AUTHORS:
Edith J. Singini[1,2]
Yannick Nuapia[2,3,4]
Luke Chimuka[4]
Ida M. Risenga[2]

AFFILIATIONS:
[1]Department of Botany, Rhodes University, Makhanda, South Africa
[2]School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Johannesburg, South Africa
[3]Department of Pharmacy, School of Healthcare Sciences, University of Limpopo, Polokwane, South Africa
[4]Molecular Sciences Institute, School of Chemistry, University of the Witwatersrand, Johannesburg, South Africa

CORRESPONDENCE TO:
Edith Singini

EMAIL:
ej.singini@gmail.com

DATES:
Received: 18 Jan. 2022
Revised: 23 Aug. 2022
Accepted: 25 Aug. 2022
Published: 31 Jan. 2023

HOW TO CITE:
Singini EJ, Nuapia Y, Chimuka L, Risenga IM. The effect of elevated temperatures on trichomes, essential oil composition and yield of *Lippia javanica*: A chemometric approach. S Afr J Sci. 2023;119(1/2), Art. #13090. https://doi.org/10.17159/sajs.2023/13090

ARTICLE INCLUDES:
☒ Peer review
☒ Supplementary material

DATA AVAILABILITY:
☐ Open data set
☐ All data included
☒ On request from author(s)
☐ Not available
☐ Not applicable

EDITOR:
Teresa Coutinho

KEYWORDS:
Lippia javanica, abiotic stress, essential oil, multivariate analysis, response surface methodology

FUNDING:
South African National Research Foundation (grant no. SFH180514329259); University of the Witwatersrand (grant no. 900005000001)

The effect of elevated temperatures on trichomes, essential oil composition and yield of *Lippia javanica*: A chemometric approach

Extreme changes in climate, especially in temperature, could have implications for herbal plants in various world regions. Medicinal plants often produce a wide variety of natural phytochemicals to enhance their defence and survival mechanisms against harsh environmental conditions, and when these mechanisms fail, plants consequently die. We investigated the impact of high temperatures coupled with the specific duration of exposure on the yield and composition of essential oils and trichomes in leaves of *Lippia javanica*. Plants were exposed to increasing temperatures (25 °C to 47 °C) for different durations (48 h to 144 h). Response surface methodology was applied to assess the interaction between temperature and length of exposure on the essential oil yield, trichome length, and trichome diameter. Essential oils were recovered from the control and treated leaf samples using hydrodistillation and volatile compounds were identified through gas chromatography–mass spectrometry (GC-MS). Multivariate analysis modelling allowed different clustering patterns to be detected. That is, increasing temperatures raised the oil yield, trichome length, and diameter from 1.007 mg/100 g to 3.58 mg/100 g, 50 μm to 160 μm, and 25 μm to 60 μm, respectively. Significant chemical differences between the essential oils were confirmed by the principal component and orthogonal projections to latent structures, which identified separate clusters for the control and treated samples. The current findings indicate that *L. javanica* has coping mechanisms against high temperatures.

Significance:

- High temperatures significantly alter the trichome morphology and secretion of essential oils in *L. javanica,* which adversely affects the shrub's medicinal properties. Regardless of climate change, this finding could have major implications for indigenous people who continue to use the shrub for therapeutic purposes.
- *L. javanica* showed coping mechanisms against high temperatures for a maximum of six days; however, a prolonged exposure would be more detrimental. As a result, climate change will negatively influence the plant's developmental and defence mechanisms.

Introduction

The fundamental cause of climate change has been identified as the worldwide rise of greenhouse gases.[1,2] The increase in greenhouse gases results in increased temperatures which significantly impact the secretion of secondary metabolites in medicinal plants.[3] Hence, plants exposed to abiotic stresses show crucial variations in their secondary metabolites.[4] Moreover, plant responses to high temperatures are complex, and include deleterious effects and adaptive traits.[5] The most basic morphological traits and biological processes key to plant growth are sensitive to temperature.[3,4] As temperatures increase, the net photosynthetic rate is negatively affected, which leads to a decline in plant performance.[4] Phenology is also driven by temperature[6,7]; thus, any moderate increase in temperature leads to the escalation in developmental processes resulting in the early blooming of plants[8].

South Africa is one of the countries in the world that has experienced a drastic increase in temperatures over the last 40 years.[9] This means that medicinal plants are now exposed to higher temperatures than previously. *Lippia javanica* (Burm. F.) Spreng, commonly known as the fever tree, is a southern African indigenous medicinal plant in the Verbenaceae family and is widely distributed in the warmer eastern and northern provinces of South Africa. The shrub is used by indigenous people and herbal/traditional healers as an inexpensive, safer, and more desirable alternative for treating numerous ailments.[10-12] The shrub is used to treat chronic respiratory diseases[11,13,14], skin disorders[12,15,16], and a wide range of immune-suppressant ailments such as malaria and HIV/Aids[13,14,17]. The commonly used parts of the shrub are the leaves and stems, but sometimes roots may also be utilised.[18] Leaves and stems of *L. javanica* are together used as inhalants, teas, food additives or leafy vegetables, and for topical formulations[12,19], whereas roots are used as an antidote against eye infections and food poisoning[12]. These different plant parts are commonly used because they contain high concentrations of secondary metabolites or phytochemicals, which are secreted in response to physiological and ecological pressures such as pathogens, insects, temperature extremes, and UV radiation.[20-22]

Plant leaves have been identified as the most flexible and adaptive part of a plant in response to changing environmental conditions[23]; thus the histology of the leaf reveals more dynamics than that of the root and stem[24,25]. Leaves of *L. javanica* contain trichomes which are specialised hairs on the adaxial and abaxial leaf surfaces.[25-27] Glandular trichomes are an essential organ in the Verbenaceae family.[26] These trichomes secrete and store phytochemicals that contain medicinal properties.[25-27] The density of trichomes on leaves is dependent on the environmental conditions to which the plant is exposed.[25] The harsher the environmental conditions, the greater the density of trichomes, because trichomes increase the production of essential oils, flavonoids and phenolics which increase the plasticity of the plant.

Research studies have reported that leaves of *L. javanica* are an excellent source of essential oils.[11-14] Chagonda and Chalchat[11] found that *L. javanica* has antiviral, antioxidant, antidiarrhoeal, antitrypanosomal, anti-inflammatory, antibacterial and anticonvulsant essential oil properties. The shrub has aromatic ingredients that have a range of commercially valuable properties.[12] For example, *L. javanica*'s essential oils incorporated in candle wax provide mosquito repellent properties (repelling no less than 95% of mosquitoes) better than most available commercial

products (which repel only 42% of mosquitoes).[12] This suggests that *L. javanica* is used as a source of revenue in both the formal and informal sectors.

Numerous studies have been conducted on the wide range of chemical extracts used in traditional healing.[21,28] Most studies have focused exclusively on the phytochemistry and trichome density of *L. javanica*.[19,25,26] However, no studies investigated the impact of high temperatures on the essential oil yield and related histological changes in *L. javanica*. Therefore, in the present study, we used a chemometric approach to evaluate the effects of temperature variation and time as abiotic agents impacting the production of essential oils in the leaves of *L. javanica*.

Materials and methods

Plant material and chemicals

Samples of *L. javanica* (grown for ~2 years) were obtained from Mountain Herb Estate in Hartbeespoort (25.7236° S, 27.9653° E) and maintained in the greenhouse at the University of the Witwatersrand (26.1929° S, 28.0305° E), South Africa. Before moving samples into the climate test incubator (Conviron - CMP6010, Canada), they were watered twice a day for a month and were kept in the greenhouse at 25 °C/20 °C (day and night simulation). All the chemicals used in this investigation were of analytical grade. Hexane and anhydrous sodium sulfate were purchased from Merck (Johannesburg, South Africa).

Design of experiment

Plants were exposed to high temperatures (25 °C, 36 °C and 47 °C) in a climate test incubator (Conviron) for 6 days. Leaves were then harvested episodically after 48 h, 96 h and 144 h (n=30). The harvested leaves were air dried to investigate the effects of temperature (T) (25–47 °C) and time (t) (48–144 h) on the production of essential oils (Supplementary figure 1) and related histological modifications of *L. javanica*. A central composite design, MODDE 13.1 (Sartorius Stedim Biotech, Malmö, Sweden), was used to generate a full-factorial design with 12 experiments and three centre-point replicates (Table 1).

Table 1: Essential oil yield, trichome height, and trichome diameter of *Lippia javanica* at set experimental conditions

Run order	Temperature (°C)	Time (h)	Yield (%)	Trichome diameter (μm)	Trichome height (um)
9	25	48	0.91	25	54
6	25	96	0.95	25	54
10	25	144	0.97	25	54
7	36	48	1.45	30	83
8	36	96	1.78	36	89
2	36	144	1.89	39	98
1	47	48	2.18	44	130
4	47	96	2.45	47	134
12	47	144	3.58	54	139
5	36	96	2.02	39	95
3	36	96	1.97	35	86
11	36	96	2.45	37	90

The temperature range was chosen based on the average temperatures obtained in all six South African provinces where *L. javanica* occurs (Mpumalanga, Limpopo, Gauteng, North West, Eastern Cape and KwaZulu-Natal). The control was kept at 25 °C, while 36 °C was based on current average summer temperatures, and 47 °C was assumed from the predicted increase in temperatures in the next 30 years.[1,2] Humidity (55%), soil type (loam), and light conditions (under 12 hours of light with a photon flux density of 100 μmol/m^2/s) in the Conviron were kept constant, and plants were watered once a day throughout the treatments.

Extraction of essential oils

Essential oils were extracted from the air-dried harvested leaves of the control and treated samples using a Clevenger-type apparatus for the hydrodistillation process.[27] A volume of 1 L of water was added to 300 g of leaf samples for 4 h. The recovered essential oil was then isolated and dried with anhydrous sodium sulfate, filtered through a small cotton-wool plug, and shifted to a pre-weighed amber vial.[27] The total yield was calculated using the formula below:

$$\text{Percentage (\%) yield} = \left[\frac{B-A}{C}\right] X\ 100$$

where:

A = Mass of empty bottle (g)

B = Mass of bottle plus oil extracted (g)

C = Mass of distilled material (g)

Essential oil analysis (GC-MS/FID)

Before injection into the chromatogram, 15% (v/v) of essential oil samples in hexane were prepared before the analysis. They were scrutinised using a Hewlett-Packard G1800A GCD system gas chromatograph (GC) combined with flame-ionisation detection (FID) and a mass spectrometry (MS) detector. An Innowax FSC column (60 m × 0.25 mm diameter) was utilised, and helium was the carrier gas, with a 0.8 mL/min flow rate.[27] The GC-MS was maintained at an oven temperature of 60 °C for the first 10 min, and then the temperature was programmed to rise to 220 °C at a rate of 10 °C/min, and it was kept constant at 220 °C for 10 min. Then again, temperature was programmed to rise to 240 °C at a rate of 10 °C/min. Split flow was adjusted to 50 mL/min, whereas the injector and detector temperatures were adjusted to 250 °C. The mass spectra/ionisation was taken at 70 eV, and the mass range was from 35 m/z to 425 m/z. Qualitative characterisation of *L. javanica* essential oil samples was performed using a GC-MS.

The US National Institute of Standards and Technology (NIST) Mass Spectral Library was used to characterise the shrub's essential oil compounds. Using literature and databases, essential oil components were tentatively identified by comparing the retention indices, molecular weight, and mass fragmentation patterns. All matches reported in our study had match quality >90% with respect to the experimental spectrum. The first step was a spectrum comparison in all the matches, which offers a range of potential matches. The second step was a post-search filter which rejected retention indices inconsistent with indices of unknown components. Using these calculated retention indices and interactive search filters built into the library decreases the chances of incorrect identifications.

Unsupervised and supervised machine learning exploratory approach

The complex total ion chromatogram data set (Supplementary figure 2) obtained from the essential oil GC-MS analysis was processed using commercial Leco Chroma TOF software. Total ion chromatogram data were aligned, and the baseline was corrected and smoothed. A MS Excel spreadsheet including all the main ion fragments for each peak was made and used for further assessment through SIMCA-17 (Umetrics AB, Malmo, Sweden). Various scaling approaches were applied before creating the unsupervised principal component (PCA) model. The PCA model was then used to investigate the relationship between variables and correlation for each variable to the principal components. The score plot was employed to identify similarity between individual clusters. The graphical output of this clustering tool shows the group-inclusive associations between classes and the value of the clustering criterion related to each treatment of the plant.[29] The essential oils variability obtained through the clusters on the PCA scores plot was further assessed by the supervised orthogonal projections to latent structures discriminant analysis (OPLS-DA) model.

The samples were assigned classes based on the treatment of the plants. OPLS-DA loadings plots identified chemical markers driving the observed variability within the control and treated samples. The data set was randomly subdivided into the testing set (30%) and training set (70%). The OPLS-DA model was developed using the training data set, whereas the testing data set was used for cross-validation, validation, and prediction ability of the model.[30] The variability of the essential oil compounds within the control and treated groups were identified using OPLS-DA scores and S-plots.

A multiple linear regression was applied to calculate the fitting model and response surface. The R^2 and Q^2 values indicate the adequacy of the models (where R^2 shows the model fit and Q^2 shows an estimate of the future prediction precision), predicted vs. observed plot, and coefficient plots. A partial least squares regression was employed to assess the response surface and fitting model response surface. The adequacy of the models was evaluated using Q^2 (an estimate of the future prediction precision) and R^2 (the model fit values).

Results and discussion

Response surface methodology optimisation

The response surface methodology was used to assess the impact of temperature and time on the yield of essential oils and related histological modification of *L. javanica*. The model was achieved based on the experimental design defined by the central composite design. These experiments were carried out at all possible level combinations of temperature and time, and the response was given as the essential oil yield, trichome diameter, and trichome height (Table 1). Thereafter, the regression models were obtained by fitting the second-order polynomial equation to the experimental data set. The fitted model showed a total explained variance of 84% to 94% (R^2 = 0.84–0.94) and cross-validated predictability of 65% to 97% (Q^2 = 0.65–0.97), where R^2 shows the model fit and Q^2 offers an estimation of the future prediction and precision. The linearity of the predicted versus observed values plot (Figure 1) underlined the validity of the model and its capability to predict the best condition of the extraction within the range of the design.

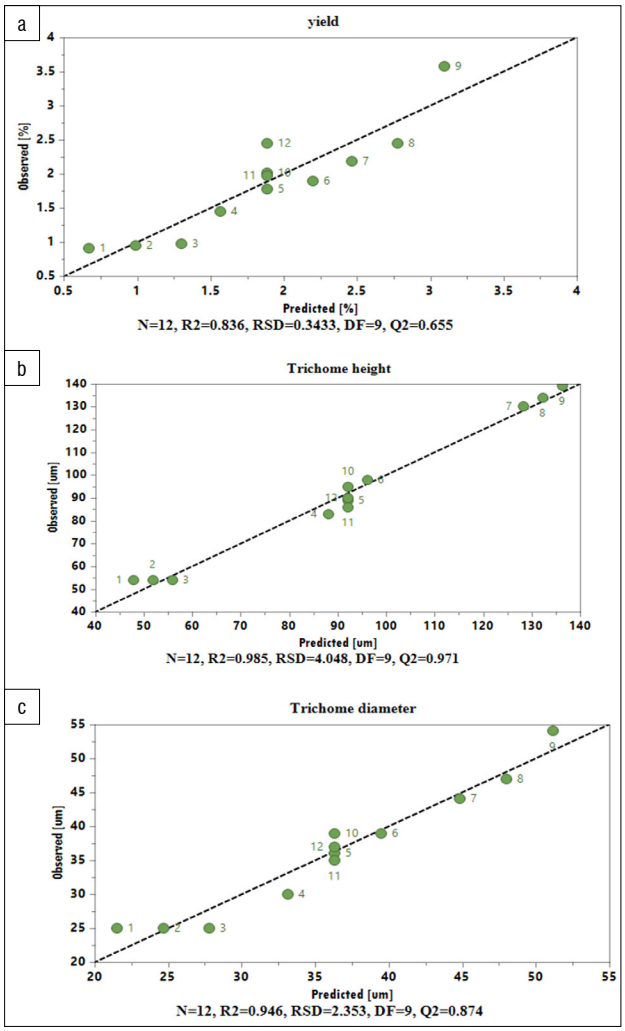

Figure 1: The linearity of the predicted versus observed values plots for (a) yield, (b) trichome height and (c) trichome diameter of *Lippia javanica*.

The coefficients plot (Figure 2) revealed that high temperatures significantly affect the yield of essential oils, trichome height, and trichome diameter (p=0.002); in contrast, time does not significantly influence plant stress. The increase in temperature from 25 °C to 47 °C increased essential oil yield, trichome length, and trichome diameter from 1.007 mg/100 g to 3.58 mg/100 g, 50 μm to 160 μm, and 25 μm to 60 μm, respectively. This finding suggests that an increase in essential oil secretion, trichome height and diameter is a defence mechanism for plants exposed to environmental stress. Our data show that time did not affect plant stress; nonetheless, it is well recognised that the longer a plant is exposed to environmental stress, the more harmful the conditions are to the plant.

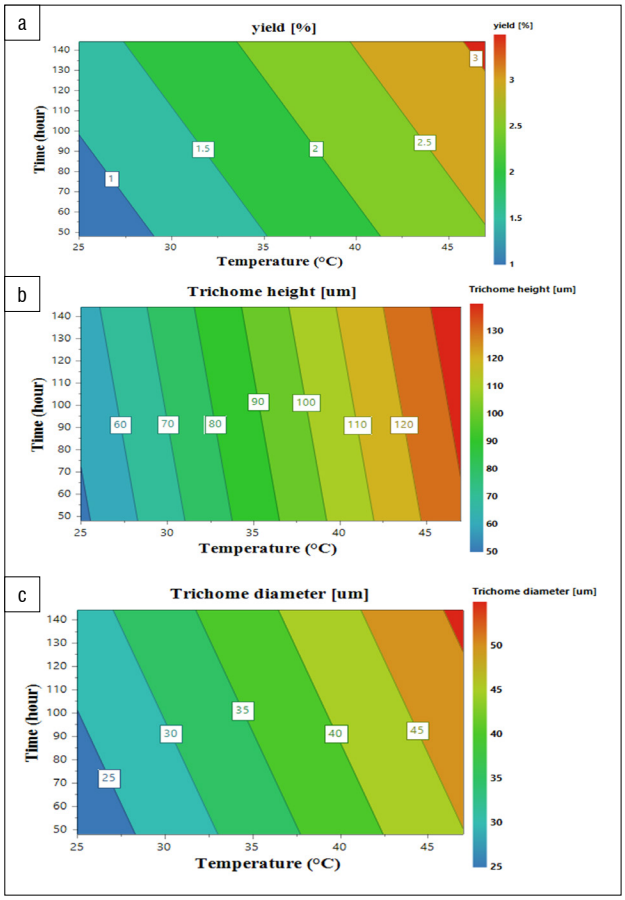

Figure 2: Contour plots showing the direct and interaction impacts of temperature and time on (a) essential oil yield, (b) trichome height, and (c) trichome diameter of *Lippia javanica*.

Several studies have shown that the increase in glandular trichome length and diameter are directly correlated to the yield of essential oils produced by the plant (Supplementary figure 1).[31,32] Moreover, other studies have reported that both essential oil yield and the quality of aromatic compounds are primarily affected by environmental factors such as altitude, light intensity, and seasonality.[11,28,33] Our findings are consistent with these studies because exposing *L. javanica* to high temperatures increased the length and diameter of trichomes, which was associated with the increase in essential oil yield (2.67% more than the control at 47 °C/144 h).

Our results clearly show that the production of essential oils in *L. javanica* is temperature sensitive (Supplementary figure 1) and that the increase in the secretion of essential oils is an indicator of environmental stress. However, it is unclear how these environmental factors affect the quality of essential oils. Al-Gabbiesh et al.[34] stated that plants exposed to environmental stresses reveal a higher concentration and yield of essential oils than those cultivated under ambient conditions. In this context, however, exposure to environmental stresses significantly reduces plant growth because carbon meant to be allocated to growth is redirected to the production of secondary metabolites for plant

survival.[4,34] Therefore, environmental stress could increase the toxicity of volatile compounds, which may be harmful to humans. The toxicity level may also depend on the duration of exposure to environmental stress.

Characterisation and identification of essential oils by GC-MS

The GC-MS analysis yielded 50 essential oil components (Supplementary table 1). The essential oils extracted from the control and treated samples displayed differences in their chemical composition. The primary essential oils found in the control samples were linalool, carvone, piperitenone and tagetenone, while the treated samples were contained eucalyptol, camphor, fenchone eucalyptol, p-cymene, caryophyllene and terpinen-4-ol. The largest relative peak area of the major compounds revealed significant chemical variations within the control and treated samples (Supplementary figure 3).

All samples fell within the Hotelling's T^2 ellipse on the scores plot (Figure 3), suggesting the nonexistence of strong outliers, and therefore all data were included. The total variation across the data was 99.7% (R^2X_{cum} = 0.997), whereas Q^2_{cum} was 0.987, indicating a good model. The scores scatter plot of PC1 against PC2 (Figure 4) shows four clusters corresponding to the control and treatments. About 56.8% and 31.1% of the sample variability were explained by PC1 and PC2, respectively (Table 2). An OPLS-DA model was created from the data for the control and treated samples. Centre-scaled models generated the best statistics for the selected data set compared to other scaling approaches (Table 2).

Table 2: Centre-scaled principal components analysis (PCA) and orthogonal projections to latent structures discriminant analysis (OPLS-DA) model statistics output

Statistical parameter	Output
PCA Model	
Number of principal components	3
R^2Xcum	0.997
Q^2cum	0.987
% Variation PC1	56.8
% Variation PC2	31.1
OPLS-DA Model	
Predictive components (P)	1
Orthogonal components (O)	1
R^2X (P1)	0.502
R^2X (O1)	0.416
R^2Y	0.919
Q^2Y	0.987

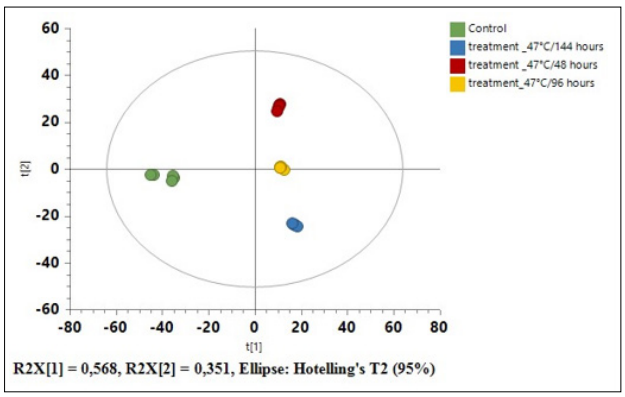

Figure 3: Principal component analysis (PCA) scores scatter plot of *Lippia javanica* exposed to 25 °C (control) and 47 °C (for 48, 96 and 144 hours).

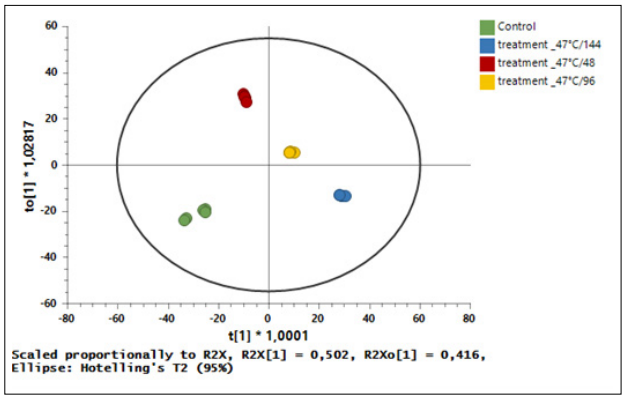

Figure 4: Orthogonal projections to latent structures discriminant analysis (OPLS-DA) scores scatter plot of *Lippia javanica* exposed to 25 °C (control) and 47 °C (for 48, 96 and 144 hours).

Four groups were distinguished in the corresponding OPLS-DA score plots (47 °C/48 h, 47 °C/96 h and 47 °C/144 h) (Figure 4), confirming the chemical variation of essential oils found in *L. javanica* at elevated temperatures. Major essential oil constituents (linalool, – (-) carvone, tagetenone and piperitenone) in the control disappeared in the high temperature treatments (Figure 5). However, eucalyptol, camphor, and p-cymene were major essential oils not previously present in the control and were produced when plants were exposed to high temperatures.

Several essential oil constituents are not produced at certain temperature ranges; thus, temperature alterations significantly impact the essential oil composition of plants. A study by Lee and Ding[35] found that the specific ratio of essential oil constituents determines the therapeutic and wellness-enhancing properties of the oil. However, a study by Nakatsu et al.[36] suggested that, although there are differences in the composition of essential oils of various plants, there is considerable overlap in their overall properties. For example, *Tetradenia riparia* and *Virola surinamensis* have different essential oil profiles, but they are both reported to treat malaria symptoms.[34] Therefore, the changes observed in the oil constituents of *L. javanica* after exposure to high temperatures may not suggest changes in the biological activity of the shrub. More research, however, is required to ascertain any changes in the biological activity of *L. javanica* and the implications of the additional compounds secreted in response to high temperatures.

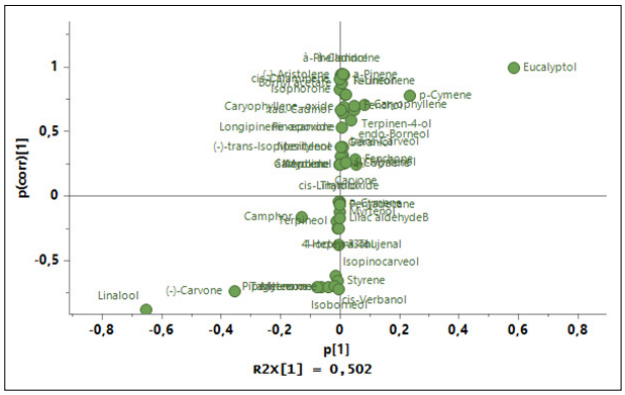

Figure 5: S-plots of essential oil components of *Lippia javanica*.

Conclusion

Temperature was the most critical factor influencing the production of essential oils, trichome length and trichome diameter in *L. javanica*. A significant degree of chemical variation was evident between the control and treated samples. Four essential oil compounds successfully identified within the control were not present in the treated samples and three compounds detected in the treated samples were absent in the control. This variation suggests resistance against high temperatures and

other physiological and ecological pressures. The increase in defence essential oil compounds could also indicate an increase in the medicinal properties of the shrub in treating various ailments. More studies should be conducted to investigate the pharmacological activities and toxicity levels of *L. javanica* exposed to high temperatures in order to elucidate any changes in the medicinal properties of the shrub.

Acknowledgements

This research was supported by the South African National Research Foundation (NRF): Freestanding, Innovation and Scarce Skill Development Fund (grant number SFH180514329259), a NRF Thuthuka grant awarded to I.M.R. which funded running costs, as well as a Post Graduate Merit Award (grant number 900005000001) to E.J.S. from the University of the Witwatersrand. We thank Dr Ellis Ayayee, Dr Tsitsi Maponga, and Prof. Ed Witkowski for their support throughout this research.

Competing interests

We have no competing interests to declare.

Authors' contributions

E.J.S.: Sample analysis, data collection, writing – initial draft. Y.N.: Methodology, supervision, proofreading the draft. L.C.: Validation, project leadership, supervision. I.M.R.: Conceptualisation, validation, supervision, funding.

References

1. Nowak DJ. The atmospheric system: Air quality and greenhouse gases. In: Hall M, Balogh S, editors. Understanding urban ecology. Cham: Springer; 2019. p. 175–199. https://doi.org/10.1007/978-3-030-11259-2_8
2. Mikhaylov A, Moiseev N, Aleshin K, Burkhardt T. Global climate change and greenhouse effect. Entrep Sustain Issues. 2020;7(4):2897–2913. https://doi.org/10.9770/jesi.2020.7.4(21)
3. Hendawy SF, Hussein MS, El-Gohary AE, Soliman WS. Chemical constituents of essential oil in chervil (*Anthriscus cerefolium* L. Hoffm.) cultivated in different locations. J Essent Oil-Bearing Plants. 2019;22(1):264–272. https://doi.org/10.1080/0972060X.2019.1587316
4. Caretto S, Linsalata V, Colella G, Mita G, Lattanzio V. Carbon fluxes between primary metabolism and phenolic pathway in plant tissues under stress. Int J Mol Sci. 2015;16(11):26378–26394. https://doi.org/10.3390/ijms161125967
5. Chaves MM, Pereira JS, Maroco J, Rodrigues ML, Ricardo CPP, Osório ML, et al. How plants cope with water stress in the field. Photosynthesis and growth. Ann Bot. 2002;89(7):907–916. https://doi.org/10.1093/aob/mcf105
6. Muhl QE, Du Toit ES, Robbertse PJ. *Moringa oleifera* (horseradish tree) leaf adaptation to temperature regimes. Int J Agric Biol. 2011;13(6):1021–1024.
7. Bita CE, Gerats T. Plant tolerance to high temperature in a changing environment: Scientific fundamentals and production of heat stress-tolerant crops. Front Plant Sci. 2013;4:1–18. https://doi.org/10.3389/fpls.2013.00273
8. Jump AS, Peñuelas J. Running to stand still: Adaptation and the response of plants to rapid climate change. Ecol Lett. 2005;8(9):1010–1020. https://doi.org/10.1111/j.1461-0248.2005.00796.x
9. Kruger AC, Sekele SS. Trends in extreme temperature indices in South Africa: 1962–2009. Int J Climatol. 2013;33(3):661–676. https://doi.org/10.1002/joc.3455
10. Mander M, Ntuli L, Diederichs N, Mavundla K. Economics of the traditional medicine trade in South Africa: Health care delivery. S Afr Health Rev. 2007;1:189–196.
11. Chagonda LS, Chalchat JC. Essential oil composition of *Lippia javanica* (Burm.f.) Spreng chemotype from Western Zimbabwe. J Essent Oil-Bearing Plants. 2015;18(2):482–485. https://doi.org/10.1080/0972060X.2014.1001140
12. Maroyi A. *Lippia javanica* (Burm.f.) Spreng.: Traditional and commercial uses and phytochemical and pharmacological significance in the African and Indian subcontinent. Evid Based Complement Alternat Med. 2017;2017, Art. #6746071. https://doi.org/10.1155/2017/6746071
13. Manenzhe NJ, Potgieter N, Van Ree T. Composition and antimicrobial activities of volatile components of *Lippia javanica*. Phytochemistry. 2004;65(16):2333–2336. https://doi.org/10.1016/j.phytochem.2004.07.020
14. Mujovo S, Hussein A, Meyer JJM, Fourie B, Muthivhi T, Lall N. Bioactive compounds from *Lippia javanica* and *Hoslundia opposita*. Nat Prod Res. 2008;22(12):1047–1054. https://doi.org/10.1080/14786410802250037
15. Abubakar EMM. The use of *Psidium guajava* Linn. in treating wound, skin and soft tissue infections. Sci Res Essays. 2009;4(6):605–611.
16. Mahlangeni NT, Moodley R, Jonnalagadda SB. Elemental composition of *Cyrtanthus obliquus* and *Lippia javanica* used in South African herbal tonic, Imbiza. Arab J Chem. 2018;11(1):128–136. https://doi.org/10.1016/j.arabjc.2014.07.007
17. Dlamini TP. Isolation and characterisation of bio-active compounds from *Lippia javanica* [Master of Technology dissertation]. Johannesburg: University of Johannesburg; 2006.
18. Viljoen AM, Subramoney S, Vuuren SFV, Başer KHC, Demirci B. The composition, geographical variation and antimicrobial activity of *Lippia javanica* (Verbenaceae) leaf essential oils. J Ethnopharmacol. 2005;96(1–2):271–277. https://doi.org/10.1016/j.jep.2004.09.017
19. Asowata-ayodele AM, Afolayan AJ, Otunola GA. Ethnobotanical survey of culinary herbs and spices used in the traditional medicinal system of Nkonkobe Municipality, Eastern Cape, South Africa. 2016;13:237–242. https://doi.org/10.4314/ajtcam.v13i2.28
20. Pereira DM, Valentão P, Pereira JA, Andrade PB. Phenolics: From chemistry to biology. Molecules. 2009;14(6):2202–2211. https://doi.org/10.3390/molecules14062202
21. Sulaiman CT, Balachandran I. Total phenolics and total flavonoids in selected Indian medicinal plants. Indian J Pharm Sci. 2012;74(3):258–260. https://doi.org/10.4103/0250-474X.106069
22. Matshediso PG, Cukrowska E, Chimuka L. Development of pressurised hot water extraction (PHWE) for essential compounds from *Moringa oleifera* leaf extracts. Food Chem. 2015;172:423–427. https://doi.org/10.1016/j.foodchem.2014.09.047
23. Nevo EN, Pavlílek TP, Beharav AB, Bolshakova MAB, Martyn GIM, Musatenko LIM, et al. Drought and light anatomical adaptive leaf strategies in three woody species caused by microclimatic selection at "evolution canyon", Israel. Isr J Plant Sci. 2000;48(1):33–46. https://doi.org/10.1092/RNPF-9HJE-8J3L-B5F1
24. Rebecchi L, Altiero T, Guidetti R. Anhydrobiosis: The extreme limit of desiccation tolerance. Invertebr Surviv J. 2007;4(2):65–81.
25. Martínez-Natarén DA, Parra-Tabla V, Dzib G, Calvo-Irabién LM. Morphology and density of glandular trichomes in populations of Mexican oregano (*Lippia graveolens* H.B.K., Verbenaceae), and the relationship between trichome density and climate. J Torrey Bot Soc. 2011;138(2):134–144. https://doi.org/10.3159/TORREY-D-10-00007.1
26. Combrinck S, Du Plooy GW, McCrindle RI, Botha BM. Morphology and histochemistry of the glandular trichomes of *Lippia scaberrima* (Verbenaceae). Ann Bot. 2007;99(6):1111–1119. https://doi.org/10.1093/aob/mcm064
27. Serrato-Valenti G, Bisio A, Cornara L, Ciarallo G. Structural and histochemical investigation of the glandular trichomes of *Salvia aurea* L. leaves, and chemical analysis of the essential oil. Ann Bot. 1997;79(3):329–336. https://doi.org/10.1006/anbo.1996.0348
28. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotechnol. 2005;4(7):685–688. https://doi.org/10.5897/AJB2005.000-3127
29. Maree J, Kamatou G, Gibbons S, Viljoen A, Van Vuuren S. The application of GC-MS combined with chemometrics for the identification of antimicrobial compounds from selected commercial essential oils. Chemom Intell Lab Syst. 2014;130:172–181. https://doi.org/10.1016/j.chemolab.2013.11.004
30. Eriksson L, Andersson PL, Johansson E, Tysklind M. Megavariate analysis of environmental QSAR data. Part I – A basic framework founded on principal component analysis (PCA), partial least squares (PLS), and statistical molecular design (SMD). Mol Divers. 2006;10(2):169–186. https://doi.org/10.1007/s11030-006-9024-6
31. Navarrete A, Wallraf S, Mato RB, Cocero MJ. Improvement of essential oil steam distillation by microwave pretreatment. Ind Eng Chem Res. 2011;50(8):4667–4671. https://doi.org/10.1021/ie102218g

32. Talebi SM, Behzadpour S, Yadegari P, Ghorbanpour M. Trichome structures and characterization of essential oil constituents in Iranian populations of *Salvia limbata* C.A. Meyer. Iran J Sci Technol Trans A Sci. 2021;45(1):41–54. https://doi.org/10.1007/s40995-020-01017-9

33. Tozin LRS, Marques MOM, Rodrigues TM. Glandular trichome density and essential oil composition in leaves and inflorescences of *Lippia origanoides* Kunth (Verbenaceae) in the Brazilian Cerrado. An Acad Bras Cienc. 2015;87(2):943–953. https://doi.org/10.1590/0001-3765201520140376

34. Al-Gabbiesh A, Kleinwächter M, Selmar D. Influencing the contents of secondary metabolites in spice and medicinal plants by deliberately applying drought stress during their cultivation. Jordan J Biol Sci. 2015;8(1):1–10. https://doi.org/10.12816/0026941

35. Lee YL, Ding P. Physiological production of essential oils in plants – Ontogeny, secretory structures and seasonal variations. Pertanika J Sch Res Rev. 2016;2(1):1–10.

36. Nakatsu [xc*] T, Lupo AT, Chinn JW, Kang RKL. Biological activity of essential oils and their constituents. Stud Nat Prod Chem. 2000;21:571–631. https://doi.org/10.1016/S1572-5995(00)80014-9