency potential of the chemotypes of the volatile constituents of T. vogelii

Repellency potential of TV4 Kyc (Farnesol chemotype) and TV4 Muya (Springene chemotype) was also evaluated and results indicate that there was no much difference in their repellency effect against *S. zeamais* (Figure 6). The preference index of TV4kyc (farnesol type) oil for gainst *S. zeamais* ranged between 0.0 to -0.7, 0.0 to -0.5 and -0.3 to -1 for 0.03, 0.16 and 0.31 µL of oil per cm^3 of air respectively. That of TV4muya (springene type) could vary between 0.1 to -0.4, -0.2 to -0.5 and -0.4 to -1 for 0.03, 0.16 and 0.31 µL of oil per cm^3 of air treatment. These results indicate that the oils had repellency effect against the maize weevil based on the preference index scale of *S. zeamais*. Chemotype3 (mixed) showed lower effect. The composition of farnesol and springene was very low; partly explain the less repellency activity for this chemotype. These observations underline the vital role that both farnesol and springene play in the repellency potential.

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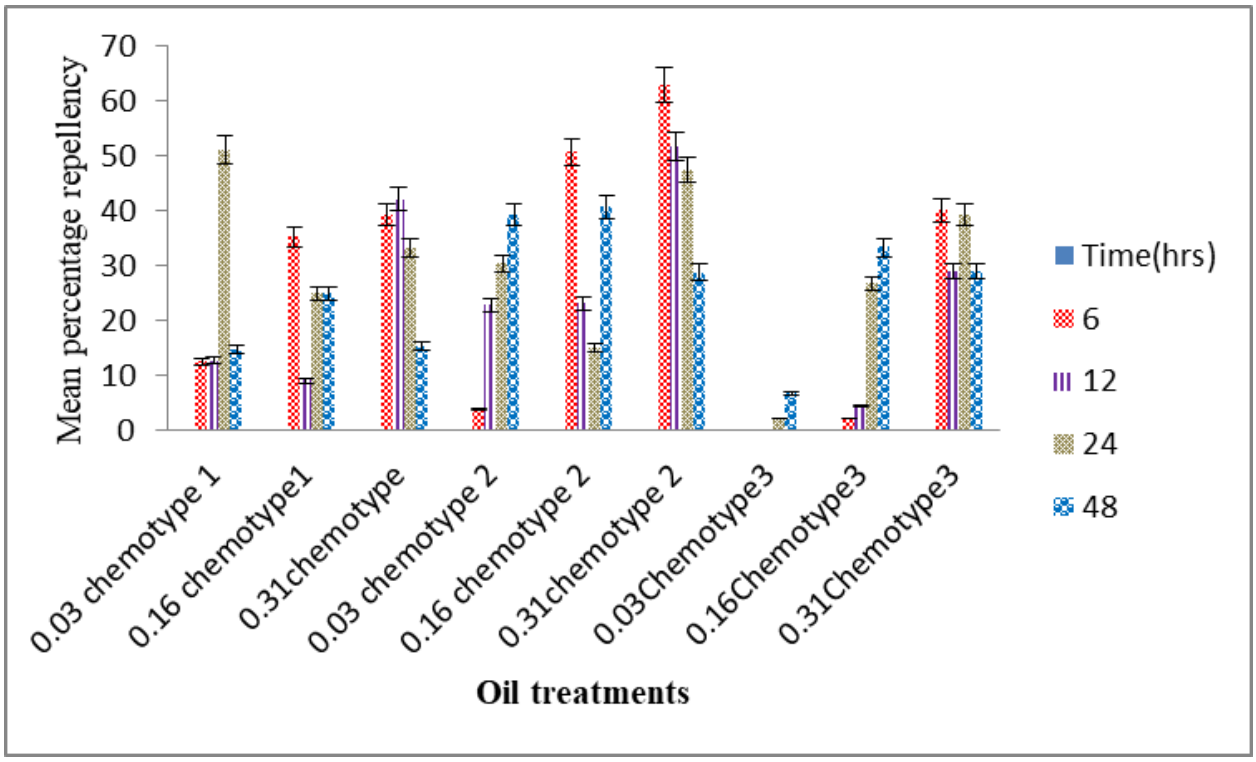

Figure 6: Mean percentage repellence of two varieties of *T. vogelii* against *S. zeamais*

The repellency potential of farnesol was also evaluated against the weevil (as shown below in figure 3.7) and results show that the farnesol is effective at higher concentration as a repellent against *S. zeamais*. The preference index evaluated for 0.31µL of farnesol per cm^3 of air varied between 0.0 to -0.4 while that of the lower concentration ranged between 0.1 to 0.6 and -0.2 to 0.3 for 0.16µL and 0.03µL of farnesol per $cm^3$of farnesol respectively. This implied that at lower concentrations of farnesol had very limited repellent effect against *S. zeamais*. Repellency activity roughly increased with increase in the amount of farnesol.

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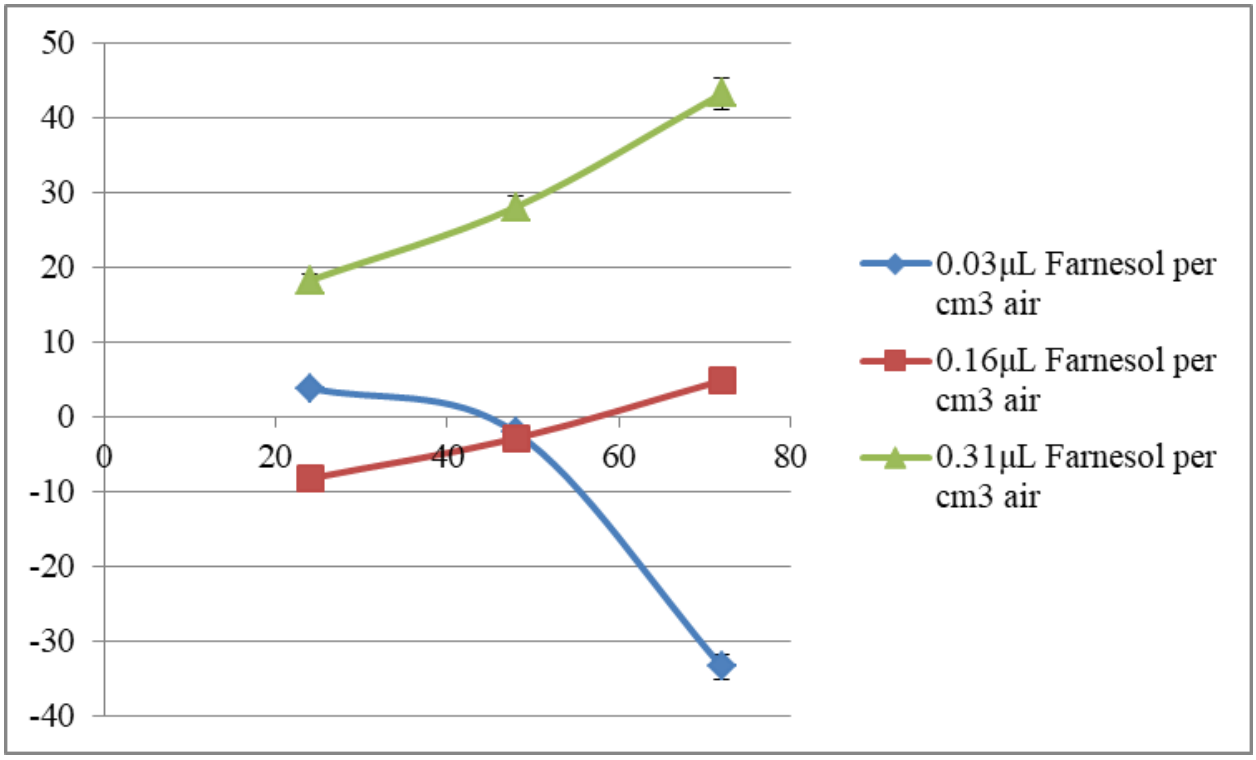

Figure 7: Mean percentage repellence of farnesol standard against *S. zeamais*

The implication of this repellency effect of farnesol isomers is that the farnesol type could have it repellency activity increased with increase in the amount of farnesol in the oil and probably would have an advantage over the springene type. In both cases however, the synergitic or complementary part of all other compounds found in the same oils play a crucial role in overall repellent and insecticidal effect of this oil.

The implications of this chemical variation on the fumigant toxicity were studied and results indicated a significant difference between the farnesol chemotype and the springene chemotype ($P < 0.05$, Fisher LSD test) at a lower dose (Table 4). Springene chemotype could exhibit a higher fumigant effect probably due to synergistic factors. However at a higher concentration; the effect is not so different for the three chemical varieties.

3.4 Conclusion

Investigation of chemical varieties in the essential oils of *T. vogelii* species from the eastern part of Uganda has revealed three chemotypes based on the profiles of farnesene compounds;

one that possesses the farnesol, and the other that has the springene and β-farnesene type; all from the farnesene family and a mixed chemotype of the two. The geographical and seasonal variation could not affect the amount of the oil significantly however; the composition and the constituents of the oils were affected by the harvest period. Evaluation of the repellency effects on these chemical varieties of *T. vogelii* showed that chemotype 1 and 2 were similarly active but more than chemotype 3, against *S. zeamais*. At a lower concentration, there was no significant repellency effect between chemotype 1 and 2. At higher concentrations though, the effect could change. Notwithstanding, the repellent effect of these varieties of *T. vogelii* still holds. The complementary part of all other compounds found in the same oils plays a crucial role in the overall repellent effect of this oil. However, more study is needed that aims to optimize and standardize the chemical varieties and harvesting period needed for recommendation to smallhold farmers especially under field conditions before it can be adopted more widely.

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