CHARACTERISATION OF ESSENTIAL OIL COMPOUNDS AND OPTIMISATION OF WATER AND POTASSIUM FOR PRODUCTION OF *LANTANA CAMARA* (L.) FOR *TUTA ABSOLUTA* MANAGEMENT

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Characterisation of Essential Oil Compounds and Optimisation of Water and Potassium for Production of *Lantana camara* (L.) for *Tuta absoluta* Management

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

I dedicate this thesis to my loving Children, Liam Anita Wanjiku, Eileen Wambui and Lynn Naliaka; mother, Lenah Naliaka; brothers, sisters and friends for their encouragement, moral support, prayers and love. To my supervisors, who inspired me throughout this study. Finally, to men and women who cherish the knowledge of science. GOD bless you ALL.

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ABSTRACT

Tuta absoluta (Meyrick) is a serious threat to the sustainable production of tomatoes (*Solanum lycopersicum* L.) in Kenya. Tomato is one of the most important vegetables grown in Kenya, playing a critical role in meeting food nutritional requirements and creating employment. The pest causes up to 100% loss of production leading to reduced income and loss of livelihood. In the effort to control the pest, synthetic insecticides have been rampant, posing serious environmental and health risks. *Lantana camara* on the other hand is an invasive weed commonly found in tropical and sub-tropical ecosystems, including Kenya. It is a multipurpose plant with copious secondary metabolites and numerous biological activities that can be exploited in the control of *T. absoluta*. However, the synthesis of the secondary metabolites is significantly influenced by the ecological aspects and agronomic practices during growth and development, thereby inducing variations in quantity, quality, and distribution of the active compounds. The objectives of this study were to; characterise the secondary metabolite profiles of the leaf essential oils of *Lantana camara* from six different agro-ecological zones (AEZ) and soils in Kenya, evaluate the efficacy of the leaf essential oils of *L. camara* against *T. absoluta*, and assess the influence of potassium nutrients and water application rates on the oil yield and secondary metabolite profiles of *L. camara*. The leaf and soil samples and corresponding monthly climatic data were sampled at 8-9 a.m. from six representative AEZs namely; Lower Highland-Njoro (LH-NJ), Upper Midland 1-Kakamega (UM1-KK), Upper Midland 2-Kandara (UM2-KA), Upper Midland 3-Embu (UM3-EM), Lower Midland-Kiboko (LM-KI) and Coastal Lowland-Mtwapa (CL-MT) located in six counties of Kenya during the wet (May) and dry (September) seasons (2018, 2019 and 2020). The essential oils were extracted from the plant leaf samples by steam distillation and analysed through GC-MS (Gas Chromatography-mass Spectrometer). The GC-MS data were analysed with environmental variables (soil and climate data) using unimodally constrained and unconstrained ordination methods for untargeted metabolomics analysis. Tomato (Rio Grande VF) plants (*L. esculentum*) were grown in the greenhouse conditions in 2-litre pots, inside large insect-proof cages and used for rearing *Tuta absoluta* and for performing the bioassays experiments. The larvicidal and repellent activity of essential oils against *T. absoluta* was performed using six different oil treatments from the six AEZs collected in May 2018. The larvicidal activity was performed using the leaf-dip bioassay protocol, while the repellency activity was performed using the repellent response method for phytophagous pests, and data were analysed using the ANOVA test and Probit analysis. Lastly, a greenhouse experiment studied the effects of potassium supplementation and water regimen on essential oil content and *Lantana camara* composition. Cuttings were collected from Lower Midland-Kiboko (LM-KI) and propagated under the greenhouse. Potassium was supplied at five rates as muriate of potash (MOP) (0.00g (F1), 0.25g (F2), 0.51g (F3), 0.76g (F4) and 0.81g (F5)) and three watering level (10% (W1), 40% (W2) and 80% (W3)) field capacity. The results showed that regional and seasonal variability was observed for secondary metabolites (SMs) in the leaf essential oil, which correlated to soil attributes and climatic factors. The study highlights the seasonal-geographic metabolism relationship for *L. camara* and the combined analytical method to obtain data that contributes to understanding the environmental factors' influence on the secondary

metabolites' accumulation and synthesis. The bioassay test showed that *L. camara* essential oil has good larvicidal activity with higher mortality (89%) on the 2nd instar larvae with a higher concentration (0.01µl/µl oil/o.1% Tween 20). The repellence test also showed a higher average repellence (93.44%) effect with a higher concentration (0.01µl/µl oil/0.1% Tween 20) of the essential oils. The potassium nutrition supplementation and water regime treatments affected the essential oil yield from the leaves. The highest essential oil content (0.76%) was obtained in the lowest moisture (10% VMC (W1)) and potassium supplement levels (0.00g (F1)). Whereas the highest moisture (80% VMC (W3)) and potassium supplement levels (0.81g (F5)) produced the lowest essential oil content (0.34%). Sixty-eight major compounds were identified in the essential oil from the grown plants. The metabolites synthesised with low moisture content comprised most of the metabolites detected at five different potassium treatments (79.36-94.46%) compared to those treated with optimum moisture level and potassium treatment (69.77-63.24%). The potassium and watering treatments affected the production of the secondary metabolites. The results showed that increasing the water and potassium levels showed negative and positive correlations on different metabolites, indicating the effect is metabolite-dependent. The research has shown that the essential oil of *Lantana camara* may be a sustainable, eco-friendly alternative for synthetic insecticide in the *T. absoluta* management program. Due to the variability in active compounds, one could choose the location of plant growth for a particular compound or add/omit nutrients to modify the secondary metabolites. Therefore, this exploratory analysis of *L. camara* was able to deduce that the environmental and soil variables and water and fertilizer can modulate metabolite accumulation. Lastly, developing agro techniques for local production is essential for assuring the production of desired metabolites in *L. camara* plant leaf essential oil for exploitation in commercial production for pest management.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Leaf-mining moths are a group of insects belonging to the Lepidoptera order, known for their internal feeding habits (Kawahara et al., 2017). These moths are found in several families and characterized by their ability to create serpentine mines in leaves, bore into fruit, or create galls and roll leaves to feed (Kawahara et al., 2017). Unfortunately, some invasive species of these moths cause significant economic damage for instance, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), commonly known as tomato leaf miner (Biondi et al., 2018; Campos et al., 2017; Guedes et al., 2019). Various biological and ecological characteristics of this species have contributed to its invasiveness and high feeding damage potential to crops (Ponti et al., 2021). This pest can successfully develop on several crop and weed solanaceous species, such as tomato, eggplant, potato, horse nettle, and nightshade as well as on non-solanaceous plants to a lesser extent, making it challenging to avoid in tomato crops (Biondi et al., 2018; Campos et al., 2017).

The tomato (Lycopersicon esculentum Mill.) plant, is a member of the Solanaceae family of flowering plants (Sato et al., 2012). Tomatoes are vital for horticulture and sustainable development, cultivated globally, contributing significantly to both household and national food and nutritional security (FAO et al., 2023). Tomato production also plays a significant role in economic development due to its high economic returns and ability to generate employment along the value chain (Aigbedion-Atalor et al., 2020). Tomato is among the most extensively cultivated and consumed vegetables in Kenya (HCD, 2023) . Besides meeting the nutritional food requirement, tomato production serves as a reliable source of employment and income, contributing to improving livelihoods, and economic growth (Kinyanjui et al., 2021). Unfortunately, tomato production is under threat from increased damage by the tomato leaf miner *T. absoluta* (Rwomushana et al., 2019).

Tomato leaf miner is a significant threat to tomato production in Kenya because of the extensive damage it elicits. Its prevalence was documented in the Northern part

of Kenya in early 2014 for the first time (IPPC, 2014). Following the first outbreak of tomato leaf miners in Kenya's main tomato growing areas, the typical response was to employ chemical control measures as a primary defense (Rwomushana et al., 2019), because other effective pest control alternatives were lacking at that time and developing non-chemical control options takes time (Guedes et al., 2019; Silva et al., 2019). The growers used synthetic insecticides available in the local market to manage the tomato leaf miner, but it has been largely ineffective (Rwomushana et al., 2019). Newly introduced areas see increased insecticide use to limit invasive pest spread and yield loss (Desneux et al., 2022).

The excess use of synthetic pesticides has amplified the threat of pest resistance development and pest resurgence (Desneux et al., 2022), and toxicological implications to human and animal health and environmental pollution (Damalas & Koutroubas, 2018; Khan & Damalas, 2015). Poor pest management practices cause genetic resistance in pest populations, leading to unbalanced insecticide use and ultimately affecting the control of tomato leaf miners worldwide (Biondi et al., 2018; Roditakis et al., 2018). Guedes *et al.*, (2019) have recorded up to 60 instances of insecticide resistance to 24 insecticides worldwide in the Arthropod Pesticide Resistance Database (https://www.pesticideresistance.org). Insecticides such as organophosphate and pyrethroid, abamectin, indoxacarb, Spinosad, diamide and cartap (Guedes et al., 2019; Silva et al., 2019), and a possibility of pesticide resistance in Kenyan tomato growing regions due to the excess use of synthetic pesticides. The practical implications of the sustainable use of synthetic pesticides and the prospects necessitate the exploration of novel, sustainable alternatives for the management of tomato leaf miners. Natural insecticides (biopesticides) are thus compatible as they are eco-friendly owing to their rapid environmental degradation (Souto et al., 2021).

Biopesticides are derived from natural products, including potassium bicarbonate, plant extracts, and essential oils. Over the past few years, plant-based essential oils (EOs) have become a popular choice as a substitute for synthetic insecticides for controlling insect pests (Stevenson et al., 2017). These insecticides are natural as they are derived from plants and contain a variety of bioactive chemicals (Javier et al., 2017; Pavela & Benelli, 2017).

In addition, essential oils have specific toxicity to control pests without destroying beneficial organisms. This is because secondary metabolites act like the defenses used by the plant to protect against attack by external agents; hence, their biosynthesis is increased only under conditions of stress. The essential oils (EOs) exhibit a wide range of actions against insects: they can act as repellents and antifeedants (Z. Yuan & Hu, 2012), attractants, or; they also may inhibit respiration, inhibit oviposition and decrease adult emergence by ovicidal and larvicidal effects (Dar et al., 2021; Murugesan et al., 2016). It is imperative to consistently explore plant species with potent pesticides, notably *Lantana camara*, which is abundant and invasive in Kenya and commonly known as Lantana.

Lantana is a source of essential oils and compounds with unique biological activities (Javier et al., 2017; Murugesan et al., 2016), which are a promising source for a new biopesticide (Begum et al., 2014). Despite having these valuable properties, they have remained neglected and are less known to agro-industries (Murugesan *et al*., 2016). Recent studies have discovered that Lantana oils have insecticidal properties (Murugesan et al., 2016; Rajashekar et al., 2014). (Z. Yuan & Hu, 2012) reported that the lantana oil leaves have repellent and antifeedant properties and acetylcholine inhibition against insect pests. In their study, Murugesan *et al*., 2016) on Lepidopteran larvae, indicated that the efficacy of essential oil is significant in terms of insecticidal properties. On the other hand, (Javier *et al.,* 2017) reported that the essential oils showed remarkable insect growth regulatory activities against *S. litura*. Chau, Tu and Quoc, (2019) also found significant insecticidal and antifeedant activities against *S. litura* and *P. xylostella* suggesting a future exploitation for the isolation of active compounds to develop a new botanical formulation and Deshmukhe, Hooli and Holihosur, (2011) also concluded that it has the potential to be exploited as a botanical insecticide for cutworm management.

The biological activities of the essential oils from Lantana are derived from the various SMs, including monoterpenoids, sesquiterpenoids, triterpenes, iridoid glycoside, flavonoids, alkaloids and phenolic compounds (Adjou et al., 2012). The presence and concentration of the active compounds and their effectiveness vary considerably (Nea et al., 2020), a phenomenon that may limit the widespread commercial exploitation of the plant. The differences in the active compound profile

could be due to the environmental conditions in the growing areas (Moustafa et al., 2016). Prior studies have found that plants of the same species grown under diverse ecological conditions exhibit significant variations in SM accumulation (Nea et al., 2020). For instance, similar variation has been observed among Lantana plants obtained from various agroecological locations in Kenya (Musyimi et al., 2017; Syombua, 2015). Therefore, studying these variations is helpful in the chemical characterisation of plants of the same species collected from different agroecological zones (AEZs) and growing in different types of soils.

Plant nutrients can alter many medicinal plants' essential oil yield and quality (A. K. Khalid, 2015; Sharafzadeh, 2012). Yadegari, (2015) further showed that essential oil production may be affected positively or negatively by the form, type and amount of fertilisers used during production. The interaction of these elements is more robust than their action (Nurzyńska-Wierdak, 2013). For example, the concentration of germacrene D in basil essential oil is dependent on the rate of nitrogen applied and the interaction between Nitrogen (N) and potassium (K) (Nurzyńska-Wierdak et al., 2011). This relationship is significantly translated into quantitative and qualitative changes in volatile substances in essential oil plants (Puttanna et al., 2010).

Water stress can depress plants' growth and development by altering their biochemical properties (Zobayed et al., 2007). Various studies have shown that medicinal plants exposed to water stress produced a higher concentration of SMs than those cultivated under well-watered conditions (Alinian et al., 2016; Kleinwächter & Selmar, 2015). The combined effects of water availability, nutrient application and environmental factors on medicinal plants are essential for developing field cultivation for this plant to produce high-quality and quantity metabolites for pest management.

1.2 Statement of The Problem

Agricultural pests and diseases are among the many factors affecting food production and food security in Africa, leading to total crop failures (FAO et al., 2023). Crops are often threatened by pests, which can harm their growth and overall quality. To protect crops from pest attacks, farmers frequently rely on quick pest management

options, such as synthetic chemicals (Silva et al., 2019), driven by the need to increase food production for the growing population (Sarkar et al., 2021). The production of tomatoes has been hampered by the increase of infestation from *Tuta absoluta* (tomato leaf miner) leading to increased use of synthetic pesticides (Desneux et al., 2022).

In Kenya, tomato leaf miner infestation is mainly managed by synthetic chemical insecticides (Kinyanjui et al., 2021; W. P. Nderitu et al., 2018; Victor & Mwangi, 2019). The pesticides used for controlling these pests are very costly to farmers. This leads to the misuse of synthetic pesticides resulting in harmful effects on humans and the environment and toxicity to non-target organisms (Özkara et al., 2016), thus impacting negatively on the biodiversity (Damalas & Koutroubas, 2018) and the continuous usage has led to the rapid development of pest resistance (Guedes et al., 2019; Roditakis et al., 2018) to the few reasonably priced insecticides and pest resurgence (Kumar et al. 2021). Most synthetic pesticides do not easily biodegrade, accumulating in the environment polluting soil, and groundwater, and depleting the ozone layer posing a persistent threat to non-target organisms in many aspects (Ruomeng et al., 2023). Furthermore, the use of synthetic pesticides has negatively affected farmers involved in export trade especially of tomato produce (Sarkar et al., 2021). The disadvantages associated with the misuse and overuse of synthetic pesticides, coupled with the increasing demand for organically produced foods have stimulated the search for alternative pest management options (Jacquet et al., 2022).

Rich indigenous knowledge exists among the farmers, and many have used plants known to control pests for decades and are widely recognised as an alternative to synthetic pesticides (Grzywacz et al., 2014). However, little progress has been made towards developing new products more effective based on scientific research (Benelli et al., 2018). With the few available biopesticides, the prices are higher, so farmers are not able to afford fewer toxic alternatives. Nevertheless, biopesticides have not been fully adopted due to challenges in formulation and commercialization which are attributed to a lack of chemical data and positive controls (Isman, 2017; Pavela & Benelli, 2016). Therefore, the production of affordable and available alternatives is vital for more sustainable pest management.

The lantana plant has a long history of use for medicinal purposes in Africa (Anjarwalla & Belmain, 2016). While research shows that it is one of the plants with great potential for use in pest management, for both pre- and post-harvest against a broad range of insects (Murugesan et al., 2016) it remains unexploited in Kenya. There are very few reports that document the biological properties of this plant against pests scientifically. Also, no significant advances have been made in this genus to exploit or enhance its utility at subsistence and commercial levels. The gap in the exploitation of the lantana plant as a biopesticide lies in the usage, what pests to use against, concentration rates, and the use interval. The big question is, how effective is the lantana plant in the management of tomato leaf miners? Therefore, the objective is to research its use as a natural biopesticide to encourage the development of new affordable oil-based biopesticides for the management of tomato leaf miners.

Most medicinal plants used as biopesticides are collected from the wild, and their metabolite pools continue to be shaped by natural, external environmental factors and agronomic management (Borges et al., 2017; Pereira et al., 2019). The natural variability of these factors could determine their quality in terms of usage for pest management. Lantana plant essential oil collected from various regions shows marked differences in the accumulated secondary metabolites (Nea et al., 2020). However, does this shape the metabolite pool of the precise needs in terms of type, quantity, quality and optimum bioactivity? Do high concentrations of particular classes of compounds translate to better activity? Experiments on the biological activity of lantana plant efficacy on potential pests as affected by abiotic factors are still scarce. This differences in quality could be a limitation for exploitation of this plant as an alternative biopesticide for pest management.

Domestication and improvement of identified wild lantana plants through propagation to improve the content of the active compounds, in addition to developing appropriate husbandry practices, including plant nutrition and watering practices are not detailed. Findings on secondary metabolite accumulation and concentration as influenced by nutrient and water rates are lacking. Therefore, the question remains, do we have room to manipulate these factors to favour the desired

types and amounts of secondary compounds accumulated in the plant? Would domestication of this plant lead to high yield of the target pesticidal compounds.

Lastly, biopesticide commercialisation is highly dependent on the availability of plant sources in large quantities and the cultivation of the plants. Also, cultivation of plants to produce botanical pesticides requires vast land, thus highly competitive with food production for arable lands. Considering the huge volumes of lantana plant material that will be needed to produce the biopesticide, indiscriminate collection of lantana plants in the wild will thus decrease its essential oil quality. This greatly limits their commercial production, and the overexploitation of source plants will raise concerns about their sustainability, highlighting the need for advanced research to increase production and productivity.

Answers to these questions could ensure improved quality of raw materials for adopting commercial propagation of lantana for the tomato leaf miner pest management.

1.3 Justification

Continuous use of synthetic pesticides has resulted in negative effects such as pesticide resistance, pollution, health hazards and loss of biodiversity, while the adoption of biopesticides results in a healthy environment and sustainable agriculture (Mkindi et al., 2017; Stevenson et al., 2017). Therefore, making alternatives affordable and available with fewer external inputs, is crucial for a transformation to a more sustainable pest management model.

Pesticidal plants are widely available at minimal or no cost and have been used for centuries. Pesticidal plants are broadly safer to use and handle than synthetic pesticides, are environmentally benign, and typically less harmful to beneficial insects (Grzywacz et al., 2014). The swift biodegradable nature of biopesticides is good and provides a safer alternative. The biopesticides present other modes of action including suffocation, mating disruption, antifeeding and desiccation (Ghodake *et al*., 2018). However, experiments on the biological activity of lantana on potential pests are still scarce. This study, therefore, seeks to bridge this

knowledge gap to facilitate the adoption of the lantana plant for the management of tomato leaf miner.

The precise knowledge of optimum conditions for plant growth and biosynthesis of desired secondary metabolites is necessary to be investigated. Since the content of bioactive compounds may is affected by environmental factors. Therefore, exploratory research to understand the influence of environmental factors on the accumulation of the secondary metabolites on lantana plant is necessary. This knowledge would assist in the manipulation of the plant's environment during growth and development to enable the accumulation of desired compounds.

Additionally, agronomic practices applied for its commercial production and the findings on secondary metabolite concentrations, as affected by nutrient and water supply, are lacking, and their impact on constituent accumulation has not been investigated. Hence fundamental research on secondary metabolites synthesis is required, serving as a basis for more practical investigations targeting the increase in compound concentration in this plant. Moreover, water and nutrient supply may be controlled more precisely during cultivation to steer both the primary and secondary metabolism of this plant for their propagation of high-quality raw material. Therefore, justifying raw material production with more desirable properties for pest management.

More research is required to improve the exploitation of lantana plant through domestication to improve the content of the active compounds, in addition to developing appropriate husbandry practices, including plant nutrition and agronomic practices such as watering. Producing biopesticides requires a significant amount of plant material. To avoid competing with food crops, it is recommended to cultivate source plants on marginal lands not suitable for arable agriculture. This way, largescale production of lantana plants can generate income to sustain the livelihoods of communities in semi-arid areas.

The research study provides information on the effect of essential oils from Lantana on a selected pest. Understanding the insecticidal properties of the plant informs the public about its usefulness in controlling agricultural pests. This information is helpful in incorporating the product into an integrated pest management program.

Therefore, it is critical to develop a new biopesticide product to deal with the resistance of tomato leaf miners and other pests. Sustainable use of pesticidal plants can be achieved by improving their propagation, harvesting, and conservation. Increased use of pesticidal plants can contribute to increasing agricultural productivity, sustainable livelihoods, and reduced environmental pressure.

The integration of natural pest control products in managing tomato leaf miner will enhance the quality and safety of tomato produce. This will increase its acceptability in niche markets, thereby boosting international trade. Additionally, it will aid in the conservation of biodiversity, and protection of the environment and human health.

1.4 Hypotheses

H1: The leaf essential oils of *L. camara* from different agroecological zones and soil types do not differ in terms of secondary metabolite profiles.

H2: The leaf essential oil of *L. camara* has no insecticidal activity against *Tuta absoluta.*

H3: Plant nutrients and water levels do not affect the secondary metabolite profiles of essential oils from *L. camara* inder controlled environment.

1.5 Objectives 1.5.1 Overall Objective

The general objective of this study is to characterise the essential oil compounds and optimise water and potassium supplementation for the production of *Lantana camara* for *Tuta absoluta* management.

1.5.1 Specific Objectives

1) To characterize the secondary metabolite profiles of the leaf essential oils of *Lantana camara* from various agroecological zones and soils in Kenya.
2) To evaluate the efficacy of the leaf essential oils of *Lantana camara* from various agroecological zones of Kenya against *Tuta absoluta.*
3) To assess the influence of Potassium and water application rates on secondary metabolite profiles of the *Lantana camara* plant.

CHAPTER TWO

LITERATURE REVIEW

2.1 Description, Origin and Distribution *Tuta absoluta*

The tomato leaf miner *Tuta absoluta* (Lepidoptera: Gelechiidae), recently reinstated as *Phthorimaea absoluta* Meyrick (Corro Chang & Metz, 2021). A multivoltine moth, exhibiting high reproductive potential that promotes rapid growth of its population (Biondi et al., 2018).

Tomato leaf miner has a life cycle of four stages: the egg, the larvae, the pupa and adult, with a sexual dimorphism from pupae stage and adults are nocturnal (Cherif & Verheggen, 2019). The female pupae are distinguished by two small tubercles present on the eighth abdominal segment and are heavier and bigger than males (Genç, 2016). The female release sex pheromones; a mix of tetradecatrienyl acetate (90%) and tetradecadienyl acetate (10%) to attract male for mating (Attygalle et al., 1996).

The reproduction mechanism involves sexual reproduction and deuterotoky parthenogenetic process in which males and females are produced (Caparros Megido et al., 2012). The life cycle of the tomato leaf miner is characterized by: Oviposition taking place on leaves, veins, stems, sepals and fruits. A female laying around 260 eggs during its life cycle. Deposited eggs are oval-cylindrical (0.4mm length; 0.2mm diameter), hatching in about 7 days; The larvae stage lasts 8 days with four instars; the pupa stage lasts 10 days: pupa is brown (4.3 mm in length and 1.1 mm in width); the adult stage, female lives 10-15 days and male lives 6-7 days (Huda et al., 2020; Yadav et al., 2022).

Tomato leaf miner is native to South America countries including Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador, Paraguay, Peru, Uruguay, and Venezuela (Biondi et al., 2018; Desneux et al., 2011). It was identified for the first time in 1917 in Peru and documented as tomato pest (Zappalà et al., 2013). Tomato leaf miner is spreading rapidly across regions from Europe, Mediterranean, Middle East, South Asia and Africa (Campos et al., 2017; Ponti et al., 2021).

In Africa, it was reported for the first time in 2008 in the northern African countries; Algeria, Tunisia and Morocco and thereafter, it was reported in Libya in 2009 (Campos et al., 2017). Tomato leaf miner has been reported in 41 of the 54 African countries (Mansour et al., 2018; Rwomushana et al., 2019) and still spreading to central and southwestern African countries. In Kenya, the pest was formerly reported in 2014 in tomato fields at the major growing zones of tomato (IPPC, 2014). Since then, the pest has spread very fast and is threatening tomato production in the country (P. W. Nderitu et al., 2020; Victor & Mwangi, 2019).

2.2 Host Plant Range and Impact of *Tuta absoluta*

Tomatoes have been reported as the primary host of tomato leaf miner; however, this pest can feed and develop on other Solanaceae plants, such as potatoes (*Solanum tuberosum* L.), eggplants (*Solanum melongena* L.), and tobacco (*Nicotiana tabacum* L.) (Caparros Megido et al., 2012; Rwomushana et al., 2019). Other host solanaceous species, including sweet peppers (*Capsicum annuum* L.), sweet cucumber (*Solanum muricatum* Aiton), and several other solanaceous and nonsolanaceous weeds, such as black nightshade (*Solanum nigrum* L.), feld bindweed (Convolvulaceae: *Convolvulus arvensis* L.) and lambsquarters (Amaranthaceae: *Chenopodium album* L. (Biondi et al., 2018; Campos et al., 2017; Soares et al., 2019). A recently compiled list of plant species harboring tomato leaf miner was updated including 44 species from nine botanical families and 15 species of economically important crops, and spontaneous weeds in both cases (Cherif & Verheggen, 2019). Though several new plant species have been identified as hosts following the arrival of the tomato leaf miner in Europe, Asia and Africa, Solanum species is the more suitable host plants (Cherif & Verheggen, 2019; Desneux et al., 2011). The tomato leaf miner uses other solanaceous plant species for oviposition, to ensure its survival throughout the year, even in the absence of tomato crops. (Cherif & Verheggen, 2019).

Economic losses caused by tomato leaf miner includes a reduction in production, since the moth infest the whole plant, resulting in severe yield losses (Biondi et al., 2018; Desneux et al., 2011, 2022); more chemical or non-chemical control practices have to be included in the management of this pest which has resulted in the development of high insecticide resistance therefore, additional management costs

(Campos et al. 2017; Biondi et al. 2018); this further leads to the risk of restricted trade or ban the importation of tomato from the infested countries (Desneux et al. 2011).

The impact of the tomato leaf miner in new environments is exemplified by the situation in Kenya (Aigbedion-Atalor et al., 2019). The spread of this species has led to a decrease in tomato yield and an increase in production costs (Nderitu et al. 2020), as well as a greater vulnerability for human and environmental well-being. This is due to a three-fold increase in the use of pesticides to control the pest (Aigbedion-Atalor et al., 2019).

2.3 Management and Control of *Tuta absoluta*

The tomato leaf miner is a pest that can be found in both its native and exotic ranges, and several approaches have been developed to monitor and control it (Rwomushana et al., 2019). In Africa, the most common methods for managing the pest include using pesticides, pheromone traps, destroying infected plants, staking, organic pesticides, and crushing larvae. Other approaches that can be used include mating disruption, microbial pesticides, botanicals, netting technology, biocontrol, and integrated pest management (IPM) strategies (Rwomushana et al., 2019).

2.3.1 Chemical Control

Chemical control remains the primary management option to control tomato leaf miner (Guedes et al., 2019; Han et al., 2019; Roditakis et al., 2018). Chemical pesticides continue to be important in the management of tomato leaf miner since they are the first line of defense, providing a quick fix to pest pressure (Tarusikirwa et al., 2020). The chemical method is more effective and faster as compared to other methods but it is harmful to the environment. Inappropriate use of these chemical pesticides leads to the development of resistance among the pests causing substantial outbursts, which increases the cost of cultivation and heavy losses (Ghodake et al., 2018). If used correctly and with a combination of other pest management strategies, they can be incorporated into a sustainable, integrated pest management program

(Colmenárez et al., 2022). Though, overapplication of insecticide can lead to resistant populations (Roditakis et al. 2018).

These insecticide classes include organophosphates (chlorpyrifos), pyrethroids (deltamethrin, lambda-cyhalothrin, bifenthrin, permethrin), oxadiazines (indoxacarb), spinosyns (spinosad, spinetoram), avermectins (abamectin, emamectin benzoate), pyrroles (chlorfenapyr), benzoylureas (diflubenzuron, lufenuron, novaluron), diamides (chlorantraniliprole, flubendiamide), diacylhydrazines (chromafenozide, methoxyfenozide, tebufenozide), semicarbazones (metaflumizone), tetranortriterpenoids (azadirachtin) and nereistoxin analogs (cartap) (IRAC, 2014). Tomato leafminer was initially controlled using organophosphates (OPs); these were gradually replaced by pyrethroids and, more recently a broader suite of chemicals including abamectin, acylurea, insect growth regulators, tenbufenozide, and chlorfenapyr were introduced (Guedes et al., 2019). The use of insect growth regulators insecticides and, more recently, novel chemical molecules such as spinetoram, cyantraniliprole, flubendiamide, and spinosad, has taken the place of such insecticides as organophosphates, pyrethroids, cartap, and abamectin, thereby diminishing their effects on the environment (IRAC, 2014).

In Kenya two brands of insecticides, Belt SC 480 (Flubendiamide 480 g/L) and Tihan OD 175 (Flubendiamide 100 g/L + Spirotetramat 75 g/L) from Bayer East Africa Limited were earlier recommended by Kenya Agriculture and Livestock Research Organization (KALRO). Neem oil is recommended and applied to cover all parts of the plant since the pest feeds on any part. Today the most effective insecticide used in Kenya against the pest is Corragen@205C (3-bromo-N- [4-chloro-2-methyl-6-(methylcarbamoyl) phenyl]-1-(3-3chloropyridin-2-yl)-1H-pyrazole-5-carboxamide) from Syngenta (Nderitu et al. 2018). Others are Warrant (Imidacloprid), Radiant (Spinosad), Tracer (Spinosad), and Thunder Nderitu et al. 2018; Victor & Mwangi 2019).

In all regions where the tomato leaf miner has invaded, increased application of different types of insecticides and increase in the number of times these chemicals are applied has been recorded (W. P. Nderitu et al., 2018; Victor & Mwangi, 2019). Resulting in the health risks of the tomato consumers, destruction of the ecosystem,

high cost of production, increased tomato prices, banning of tomato products trade and disruption of integrated pest management programs of other tomato pests (Victor & Mwangi, 2019).

2.3.2 Biological Control

There have been several studies conducted to determine which natural enemies are capable of attacking tomato leaf miner. Parasitoids and mirid predators are among the natural enemies that have been identified as potential biological control agents (Mansour et al., 2018). The strategies proposed by Mansour et al. (2018) for implementing a biological control program include mass production of natural enemies for regular releases in affected fields, adaptation of specific cultural practices for conservation biological control, and introduction of exotic natural enemies for classical biological control. Currently, augmentative biological control is the only method being used, and only in Northern Africa, where egg parasitoids and predatory bugs are being exploited (Rwomushana et al., 2019).

Field parasitism of the tomato leaf miner is low in Africa. However, some success has been reported in Tunisia with biological control, where releases of either the parasitoid *Trichogramma cacoeciae* or *T. bourarachae* resulted in a reduction of 87% and 78%, respectively, in leaf damage in greenhouse tomato (Zappalà et al., 2013). Additionally, the predatory mirid *Nesidiocoris tenuis* was shown to significantly reduce the density of tomato leaf miner eggs in Tunisian greenhouses (Chailleux et al., 2014).

Classical biological control for this pest may also become a reality in Africa in the near future. The International Centre of Insect Physiology and Ecology imported the larval parasitoid *Dolichogenidea gelechiidivoris* into Kenya, which has shown promising results as a potential classical biological control candidate against tomato leaf miner.

2.3.3 Cultural Control

Management such as ploughing, manuring, irrigation, crop rotation, solarisation, as well as specific management by the elimination of symptomatic leaves and the

destruction of infested tomato plants have all been used to control tomato leaf miner. Sequential planting with or near to other solanaceous crops that serve as shelter and food sources for tomato leaf miner such as potatoes, aubergine and pepper should be avoided and the removal of alternative reservoir hosts such as nightshades (*Solanum nigrum, Atropa belladonna, Solanum dulcamara*) (Sylla et al., 2019). In greenhouses, one of the management tactics used to reduce the initial level of populations is to keep infested greenhouses closed after harvest to prevent the migration of adults to open-field crops. Alternating host crops, mainly tomato, potato and aubergine, with non-host cultures can ensure a long-term reduction in pest pressure (Sylla et al., 2019).

2.3.4 Biopesticides

Biopesticides are competitive subclass of pesticides that are naturally occurring organisms or compounds that suppress the growth and proliferation of pests' population by diverse mechanisms of action, excluding those that interfere with pests' nervous systems (Nuruzzaman et al., 2019; Wattimena & Latumahina, 2021). They are categorized into three groups: microbial biopesticides, and biochemical biopesticides (Fenibo et al., 2021).

In the pursuit of finding effective ways to control pest insects, researchers have developed biopesticides extracted from plants (Pavela & Benelli, 2016). What sets biopesticides apart from synthetic chemical pesticides is their mode of action. The modes of action of biopesticides are diverse and can include mating disruption, antifeeding, suffocation, and desiccation, in addition to neurotoxic effects on pests (Ghodake et al., 2018). A number of biopesticides have shown promising results against the tomato leaf miner, with essential oils from citrus peel, cardamom, and ajwain demonstrating significant pest repellent and mortality efficacy (Campos et al., 2017; Chegini et al., 2018). Several plants' extracts have been used to control tomato leaf miner. For example, azadirachtin, an extract from neem (*Azadirachta indica*) seeds, is used as a contact insecticide. the neem extract has also resulted in a 24.5% egg and 86.7 to 100% larval mortality of the pest at different concentrations (Elshiekh et al., 2014). From the same study, petroleum ether extract obtained from Jatropha, achieved 18% to 25% egg and 87% to 100% larval death after being

exposed for four days in different concentrations. Other plants (garlic, basil, thyme, castor bean, eucalyptus, chinaberry, geranium and onion) have also been found to exhibit insecticidal activity with different efficacies against tomato leaf miner larvae (Abd El-Ghany et al., 2016). A research using the jojoba seeds, showed that simmondsin extracts significantly reduced (at 5%) tomato leaf miner populations and concluded that they were effective in controlling the 2nd instar larvae (Abdel-Baky & Al-Soqeer, 2017).

A challenge with the use of biopesticide, is the numerous variabilities in efficacy, especially in homemade formulations, as a result of poor standardization and quality control. Other limitations, such as optimized and authorized formulations, for the practical inclusion of essential oils into IPM programs are still occurring (Pavela & Benelli, 2016). Moreover, the compatibility of essential oils with effective biocontrol agents should be evaluated case by case (Soares et al., 2019). These reasons, together with cost, efficacy and reliability, may hamper the use of this control option.

2.4 The Lantana camara Plant

2.4.1 Taxonomy, Ecology and Morphology of *Lantana camara*

Lantana camara Linn. species belongs to the family Verbenaceae within the order of Lamiales and the genus Lantana (Gisin, 2019). Historically, the taxonomy of the genus Lantana has been very complicated, and this hinders their identification in the field (Passos et al., 2009; Urban et al., 2011). Innumerable taxonomic problems are reported and frequently classified incorrectly (Passos et al., 2009). Based on floral and carpological features, the Lantana genus has four sections: Camara, Calliorheas, Rhytocamara and Sarcolippia (Costa et al., 2010). The Lantana complex in the Camara section includes the weedy lantana referred to as *Lantana camara* Linn.

Lantana is a highly variable species with high genetic diversity (Goyal & Sharma, 2015). The widespread hybridisation, the changes in the shape of the inflorescence with age, and the colour variation of the flowers with age and maturity complicate the taxonomic identification of the species in the complex (Costa et al., 2010; Goyal & Sharma, 2015). Sanders, (2006) acknowledges that lantana that grows in the wild differs morphologically, karyologically, physiologically and ecologically from those

priced for their horticultural value, multicoloured flowers. Therefore, weedy naturalised and invasive complex constituents, referred to as *Lantana camara* L. (sensu lato), merit a deliberate taxonomic delineation (Sanders, 2006).

Lantana in Kenya is naturalised, and the distribution is still increasing, with it infesting most parts of the country (Shackleton et al., 2017). Human activities, such as logging, facilitate the spread of lantana, and other forms of habitat disturbances and climate change exacerbate the invasion (Mungi et al., 2018). Lantana has many traits that make it a good invader, including all-year flowering and fruit production, inadequate moisture and light. Adaptation to long-range dispersal by birds and mammals; high establishment rates; the ability to coppice; poisonous leaves; high phenotypic plasticity; the ability to hybridise; vegetative reproduction; and allelopathy (Shackleton et al., 2017). It occurs in habitats ranging from wastelands, rainforest edges, disturbed forests, roadsides, urban and rural homesteads, and other sites. (Enloe et al., 2018)

The plant grows luxuriantly at altitudes from the sea level to 1800 ms and thrives very well under precipitation ranging from 750 to 5000 mm per annum. It can adapt to different kinds of soils (rich and poor soils, gravel and laterite). It does well under optimal temperatures but is susceptible to low temperatures and frosts. It is drought-resistant and light-loving but cannot tolerate shades; therefore, it does not survive in dense forests (Negi et al., 2019). This broad adaptation ability to different conditions (climate and soils) has made the plant disperse widely and adapt differently to their environment.

The morphology of lantana is very distinct in different regions of its naturalised range compared to the native range (Goyal & Sharma, 2015). Lantana is a perennial, multi-stemmed, deciduous, thorny shrub growing to an average height of about 2m. The leaves are ovate and oppositely arranged. They are bright green on the upper surface and hairy and pale green below, and serrated leaf blades (Plate a). It feels like fine sandpaper (Sankaran, 2013). Flower heads have many smaller flowers. Each flower is tubular-shaped and has four spreading lobes (petals), changing colour with age (Plate b). The colours would be a combination of white, yellow, orange, red, or pink, with a robust aromatic smell that characterises the leaves and flowers. The fruit

is a tiny, one-seeded berry about 6–8 mm. It looks green when young and unripe and shiny black or purple when fully ripe (plate 2.1). The stems have bristly hairs when green (S. K. Rana et al., 2019) and are armed with small prickles (Enloe et al., 2018) (Plate 2.1e). The shallow root system has a short taproot and lateral roots branching out to form a mat (Sankaran, 2013) (Plate 2.1e).
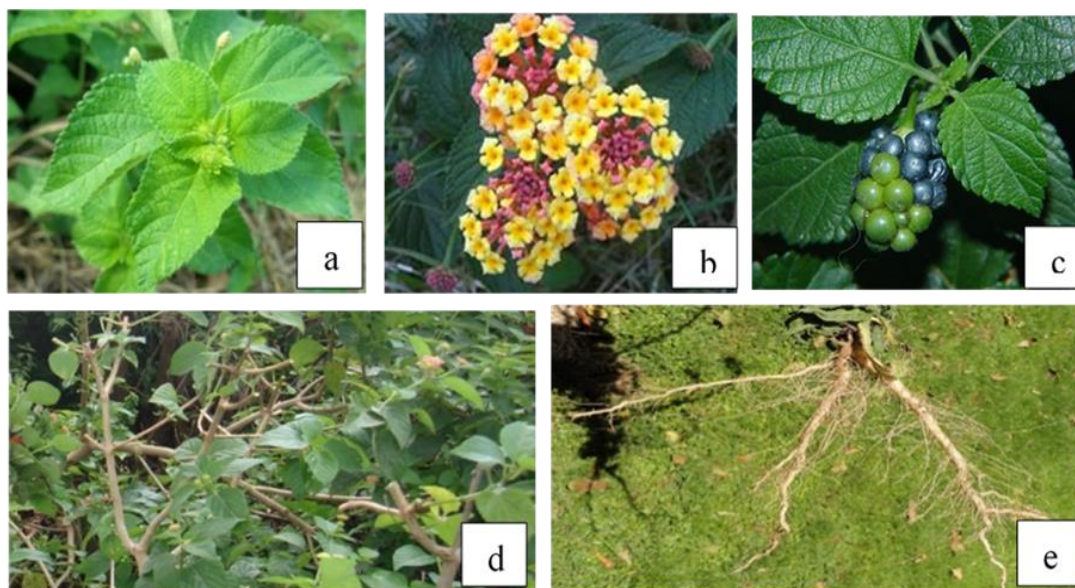
Plate 2.1: Morphology of Lantana camara Linn. a) Leaves, b) Flowers, c) Berries (Ripe and unripe), d) Stem, e) Root system

2.5 Bioactivity of *Lantana camara* Essential Oils

Plants synthesize the biologically active compound, making them an important resource for biopesticide production. Essential oils have numerous advantages as a candidate for novel biopesticides; they are easy to extract, are biodegradable and hence eco-friendly, have low persistence in soil and water, and require less stringent approval and regulatory mechanisms due to their long usage history (Gakuubi et al., 2016) .

Lantana is an important plant of the family Verbenaceae, consisting of essential oil with desirable properties making it an excellent source of commercially important oil that can find extensive use in agrochemicals ((Kaur et al., 2017) and pharmaceutical industry (Delgado-altamirano et al., 2019). The essential oil from lantana can be

among the world's top traded oils because of its abundance in a wide range of climatic regions.

Lantana is used indirectly to perform a variety of services through their essential oil as insect/pest repellent and as a pesticidal agent (Murugesan et al., 2016). The Lantana oil has the potential of being rated as superior in quality if the extraction process and propagation are standardized. It has an advantage, because of the multipurpose uses in both agriculture and pharmaceutical industries.

In recent history, the essential oils of lantana have been extensively researched for various bioactivity properties and reported to have insecticidal, antibacterial, antifungal, nematicidal, acaricidal and fumigant activity against a wide range of pests and diseases (Murugesan et al., 2016). The lantana leaves have repellent and antifeedant properties against insects (Zhonglin. Yuan & Hu, 2013). It is highly regarded as a rich plant in medicinal values and has been incorporated into people's culture.

2.5.1 Insecticidal activity of *Lantana camara* Plant Essential Oil

Insecticidal activities of lantana essential oils have been evaluated and reported. For example, (Murugesan et al., 2016) tested the essential oils on *Hyblaea puera* and *Ahevidae fabriciella* (Lepidoptera) and concluded that the essential oil expressed insecticidal and antifeedant properties. In other studies, the essential oil of lantana showed insecticidal properties against 3rd instar larvae of *Helicoverpa armigera,* causing 56% inhibition. Lantana was also found to be used effectively in the control of *Spodoptera litura* (Kasmara et al., 2018). Costa et al. (2010) conducted a study to test the larvicidal activity of lantana leaf essential oils against *Aedes aegypti* larvae and found that it has great potential as a larvicidal agent in pest control. The oil was also tested on other pest species. (Ayalew, 2020) discovered that the oil extracted by three-solvent fraction had a direct repellent and toxic effect on weevils, resulting in an increased percentage of mortality. Furthermore, a study in Kenya using lantana essential oils against *O. americanum* demonstrated its effectiveness as a bio-organic post-harvest pest control agent in beans Syombua, (2015), with Zandi-Sohani et al., (2012) agreeing that the oil can be useful as an alternative for bean protection against

C. maculatus. Another researcher found that lantana essential oil provided better protection for chickpeas from *C. chinensis* than the organophosphate insecticide malathion in their experiments, with a protection rate of 94.05% compared to 90.75% (Kumar, 2021).

Apart from that, lantana active ingredients have acetylcholine inhibition properties against insect pests (Zhonglin. Yuan & Hu, 2013). The essential oil causes rapid neurotoxicity in insect pests due to the interference with the neuromodulator octopamine, acetylcholinesterase, GABA-gated chloride channels, and inner cell membranes (Ntalli et al., 2019). In addition, they disrupt the cytoplasmic membrane and cell wall leading to lysis and intracellular compounds' leakage or uptake of inorganic phosphate and K+ leakage (Pavela & Benelli, 2016).

The biological activity of the essential oils of lantana has not been studied on tomato leaf miner. Although a lot has been done on the laboratory bioassay with many other pests with promising results, so far only one product has been isolated and patented as a modern biopesticide "Tree PAL[®]" (Murugesan et al., 2016) for the control of the nursery forest tree defoliators in India. Through the documented evidence of the effectiveness of this weed, more research is called for to validate this plant as a new-generation crop for pest management.

All these studies indicate the potential this plant has in the control of cutworms, among them is tomato leaf miner. Therefore, this study would be beneficial for the future utilization of the *L. camara* essential oil in the management of tomato leaf miner.

2.6 Plant Secondary Metabolites and Chemotype Variations

Plant secondary metabolites (SMs) are a diverse group of compounds that contribute to many important biological and ecological functions (Divekar 2022). They are secondary metabolites because the plants that manufacture them do not need them for growth and development and are not directly essential for basic photosynthetic or respiratory metabolism but necessary for the plant's survival (Divekar et al., 2022; Erb & Kliebenstein, 2020).

Plants have evolved a diverse set of defensive mechanisms that include a range of cellular, molecular, and biochemical responses that work together to counteract the deleterious effects of stressors (Fernie & Pichersky, 2015), by producing the chemical compounds. These chemicals play a crucial role in the interaction of plants with the environment, and these compounds are recently referred to as "specialized metabolites" (Yuan & Grotewold, 2020). Secondary metabolite accumulation often occurs in plants in response to different stimuli, such as osmotic stress, temperature, humidity, drought, bacterial, yeast, or fungal extracts, salicylic acid, or physical injury, among others, which are biotic or abiotic elicitors (Divekar et al., 2022; Khare et al., 2020). These secondary metabolites produced by plants are crucial because they serve a number of purposes including defense signaling molecules and additionally as bioactive compounds for medicinal usage (Isah, 2019).

Plants have a range of defence mechanism enrolled to fight under wide array of stress at the genetic or molecular level by various genes or transcriptional factors (TFs) (Li et al., 2020) . They provide a range of protection in plants against pathogens, insects, and predators according to the toxic nature of microbes and give plants characteristics such as color (Divekar et al., 2022). Some plants secondary metabolites have another function to help plants create communication with microorganisms, some have biotic stress tolerance, some mitigate abiotic stress due to drought, salinity, extreme temperature, and UV radiation (Khare et al., 2020). Secondary plant metabolites are also used in signalling and regulation of primary metabolic pathways (Li et al., 2020).

The metabolic physiology of plants is influenced by multiple stress factors, and it is difficult to attribute a particular change to a single stressor (Jan et al., 2021). Rather, plant responses to stress are complex and involve the activation of different signaling pathways depending on the type of stress encountered (Li et al., 2020). The genetic makeup of plants encodes numerous traits that collectively form an intricate immune system known as innate immunity, which enables plants to combat a variety of pathogens effectively (Anjali et al., 2023).

2.6.1 Biosynthesis of Secondary Metabolites

Secondary metabolites in medicinal plants are produced by different metabolic pathways under unfavorable conditions. Furthermore, the biosynthesis of secondary metabolites is highly interconnected/interrelated with the primary metabolism inside the plant cell (Zhao et al., 2023). The quantity and quality of these compounds are greatly influenced by the growing environment (Al-Khayri et al., 2023; Erb & Kliebenstein, 2020). The biosynthesis of these compounds is complex and dynamic, with more than one million secondary metabolites identified from terrestrial and aquatic plants (Li et al., 2020). Certainly, plants have a range of defence mechanism enrolled to fight under wide array of stress at the genetic or molecular level by various genes or transcriptional factors (TFs) (Anjali et al., 2023).

The shikimate pathway is the initial pathway for biosynthesis of aromatic amino acids; it is activated in stress conditions to produced tryptophan, tyrosine, and phenylalanine, which further enhance secondary metabolites biosynthesis (Jan et al., 2021). The accumulation of secondary metabolites in response to stress conditions is regulated at the molecular level by various genes and transcription factors (TFs) including those of the phytohormonal pathway (Li et al., 2020).

The precursors of metabolites are essentially produced in the Krebs cycle and shikimate pathway. The fundamental biosynthetic pathways of metabolites are conserved in the majority of plants, with most primary metabolites found in every tissue type (War et al., 2019). The maintenance of this metabolic core has led to the occurrence of a limited number of fundamental metabolic frameworks. Frequent glycosylation, methylation, hydroxylation, acylation, oxidation, phosphorylation, and prenylation, as well as fewer chemical alterations due to tailoring of enzymes, causes a wide range of modifications in basic structures (Jan et al., 2021). Based on biosynthesis pathways, secondary metabolites can be divided into three main groups: (1) terpenoids, (2) flavonoids, phenolic and polyphenolic compounds, and (3) nitrogen-containing alkaloids and sulfur-containing compounds (Crozier et al., 2007; Yadav et al., 2021). Amongst these three, the terpenoids or terpenes, are the largest class of secondary metabolites or specialized metabolites.

Terpenoids are a structurally diverse group of secondary metabolites in which each member has a core isoprene unit (War et al., 2019).They are synthesised through the condensation of isopentenyl diphosphate (IPP; C5) and its isomer dimethylallyl diphosphate (DMAPP; C5), and are classified by the number of five-carbon units present in the core structure (Jan et al., 2021). Major terpene classes include monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), triterpenes (C30), and tetraterpenes (C40), although lower and higher-order terpenoids (e.g., isoprene and natural rubber, respectively) also exist (Mahmoud et al., 2021). In general, the biosynthesis of terpenoids can be classified into the following four states: generation of general precursors IPP and DMAPP, production of specific isoprenyl diphosphates for various isoprenoid classes, the transformation of isoprenyl diphosphates to individual isoprenoids by terpene synthase (TPS) enzymes, and structural modifications catalyzed by other catalysts (Divekar et al., 2022).

Terpenes are important in plant defense, and others involved in primary functions, such as plant growth and development (Kumari et al., 2014). Terpenes comprise a diverse pool of compounds, with diverse functions including feeding deterrence, direct toxicity, or oviposition deterrence and can serve as attractants to pollinating insects (Divekar et al., 2022). Specialist herbivores can tolerate terpenoids and utilize them as an attractant to locate their host plants and as feeding stimulants (War et al., 2019). Terpenes indirectly protect plants by increasing the efficacy of herbivore natural enemies through the release of specific volatiles (Bergman et al., 2019). Some examples of terpenes that play an active role in plant defense are iridoids, benzoxazinoids, and volatile compounds, such as mono and sesquiterpenes, α-bisabolene and bisabolene and β-caryophellene (Mahmoud et al., 2021).

2.6.2 Plant Chemotypes

A chemotype is a chemically distinct entity in a plant, with differences in the composition of the secondary metabolites (Polatolu, 2013). Minor genetic and epigenetic changes with little or no effect on morphology or anatomy may produce large changes in the chemical phenotype (Benomari et al., 2020). Chemotypes are often defined by the most abundant chemical produced by that individual and the

concept has been useful in work done by chemical ecologists and natural product chemists (Polatolu, 2013).

A high variation in chemical defence profiles can be found within plant species, resulting in the formation of different chemotypes that vary in the composition of certain metabolites (Benomari et al., 2020; Tewes & Müller, 2020). These differences result in the formation of unique chemotypes that differ in the composition of specific metabolite classes. These differences in chemotypes are significant and offer great potential for the development of novel therapeutic agents, agricultural practices, and ecological insights. Therefore, it is crucial to explore these variations and their underlying mechanisms to harness their potential for the greater benefit of society. However, the bioactivity depends on the chemical profile of the plant (Tewes & Müller, 2020), thus careful analyses of the risks and targets should be conducted before use. For example, the composition of the essential oil of two plants of the same botanical species is not constant. Under the influence of external factors, it may even present different biochemical specificities (Benomari et al., 2020).

Some studies have shown that the presence of different chemotypes modifies the activity of the oil and its bioactivity (Benomari et al., 2020). The chemical composition of an essential oil varies with hybridization, environmental factors, and cultural practices (Polatolu, 2013; Tewes & Müller, 2020). For example, the essential oil of peppermint (Mentha x piperita L.) is rich in (-)-menthol, the enantiomer responsible for the well-known minty fragrance. However, the addition of nitrogen has been shown to increase biomass yield and delay flowering development, resulting in a higher yield of essential oil but with less menthol and more menthone. In contrast, the addition of potassium forces the plant to mature and decreases the yield of essential oil, which contains more menthol and methyl acetate (Baser & Buchbauer, 2015). This means that individuals of the same botanical species, with the same genome and phenotype, may differ in chemical composition and thus biological activities. Factors affecting the plant's composition such as time of harvest, vegetative cycle, soil quality, environmental factors, climate, altitude, and hygrometry are also to be taken into consideration (Benomari et al., 2020; Tewes & Müller, 2020; Polatolu, 2013).

2.7 Factors Affecting Plant Metabolism

The synthesis and proper accumulation of secondary metabolites are influenced spatially and temporal by the abiotic and biotic environment. The abiotic stress is responsible for the decrease in the production and yield of medicinal plants (Li et al., 2020). The plants interact with the surrounding environment during growth and development, coming in contact with different abiotic components like temperature, water, soil and nutrients. Secondary stresses generated by negative abiotic factors, such as drought or flooding, extremes of light and temperature and the presence of poor soil or toxic chemicals triggers the variation in the biosynthesis of secondary metabolites (Li et al., 2020; Y. Yuan et al., 2020; L. Yang et al., 2018). Thus, environmental agronomic management factors are crucial determinants for the biosynthesis and fluctuations in plant secondary metabolites (Verma & Shukla, 2015).

2.7.1 Temperature on Secondary Metabolite Biosynthesis

As one of the major weather variables temperatures can significantly influence the composition of secondary metabolites (Isah, 2019). Plants growing at elevated temperatures exhibit a decline in the photochemical efficiency of photosystem II, indicating increased stress (Li et al., 2020). The biosynthesis of secondary metabolites is also correlated with high temperature in plants (Verma & Shukla, 2015). Secondary metabolites can increase or decrease in response to elevated temperatures and this is dependent on the species and multiple factors. In this case high temperature downregulates or upregulates the responding genes and affects the growth and development of plants (Li et al., 2020). In such conditions of heat stress, modification of physiological and biochemical processes by gene expression changes slowly leads to the development of heat tolerance in the form of acclimation or adaptation of a plant to high temperature (Li et al., 2020; Yuan et al., 2020). Plant growth and the biosynthesis and the storage of secondary metabolites are also significantly hampered as a result of low-temperature stress (Verma & Shukla, 2015). Plants grown under low temperature exhibit significant alterations in various physiochemical and molecular processes (cellular dehydration, water uptake, and

metabolic reactions) that enable plants to survive low temperature stress, a phenomenon known as cold acclimation (Ashraf et al., 2018).

For instance, the emission of sesquiterpene compounds from seven pine species had a strong temperature dependency, in which the emission of β-caryophyllene, α-bergamotene, α-farnesene, and β-farnesene increased exponentially with temperature (Helmig et al., 2007). Similarly, the correlation between terpenoid yield and temperature was investigated for *Quercus rubra* and *Quercus alba* in warm conditions. The emission isoprene was twice in cold conditions (Bennett et al., 2010). In addition, an exponential increase with increasing temperature was observed in the emissions of many oxygenated monoterpenes except (E)-β-farnesene in *Picea abies* (Kivimäenpää et al., 2013). Though the biological properties and the chemical composition of lantana have been studied widely, there is no study on the correlation of temperatures with its chemical composition, which could be different according to environmental factors.

2.7.2 Light/Solar radiation on secondary metabolites contents

Light is indispensable to the biosynthetic course of a growing plant. The key factors related to light radiation include photoperiod (duration), intensity (quantity), direction and quality (frequency or wavelength) (Zoratti et al., 2014). Light plays a unique role in promoting plant growth and inducing or regulating plant metabolism. In response to light radiation, plants can adapt to the changes in circumstances by the release and accumulation of various secondary metabolites including triterpenoids, phenolic and flavonoids, and many of them, have high economic and utilization value due to biological properties. Photoperiod factors influence the growth and development of plants and thus regulate the biosynthesis of secondary metabolites (Yang et al., 2013). Studies showed that the duration of light radiation had a predominant role in regulating the levels of various secondary metabolites. In general, long-day sunshine can increase the level of secondary metabolites in plants (Yang et al., 2018). The exposure to full-day sunlight resulted in an increase in the contents of asiaticoside, madecassoside, flavonoids and chlorogenic acid in *C. asiatica* plants compared with those grown under shade (Moinuddin et al., 2012). These results indicate that the accumulation of triterpene and phenolic compounds

depends on duration and amount of daylight. However, no studies have been done to focus on the metabolite profiles of *Lantana camara* variation due to the influence of light and other environmental factors. Therefore, light is capable of redirecting the production and accumulation of active constituents in a plant.

2.7.3 Plant Nutrients on Secondary Metabolite Biosynthesis

Plants synthesizing essential oils are grown as medicinal, spice, aromatic, and cosmetic plants. Growing conditions, which sometimes largely determine raw material yield, also determine raw material quality and quantity. Nurzyńska-Wierdak, (2013) explain that fertilization can significantly modify both the content and composition of plant essential oils.

Supplemental plant mineral nutrition may provide a means not only to stimulate plant growth but also to influence the content and quality of secondary metabolites in medicinal plants. Plant nutrients are an important factor in determining the secondary metabolism and biological activity of a plant. Nitrogen (N), along with phosphorus (P) and potassium (K), is one mineral required by medicinal plants in large amounts for their proper growth and development. Many research works have been done on medicinal plants, and some evidence supports the influence of mineral nutrition, especially the significant macronutrients N (Lima et al., 2020; Peng & Ng, 2022; Walia & Kumar, 2021), P (Attarzadeh et al., 2020; Shiponi & Bernstein, 2021; Sun et al., 2022), and K (El Gendy et al., 2015; Sun et al., 2022), on the accumulation of secondary metabolites. In aromatic plants, terpene compounds are affected by macronutrients (Rioba et al., 2015). Variations in micronutrients can also affect the secondary metabolite profile (Rioba et al., 2015).

Variations in N, P, and K availability may influence resource allocation between primary and secondary metabolism, and consequently affect the concentration of secondary metabolites in the plant tissues (Lattanzio et al., 2009). The analysis of the metabolite content is essential to determine the effect of these minerals or their combination on the metabolism of secondary compounds. Thus, it is clear that standardization of nutritional doses particularly N, P and K for different agro-ecological conditions is essential for increasing the secondary metabolites of lantana.

The metabolite contents of lantana may vary due to the fertility status of that soil. However, precise knowledge of the relevance of defined nutrients for plant physiology, growth, and secondary metabolite biosynthesis of lantana is still lacking. Therefore, fertilization regimes should be evaluated not only for their agronomic advantage but also for the secondary metabolites profile content.

While the emphasis is on the availability of sufficient quantities of the major plant nutrients, the potential role of K nutritional supplementation must be considered. Potassium (K) is one of the critical primary nutrients and is considered the most abundant cation in higher plants which occurs in the centre of developing tissues and plays a significant role in plant physiology and enzymatic activity (Chrysargyris, Xylia, et al., 2017). However, the influence of K is another aggregate function played by nutrients in mitigating the adverse effects of abiotic stresses during the plant's growth (Hasanuzzaman et al., 2018). Several studies have demonstrated that K may play an active role in modulating secondary metabolites in plants. For example, (Ali et al., 2012) observed that total phenolics in blackberries decreased as K levels decreased. Likewise, (Troufflard et al., 2010) and Lubbe et al., (2010) also found that the content of oxylipins in *Arabidopsis thaliana* and galanthamine production in Narcissus bulbs was reduced under K-deficient plants, respectively. These results all suggest the importance of K in regulating the production of secondary metabolites in plants. Many researchers have evaluated the effects of K concentration on plant oil yields (Chrysargyris, Drouza, et al., 2017; K. Khalid, 2013). However, information on oil yield quality traits is scarce and lacks inconsistent data concerning the K fertilisation level's influence on cultivated medicinal plants. To enhance this knowledge, a study should be conducted to determine the influence of differentiated fertilisation with K on medicinal plant oil yield and secondary metabolite production (Abdelaziz et al., 2007).

2.7.4 Water on Secondary Metabolite Biosynthesis

Water availability is also one of the important factors that influence plant growth, development and production of secondary metabolites. The availability of water is a known factor related to the variation in the production of metabolites in plants (Arbona et al., 2013; Ramakrishna & Ravishankar, 2011). Water stressed plants close

their stomata and restrict photosynthesis and therefore, one might expect a negative relationship between the biosynthesis of SMs and water shortage. The effective role of water in the growth and production of several medicinal plants was observed by many investigators. (F. A. S. Hassan et al., 2013) found that the main components of volatile oil were α-pinene, 1, 8 cineol, linalool, camphor and borneol but these components were affected by irrigation. Deficit irrigation increased α-pinene, 1, 8 cineol and borneol, especially when 60% of FC was applied. On the other hand, linalool and camphor were decreased by deficit irrigation. Similarly, (Mohamed et al., 2014) in their investigation of the effect of irrigation intervals on the growth and chemical composition of *Curcuma domestica* plant, found that the chemical composition, volatile oil and curcumin in dry rhizomes increased when the plants were irrigated every week compared every two or three weeks. The effect apparently, is dependent on the severity of the water stress and varies for different compound (Niinemets, 2015).

Secondary metabolite biosynthesis in lantana is a natural process and is highly influenced by many factors. The synthesis of the metabolites may depend on the nutritional elements as well as the water present in the soil. Studies about the effect of irrigation on lantana plant yield, essential oil and accumulation of secondary metabolites have not yet been done. Therefore, this study aims to evaluate the productivity of lantana under different irrigation intervals under a controlled environment.

CHAPTER THREE

CHARACTERISATION OF THE SECONDARY METABOLITE PROFILES OF THE LEAF ESSENTIAL OILS OF LANTANA CAMARA FROM VARIOUS AGROECOLOGICAL ZONES AND SOILS IN KENYA

Abstract

Studies examining wild plants' metabolic expression variability suggest that ecological aspects significantly affect the Essential oil (EO), quantity, and quality in a plant. *Lantana camara* is a widely distributed invasive plant species globally, with immense metabolites that can become a source of novel compounds to produce biopesticides. However, the quality aspect must be considered due to the impact on the synthesised metabolites. To characterize the secondary metabolite profiles of the leaf essential oils of *L. camara* from various agro-ecological zones and soils in Kenya. Leaf samples were collected from six different agro-ecological zones of Kenya and the corresponding monthly climatic data and soil samples. The essential oil was extracted through steam distillation and the oil analyse through the GC-MS. The secondary metabolites data from leaf EO was combined with soil and climate data and analysed using unimodally constrained and unconstrained ordination methods for untargeted metabolites. Regional and seasonal variability was observed for secondary metabolism (SM) in the leaf EO, which correlated to soil attributes and climatic factors. We highlight the seasonal-geographic metabolism relationship for *L. camara* and the combined analytical method to obtain data that contributes to understanding the environmental factor's influence on the SMs' accumulation and synthesis. This research will have all-embracing implications for maximising phytochemical uniformity.

3.1 Introduction

The composition of *Lantana camara* essential oils collected from different parts of the world is identified by the main components being terpenes (sesquiterpenes and monoterpenes) and their oxygenated derivatives (Nea et al., 2020; Pereira et al., 2019). These respective groups of secondary metabolites play a significant role in the

adaptation of the plant to the surrounding environment (Pereira et al., 2019; Sampaio & Da Costa, 2018). The ecosystem influences the biosynthesis of secondary metabolites, facilitating the chemical interaction between plants resulting in the differences in metabolite plant's profile; thus, exerting their biological roles as a plastic adaptive response mechanism to their environment (Pereira et al., 2019).

The ecological and adaptive factors may also affect the accumulation and production of secondary metabolites on similar species growing in various regions. The reported metabolites from *Lantana camara* growing in various regions have demonstrated marked differences in concentration and composition. Several chemotypes have been described, comprising a cineole/sabinene/b-caryophyllene chemotype from Algeria (Zoubiri & Baaliouamer, 2012); ß-caryophyllene (9.8%), 1,8- cineole (9.4%), and ß-inene (8.2%) from Egypt; ß-caryophyllene (23.3%), α -humulene (11.5%), germacrene D (10.9%) or davanone, ß-caryophyllene and bicyclogermacrene from India (V. S. Rana et al., 2005) and bicyclogermacrene (19.4%), isocaryophyllene (16.7%), valencene (12.9%), and germacrene D (12.3%) from Brazil (Medeiros et al., 2012; Sousa et al., 2012). These results have shown that edaphic and environmental factors influence plays a significant role in accumulating and producing secondary metabolites.

The variation in secondary metabolites' production influenced by environmental conditions can characterise one species' plant populations. In this context, the metabolites may be used as a chemical marker to differentiate species found in specific geographical zones and seasons (De Souza et al., 2018; Pereira et al., 2019). Plants that produce essential oils vary considerably in their quality and quantity (composition and concentration of their constituents) due to their interaction with the natural environment.

The vast compound syntheses in the Lantana plant points out the adaptive significance of such a diversity of compounds. The variability for the essential oil's composition of *Lantana camara* may be associated with geographical pressures and distributions (Murugesan et al., 2016; Pereira et al., 2019). The accumulation and metabolism of secondary metabolites reflect the integrated influences of numerous

environmental factors on the plant during their growth and development periods besides genetic factors (Liu et al., 2016). Some metabolites are only synthesised under particular environments, or their contents significantly increase. Further, previous studies have shown that medicinal plants growing in different environments and regions produce different SMs leading to differences in their qualities (Liu et al., 2015).

Several studies examining the environmental factors that influence plant secondary metabolite biosynthesis consider these factors' effects on individual compounds. However, individual compounds hardly happen alone (Gershenzon et al., 2012). Instead, (Berini et al., 2018) explain that any influence of the compound depends on conditions in the prevailing environment because one factor cannot be extrapolated from a grouping of environmental factors in plants growing in the natural environment. Therefore, understanding how environmental factors affect a plant's metabolic profile is essential for interpreting how these changes influence the individual compounds' abundance.

Considering Lantana adaptive capacity and wide distribution, the absence of studies on this subject, and the plant's pesticidal properties, we proposed to carry out a comparative study with essential oil samples from Lantana leaves obtained from samples collected from six different geographic regions in Kenya and collected in different seasons. The approach includes comparing the data obtained by chemical profiles of these oils and abiotic factors. To determine the primary factors responsible the variations in secondary metabolites in Lantana in the different locations. This will potentially assist in identifying best harvesting seasons and regions for this wild species and improve its reasonable exploitation for particular compound for biopesticide.

3.2 Materials and Methods

3.2.1 Sampling locations and Plant materials

To determine the effects of geography, climate, and edaphic conditions on the secondary metabolite composition of *Lantana camara*, plant. The sampling sites for

the study were selected based on their Agroecological zones (AEZ) in Kenya, which shared comparable characteristics in terms of land suitability, potential production, and environmental impact. The study selected six different geographic zones of Kenya, namely: Lower Highland-Njoro (LH-NJ), Upper Midland 1-Kakamega (UM1-KK), Upper Midland 2-Kandara (UM2-KA), Upper Midland 3-Embu (UM3-EM), Lower Midland-Kiboko (LM-KI), and Coastal Lowland-Mtwapa (CL-MT). The altitudes of the sampling sites ranged from 20 to 34 m above sea level (a.s.l.) at Coastal Lowland-Mtwapa (CL-MT), 810 to 975 m a.s.l. in Lower Midland-Kiboko (LM-KI), 1980 to 2423 m a.s.l. in Lower Highland-Njoro (LH-NJ), 1536 to 1543 m a.s.l. in Upper Midland 1-Kakamega (UM1-KK), 1487 to 1508 m a.s.l. in Upper Midland 2-Kandara (UM2-KA), and 1,350 to 1416 m a.s.l. in Upper Midland 3-Embu (UM3-EM).

For sampling plants along the roadsides, a linear sampling design was implemented. The sampling unit was a 30 km long in each zone, divided into equidistant transects of 5 km. Each transect had 4 quadrats of 5 km by 5 m, and each quadrat was considered a replication unit. A GPS system from Magellan GPS 315 was used to map the sampling sites, which can be seen marked with dots on Figure 3.1B. Each transect was treated as the primary experimental unit. The same sites were used for both dry and wet season. A botanist (John Kamau from the Botany Department at JKUAT) identified the *Lantana camara* Linn species in each region and site. The identification process involved observing the qualities of the Lantana by using a field guide that had keys to plants of the region.

To obtain fresh and healthy leaves of lantana plants (Figure 3.1A), the collection was done in the morning between 0800-1000 hours during both the wet (March-May) and dry (October-December) seasons of 2018, 2019, and 2020 in each AEZ. The first four leaves were collected from the top of the plant stem, from four directions (north, south, east, and west), to ensure that the leaves were harvested from all sides of the plant. Leaves were collected from thirty (30) sub-sampling points in each site and mixed to make a composite sample. The samples were transferred to the lab within two days of collection in ventilated nylon gunny bags. The samples were washed with tap water and air-dried under room temperature (23–26 °C) in a well-ventilated

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room for two weeks until crisp (plate 3.1). Subsequently, the samples were grounded into powder using a stainless-steel grain miller (food-grade) 28,000/min (Huangcheng, China), and stored in khaki paper bags until the time of oil extraction.
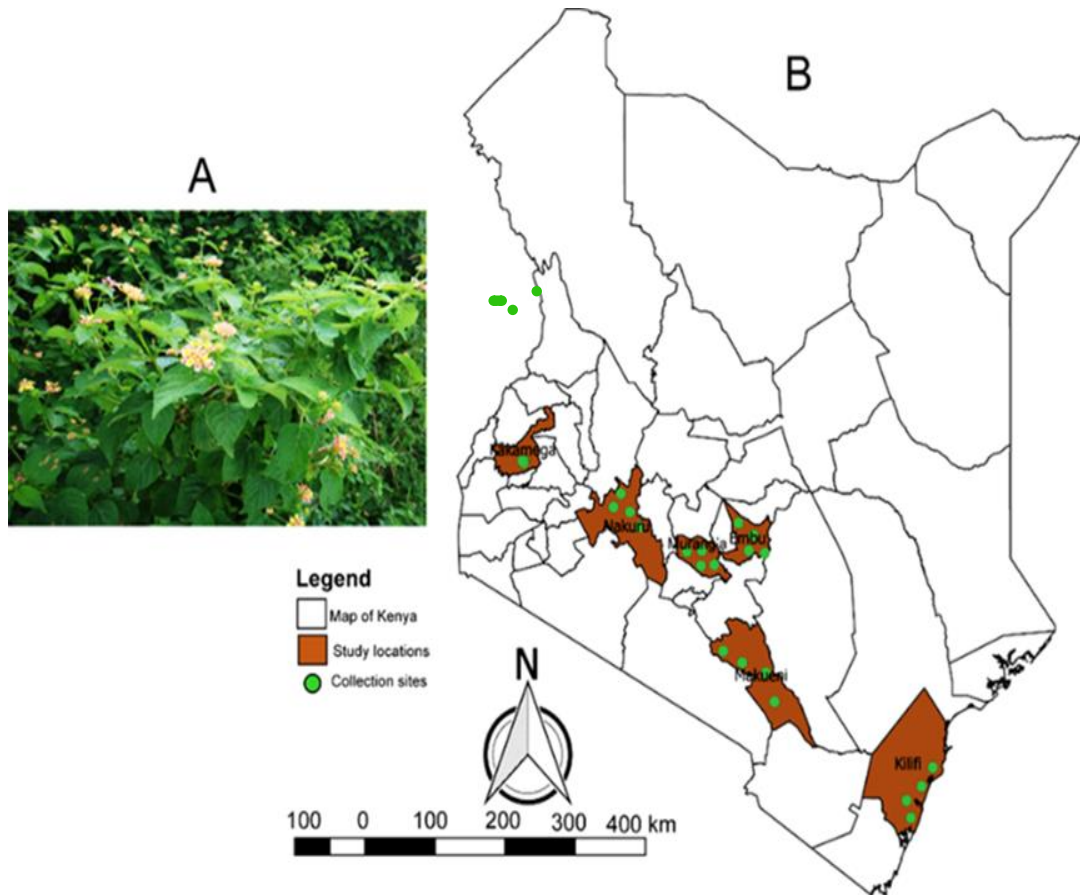
Figure 3.1: A map of Kenya showing the locations where Lantana camara plants were collected for the study.

Plate 3.1: Drying of Lantana camara Leaves.

3.2.2 Environmental data collections

The environmental data were divided into soil and climate data. Soil samples from the surface layer (0-15 cm depth and 6 cm diameter) using an alderman auger directly under Lantana plant canopies where the plant material was taken. The soil samples were mixed into a composite of 500 g (four replicates for each geographical location). Soil samples were placed in labelled plastic bags. The soil samples were dried at room temperature (20–25°C) and sieved to 2 mm. A duplicate soil sample was sieved through a 2 mm filter once again for determination of soil chemical characteristics. The critical soil parameters, including nitrogen (N), phosphorus (P), potassium (K), Total organic carbon (TOC), electrical conductivity (EC) and pH were analysed using the standard soil analysis methods.

Nitrogen (% N) was determined using Sulfuric Acid (H_2SO_4) digestion and measured with the Kjeldahl method (Kjeldahl, 1883). Phosphorus (P) of soil samples was extracted with ammonium fluoride (NH_4F, 0.03 M) and hydrochloric acid (HCl, 0.025 M) (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China), Olsen method

(Olsen & Sommers, 1982) and measured by UV–Vis spectrophotometer (Thermo Fisher Scientific, San Jose, CA, USA). The potassium (K) of soil samples was determined by extraction with Ammonium acetate (CH$_3$COONH$_4$) (1 M) (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China) saturated paste extract method (Kudsen & Peterson, 1982) and quantified by Corning flame photometer (Sherwood Scientific Ltd, Cambridge, UK). Soil pH (soil reaction) was measured in 1:2.5 soil: water suspension solution using the Consort pH meter (model C835). The Electrical Conductivity EC) was measured using the EC meter model 4510 in the soil-to-water ratio of 1:2.5. The analyses were undertaken at Jomo Kenyatta University of Agriculture and Technology Soil Chemistry Laboratory. The analysis data presented in Table 3.1.

The climate data for the collection month (during both dry and wet seasons) was obtained from nearby meteorological stations located closest to the habitats across all six study locations. MS Excel was used to pre-treat the data, which was later analyzed. The climate data, as shown in Table 3.1, included figures for monthly average temperature (aT), average precipitation (P), sunshine duration (SD), and UV index.

Table 3.1: Average Climatic and Soil parameters data for the Dry and Wet season

Seasons	Locations	Climatic data						Soil data			
		AT	P	UV	SD	pH	EC	% N	Ex. P mg/l	Ex. K mg/l	% TOC
		°C	mm	mW/m²	W/m²		mhos				
S1	UM-EM	20.0	143.9	5.0	192.8	5.9	0.4	0.5	0.3	3.7	1.5
	UM2-KA	18.3	135.1	4.3	171.9	6.1	0.5	0.3	0.3	3.0	1.4
	UM1-KK	20.5	283.6	5.0	195.9	6.0	0.3	0.4	0.3	0.7	1.2
	LM-KI	18.8	70.7	4.3	215.9	7.0	0.7	0.2	0.5	4.2	1.5
	CL-MT	18.3	135.1	4.3	171.9	6.1	0.5	0.3	0.3	3.0	1.4
	LH-NJ	18.3	230.2	4.3	188.3	6.7	0.7	0.4	0.2	4.9	2.4
S2	UM-EM	20.8	50.7	5.5	255.3	6.1	0.6	0.3	0.5	3.7	1.5
	UM2-KA	18.8	24.1	5.3	287.6	6.3	0.7	0.3	0.4	3.0	1.4
	UM1-KK	19.8	37.4	5.4	271.4	6.6	0.7	0.3	0.5	4.5	1.4
	LM-KI	20.5	50.9	5.5	280.5	7.1	0.8	0.3	0.7	4.4	1.7
	CL-MT	27.3	34.5	6.5	252.9	8.2	0.7	0.1	0.4	1.3	0.8
	LH-NJ	17.3	25.3	5.0	290.0	6.8	0.9	0.4	0.4	5.1	2.6

3.2.3 Extraction of the essential oils

The *Lantana camara EO* was isolated by steam distillation using a steam distiller apparatus (Model S 2, Deschem Science supply, China) (plate 3.2). This technique is mainly applied for the extraction of volatile plant components such as essential oils, dry or wet plant materials that are dispersed in water, and suitable for processing large quantities of plant materials, especially for industrial extractions. About, 200g of dried leaves from each sample was steam distilled separately using 2000 mL distilled water (plant material to distilled water ratio 1:10 w/v) for 3 hours at 100 °C. The mixture, being in a container connected to the condenser, is heated, and the resulting steam formed after condenses in the condenser, which is having a two-phase system consisting of extracted essential oil and water in the condensation vessel. Phases are separated using a simple separatory funnel. The distillation rate was maintained at around twenty-five drops per minute, and the continually condensed distillate was collected to a vial in the receiver arm of the steam distiller apparatus after heating for around 3 h to guarantee the optimal yield. The condensing oils were separated with a separating funnel, and the oily sample was treated with anhydrous Sodium Sulphate (Na_2SO_4) (Merck) to remove the remaining trace water and collected in amber-coloured vials (plate 3.3), labelled, and stored at 4 °C until testing with GC-MS. The extraction of the essential oil was conducted in the horticulture department laboratory (JKUAT).
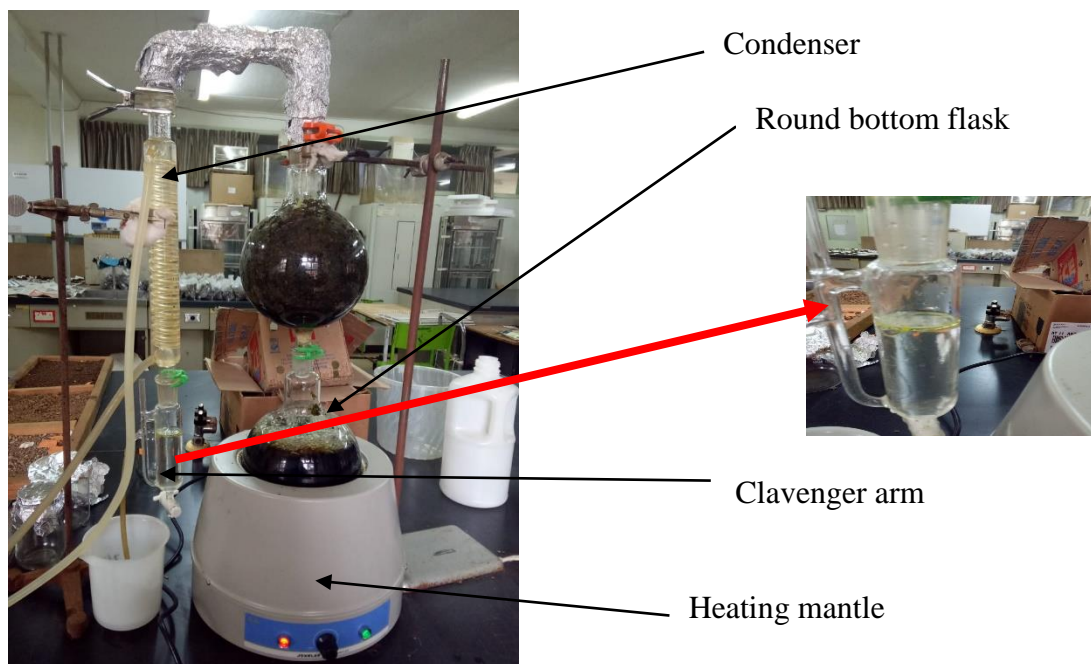
Plate 3.2: Essential Steam Distillation set-up and oil extraction process.

Plate 3.3: Vials containing essential oil extracted oils from *Lantana camara*

3.2.4 Gas Chromatography-Mass spectrometry (GC-MS) analysis

The essential oil samples were subjected to GC-MS analysis, where they were first diluted in 100% n-hexane at a dilution rate of 0.1%. Following dilution, the samples were then transferred to the auto-sampler vials for further analysis. The use of n-hexane as a

diluent is common in GC-MS analysis as it is an effective solvent for dissolving high boiling point compounds such as essential oils. This process allowed for the separation and identification of the various chemical components present in the oil samples. Agilent Technology (GC, Agilent 6890) Agilent Technologies Inc., Santa Clara, CA, USA) gas chromatography with a split detector and Mass Spectrometer Detector (MSD) coupled with an autosampler of the volatile oil was conducted with a splitless injector at 220 °C. The compounds were then separated on a nonpolar capillary HP column at an average linear flow rate of 35 cm s-1 with helium as the carrier gas. The oven temperature was held at 35 °C for five minutes and then increased to 280 °C by 10 °C/min and held for 10 minutes. The collected volatile compounds were then identified by comparing their mass spectra and retention times with the National Institute of Standards and Technology (NIST) 2017 library of mass spectra for possible identification of compounds. Samples were analyzed at the Jomo Kenyatta University of Agriculture and Technology analytical chemistry laboratory.

3.2.5 Data processing and Statistical analyses

The environmental data and chemical profiles were divided into two sets of variables: chemical (secondary metabolites) and environmental (soil and climate data) variables and preprocessed in Excel. The R software version 3.6.3 (R Core Team, 2020) and the RStudio graphical user interface (version 1.2.5033) were used to perform all the analyses. The total area of peak data was normally distributed (Shapiro-Wilk test: $p>0.05$), and their variance was homogeneous (Barlet test: $p>0.05$); therefore, an unpaired t-test was used to compare the amount SMs synthesised by lantana between the rainy and dry seasons. For the same reason, we used the analysis of variance (ANOVA) followed by the student-Neuman-Keuls (SNK) post hoc test to compare the amount of SM synthesised by lantana across the different localities during a specific season using the R software package 'Agricolae'version 1.3-3 (Mendiburu, 2020). The one-way analysis of similarity (ANOSIM) using the Bray–Curtis dissimilarity matrix to compare the chemical profiles of the different compounds synthesised by *Lantana camara* between the seasons and

across the various localities. Based on the SIMilarity PERcentage (SIMPER) analysis, we identified the 10 most significant SMs contributing to *Lantana camara* essential oil's diversity between the seasons and across the different localities. To visualise this difference, the non-metric multidimensional scaling (NMDS) plot overlaid the physicochemical and environmental variables to visualise their relationship with SM profile diversities. All statistical results were considered significant when $P < 0.05$.

3.3 Results

3.3.1 Seasonal variation in essential oil composition of *Lantana camara*

The GC-MS analysis of Lantana camara's essential oil (EO) was conducted on samples harvested from various agroecological zones across Kenya, during two distinct seasons. The study discovered that the wild population of lantana plant yielded 146 classes of secondary metabolites. The dry season produced 126 compounds, whereas the rainy season yielded only 94 compounds. (Table 3.2). the dorminant compounds identified were (E)-caryophyllene (0.02-13.09%), caryophylle oxide (1.62-9.48%), 1,8 cineole (4.94-11.01%), Lavanduly isovalerate (2.33-8.89%), Alpha-muurolene (1.34-8.02%), Spathulenol (0.28-4.5%) and Trans-Cadina-1(6),4-diene (2.11-11.03). The sesquiterpenes were dominated by the (E)-caryophyllene (0.02-13.09%), caryophylle oxide (1.62-9.48%) and produced in abundace during the dry and rainy season respectively. On the other hand, the monoterpenes were dominated by I, 8-cineole (4.95-11.01%) being produced in abundance.

The boxplot depicts there is quantitative variation of the total amount of compounds synthesised by the lantana plant between the rainy and dry seasons in each collection site (Figure 3.2). Therefore, the number of SMs synthesised by lantana varied significantly between the seasons. There also appears to be a slight increase in the median number of compounds synthesized and a greater dispersion during the wet season in, LM-KI (p=0.68, $F_{(5,18)}$= 0.68) and LH-NJ (p=0.64, $F_{(5,18)}$ = 0.69) (Figure 3.2), showing a non-significant variation between the season. From the plot median, there was lower number

of compounds in CL-MT (P=<0.0001, $F_{(5,18)}$= 10.07)) but higher in UM3-EM (P=<0.01, $F_{(5,18)}$= 4.24), UM2-KA (P=0.017, F (5,18)= 3.73) and UM1-KK (P=0.002, $F_{(5,18)}$= 5.97) during the rainy season. In comparison, there was a tremendous increase in the number of compounds observed in the plants sampled from UM3-EM and CL-MT and a reduction in UM2-KA and UM1-KK during the dry season .

Table 3.2: Composition of essential oil of the leaves of *Lantana camara* from diverse Agro Ecological zones of Kenya.

Name of compound[a]	RI	DRY SEASON						RAINY SEASON (%) [b]					
		UM3-EM	UM2-KA	UM1-KK	LM-KI	CL-MT	LH-NJ	UM3-EM	UM2-KA	UM1-KK	LM-KI	CL-MT	LH-NJ
(2E)-Hexenal	827.3	0.02±0.01	0.04±0.02	0.01±0.02	0.02±0.02	0.02±0.01	0.06±0.07	0	0.01±0.02	0	0	0	0
b-Damascenone, (E)-	1363.4	0	0	0	0	0	0	0.03±0.04	0.05±0.04	0.01±0.02	0.03±0.04	0	0
(E)-beta-Ocimene	1047.7	0.02±0.05	0.01±0.01	0	0	0	0	1.18±0.16	1.18±0.12	1.22±0.36	0.35±0.41	0.62±0.72	0.76±0.53
(E)-Caryophyllene	1419.3	13.07±3.33	5.32±0.04	13.09±1.27	5.16±5.97	10.12±7.1	8.08±5.41	2.12±0.01	2.01±0.04	1.91±0.05	4.11±0.03	3.98±0.21	01.67±0.31
(E)-Isovalencenol	826.3	0	0	0	0	0.01±0.02	0	0	0	0.05±0.1	0.02±0.03	0	0.05±0.11
(Z)-alpha-Bisabolene	1503.1	0.18±0.09	0.25±0.14	0.26±0.51	0.16±0.19	0.31±0.33	0.33±0.39	2.1±1.8	5±3.39	3.78±4.25	1.59±2.93	0.12±0.14	0.07±0.14
(Z)-beta-Ocimene	1037.8	0	0	0.02±0.04	0	0	0	0.01±0	0	0	0.13±0.26	0.77±0.54	0.32±0.37
1,8-Cineole	1022.4	0	0.97±1.86	0.14±0.27	0	0	0	11.01±1.42	9.72±1.05	6.54±3.23	4.95±5.78	7.88±5.54	7.04±4.77
14-hydroxy-(Z)-Caryophyllene	1407.7	1.33±0.58	0	0	0	0.06±0.12	0	0	0	0	0	0	0
1H-Cycloprop[e]azulene	1459.1	0	1.1±0.46	0.04±0.08	0.22±0.44	0	0.37±0.58	0	0	0	0	0	0
1-Hexadecene	1938.5	0.29±0.07	0.16±0.23	0.18±0.37	0.11±0.23	0.05±0.09	0.04±0.07	0	0	0	0	0	0
1-Octen-3-ol	965.9	0.17±0.24	0.01±0.03	0.64±1.28	0.26±0.32	0.11±0.21	0	0	0	0	0	0	0
2,2,7,7-Tetramethyloctane	958.0	2.27±2.58	2.27±3.07	1.26±2.51	1.28±1.57	0	3.46±4.66	0	0	0	0	0	0
2-Cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl-	1250.1	0.02±0.02	0	0	0.01±0.02	0	0	3.08±0.45	0	0.33±0.67	0.05±0.09	0	0
2-Cyclopenten-1-one, 3-methyl-2-(1,3-pentadienyl)-, (E,Z)-	1368.9	0	0	0	1.82±2.13	2.15±2.06	0	0	0	0	0	0	0
2-Methyl butyl-2-methyl butyrate	1026.0	0.09±0.18	0	0	0	0	0	0.18±0.13	0.01	0	0	0	0
2-Pentadecanone, 6,10,14-trimethyl-	1680.8	0.01±0.02	0	0	0	0	0	0.04±0.05	0.04±0.03	0.09±0.1	0.03±0.03	0	0.01±0.02
2-Propenal, 3-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	1178.0	0.23±0.1	0.12±0.14	0	0.11±0.09	0.17±0.22	0.09±0.09	0	0	0	0	0	0
2-Tridecanone	1478.8	0	0	0	0	0	0	0.05±0.05	0.02±0.03	0.11±0.14	0	0.02±0.04	0.03±0.04
2Z,6E-Farnesyl acetate	1704.7	0.21±0.3	0	0.01±0.02	0	0	0	0	0	0	0	0	0
2Z,6Z-Farnesol	1686.9	0.07±0.12	0	0	0	0.15±0.3	0.69±1.3	0.03±0.06	0.01	0.04±0.01	0.18±0.37	0	0.01
3-Octanol	5.843	0.45±0.33	0.57±0.1	0.03±0.05	0.63±0.42	0.31±0.24	0.17±0.15	0	0	0	0	0	0
4-methyl-3-Heptanone	868.4	0.14±0.17	0.18±0.07	0	0.1±0.21	0.08±0.09	0	0	0	0	0	0	0

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b-Calacorene	1547.1	4.38±3.9	1.16±0.97	0.11±0.21	3.02±3.92	0.34±0.32	0.4±0.27	0	0	0	0	0	0
Acetic acid	633.0	0	0	0	0	0	0	0	0.01±0.03	0.09±0.02	0	0	0.01±0.02
Allo-Aromadendrene	1459.1	3.63±2.54	2.72±3.15	0.99±1.98	4±2.74	1.84±2.9	3.57±2.42	0	0	0.31±0.38	0	0.07±0.15	0.08±0.17
Allo-Aromadendrene epoxide	1439.0	0	0	0	0	0	0.93±1.86	0.72±0.85	0	0.85±0.99	0.02±0.03	0.99±1.3	1.04±1.22
Alloaromadendrene oxide-(1)	1649.2	0.03±0.06	0.06±0.07	0	0	0	0	0	0	0	0	0	0
Allo-Ocimene	1116.4	0	0	0	0	0	0	0.13±0.02	0.14±0.02	0.1±0.13	0.06±0.07	0.1±0.13	0.07±0.05
Alpha-Bulnesene	1500.6	0	0	0	0	0	0	0.09±0.02	0.09±0.01	0.91±0.14	0.3±0.6	0.03±0.05	0.04±0.03
Alpha-Calacorene	1530.4	0.07±0.08	0.02±0.04	0	0.07±0.08	0.33±0.66	0.03±0.06	0	0	0	0	0	0
Alpha-Cedrene	1410.9	0.06±0.12	0.03±0.05	0	0	0	0	0	0	0	0	0	0
Alpha-Colocalene	1622.0	1.32±1.66	0.2±0.4	0	0.77±1.54	0.03±0.06	0.14±0.29	0	0	0	0	0	0
Alpha-Cubebene	1352.2	0	0	0.03±0.06	0	0	0	0.67±0.15	0.65±0.07	0	0.04±0.04	0.33±0.38	0.44±0.3
Alpha-Farnesene	1496.3	0	0	0.1±0.19	1.04±2.09	0.05±0.1	0.07±0.15	0.32±0.64	1.11±2.14	1.64±0.16	0	0.28±0.48	0.37±0.73
Alpha-Gurjunene	1405.6	2.63±5.11	3.4±6.74	0	0.02±0.03	2.56±5.08	0.05±0.06	0.04±0.07	0	0	0	0	0.03±0.06
Alpha-Humulene	1449.3	0	0	0	0	0.3±0.6	0.09±0.19	6.51±1.22	1.46±2.92	2.95±3.41	1.39±2.78	0.95±1.91	4.38±2.93
Alpha-Muurolene	1491.0	5.25±3.59	8.02±1.56	1.34±2.68	3.91±2.88	1.73±1.77	4.36±3.02	0	0	0	0	0.27±0.31	0
Alpha-Neocallitropsen	1493.0	0.43±0.87	1.61±1.2	0.36±0.71	0.68±0.83	0.67±1.2	1.3±1	0	0	0.02±0.05	0	0	0
Alpha-Phellandrene	1004.1	0.43±0.39	0.09±0.1	0.03±0.06	0.21±0.15	0.07±0.14	0	0.95±0.42	0.64±0.07	0.4±0.13	0.58±0.67	0.46±0.67	0.57±0.54
Alpha-Pinene	936.1	0.11±0.13	0	0	0	0	0	1.43±1.65	0.01±0.02	1.62±1.12	0.01±0.01	0.66±1.32	2.54±1.69
Alpha-Terpinene	1017.1	2.3±1.91	1.28±0.11	0	1.2±0.89	0.6±0.5	0.39±0.33	0.01±0.03	0.02±0.03	0.06±0.07	0.01±0.03	0.01±0.03	0
Alpha-Terpineol	1175.6	0.75±1.5	0	0	0	0	0.64±1.28	1.01±0.27	0.98±0.24	0.51±0.22	0.41±0.52	0.57±0.66	0.32±0.22
Alpha-Thujene	927.8	0.64±0.44	0.76±0.32	0.19±0.39	0.41±0.48	0.36±0.25	0.06±0.12	0.46±0.53	0	0.5±0.17	0	0.23±0.46	0.42±0.48
Amorpha-4,7(11)-diene	1479.0	0.44±0.52	0.73±0.5	0.11±0.22	0.36±0.42	0.26±0.45	1.16±1.3	0	0.02±0.03	0	0	0	0.06±0.09
Aromadendrene oxide-(2)	1439.0	0.12±0.16	0.15±0.21	0.11±0.23	0	1.41±2.81	0	0	0	0	0	0	0
Benzenamine, 2,3,4,5,6-pentamethyl-	1265.42	1.11±0.42	0.54±0.15	0	0.83±0.67	0.51±0.49	0.11±0.12	0	0	0	0	0	0
Beta-Alaskene	1501.8	0	0	0	0	0	0	0.24±0.47	0	0.89±0.61	0	0	0
Beta-Bisabolene	1508.4	3.95±2.89	2.95±2.67	3.07±2.7	4.29±2.92	1.59±1.21	0.7±0.52	0	0	0	0	0	0
Beta-Bourbonene	1384.2	0	0.08±0.15	0	0	0	0	0.06±0.12	0.05±0.09	0	0.31±0.44	0.07±0.08	0.16±0.12
Beta-Calacorene	1547.1	0.13±0.26	0	0	0.01±0.02	0.43±0.33	0.39±0.29	0	0	0	0	0	0
Beta-Chamigrene	1470.1	1.85±2.28	0.51±1.02	0	0	0	1.22±1.76	0	0	0	0	0	0
Beta-copaen-4-alpha-ol	1580.2	0	0	0	0	0	0	0.57±0.67	1.11±0.75	1.67±0.1	0	0	0
Beta-Copaene	1433.1	0	0	0	0	0	0	0.45±0.58	0.28±0.56	0.33±0.39	0.47±0.57	0.28±0.32	0.33±0.38
Beta-Cyclocitral	1610.3	0.73±0.12	0.77±0.19	0.08±0.17	0.46±0.34	0.35±0.37	0	0.06±0.01	0.06±0.01	0.06±0.01	0	0.01±0.03	0
Beta-Elemene	1590.9	0	0	0	0.07±0.13	0.05±0.09	0	2.04±0.28	2.02±0.29	2.26±0.37	0.78±0.9	0.61±0.72	1.21±0.82
Beta-Ionone	1466.2	0.14±0.1	0.09±0.11	0	0.09±0.06	0.14±0.24	0.04±0.05	0	0	0	0	0	0
Beta-Phellandrene	1021.3	0.04±0.07	0	0	0	0	0	2.48±4.86	0.03±0.06	0.15±0.18	0	0.02±0.05	6.38±7.56
Beta-Pinene	973.1	0	0.43±0.51	0.04±0.07	0	0.56±0.42	0.3±0.24	0.76±0.88	1.6±0.09	0	0.7±0.82	1.25±0.99	0.71±0.82
Beta-santalene	1453.0	0.04±0.03	0.05±0.04	0.02±0.05	0.05±0.06	0.03±0.03	0	0	0	0	0	0	0

Compound	RI												
Beta-Selinene	1480.7	0	0.51±1.01	0.1±0.2	0	0	0	0	0	2.54±5.07	0.05±0.06	0.02±0.02	0
Beta-Vetispirene	1484.0	3.77±0.61	4.76±1.26	0.52±1.04	1.67±1.47	0.51±0.85	1.71±1.16	0	0	0	0	0	0
Beta-Vetivenene	1547.0	0.16±0.19	0.24±0.18	0	0.27±0.18	1.26±2.28	0.09±0.18	0	0	0	0	0	0
Bicyclogermacrene	1489.8	0	0	0.48±0.95	0.89±1.77	0	0	5.02±2.94	4.83±0.32	0.02±0.05	2.07±2.4	2.65±1.83	3.73±2.5
Borneol	1166.2	0.07±0.09	0	0	0	0	0	0.88±0.18	0.84±0.16	0.5±0.21	0.39±0.5	0.37±0.43	0.39±0.26
Camphene	1068.5	0	0	0.04±0.07	0	0	0	1.46±0.67	3.09±0.18	1.04±0.37	0	1.46±1	1.36±1.01
Camphor	1515.1	0	0	0	0	0.03±0.04	0	1.98±0.36	1.91±0.26	1.13±0.45	1.01±1.18	1.13±0.76	1.1±0.74
Caryophylla-4(12),8(13)-dien5-beta-ol	2301.0	0.02±0.02	0.01±0.02	0	0.02±0.02	0	0	0	0	0	0.11±0.23	0.15±0.18	0.12±0.14
Caryophyllene oxide	1986.2	0	0.03±0.06	0.01±0.02	0.25±0.51	0.28±0.56	0.75±0.86	9.48±1.05	7.27±0.96	1.62±3.23	7.51±5.12	3.52±6.66	2.03±0.07
cis-3,5-diene-Muurola	1449.8	0	0	0	0	0	0	1.11±0.76	0	0.96±1.1	0	0	0
Cis-4-Caranone	1200.0	0	0	0	0	0	0	0	0.02±0.02	0	0	0.01±0.01	0.01±0.02
Cis-beta-Guaiene	1478.6	0.21±0.25	0.45±0.12	0.02±0.05	0.31±0.21	0.06±0.07	0.13±0.1	0	0	0	0	0	0
Cis-Cadina-1(6),4-diene	1523.9	0.15±0.13	0.17±0.12	0	0.11±0.14	0.21±0.24	0.08±0.09	0.61±0.41	0	0	0	0	0
Cis-threo-Davanafuran	1418.8	0.05±0.1	0	0	0	0	0	0	1.28±0.91	1.07±0.93	1.29±1.5	0.25±0.29	0.41±0.28
Copaen-4-alpha-ol<beta->	1577.9	0	0	0	0	0	0	0	0	0	0	0.23±0.46	0.82±0.95
Cumin aldehyde	1212.6	0.25±0.08	1.02±1.04	0.16±0.31	0.18±0.22	1.47±1.84	0.47±0.94	0.02±0.02	0.02±0.02	0	0.02±0.02	0.01±0.01	0.01±0.02
Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl	1388.0	0.59±0.28	1±0.57	0.34±0.69	0.57±0.46	0.94±0.88	3.2±3.48	0	0	0	0	0	0
Cycloisolongifolene, 8,9-dehydro-9-formyl-	1888.0	0	0	0	0	0.02±0.05	0	0.37±0.74	0.31±0.61	0.04±0.06	0.61±0.77	0.33±0.67	0.38±0.77
Cyclopentadecanone	1676.0	1.01±0.7	1.04±0.78	0	0.52±0.96	0.16±0.32	0	0	0	0	0	0	0
Cyclopropanecarboxylic acid,	2250.8	0.14±0.29	0.26±0.51	1.02±1.18	0.58±0.46	0.6±0.69	0	0	0	0	0	0	0
Cycloseychellene	1417.9	0.17±0.16	0.04±0.06	0	0.08±0.05	0.01±0.02	0	0	0	0	0	0	0
Davana ether	1497.0	0.03±0.05	0.14±0.27	0	0	0	0	0.89±0.8	0	0.31±0.61	0.25±0.43	0.23±0.46	0.39±0.77
Davanone	1570.0	0	0.02±0.05	0	0	0	0	0	0	0	0	0.03±0.07	0
Davanone B	1564.0	0	0	0.11±0.21	0	0.02±0.05	0	0.47±0.11	0.15±0.3	0.31±0.22	0.1±0.2	0.14±0.17	0.15±0.18
Decane, 2,2,5-trimethyl-	1121.0	2.57±1.44	0.49±0.19	0.06±0.13	2.48±2.35	0.65±0.59	0.33±0.38	0	0	0	0	0	0
Delta-2-Carene	11.842	0.26±0.38	1.25±2.2	0.11±0.22	0.34±0.4	0.24±0.38	0.12±0.13	6.83±6.89	0	0	0.01±0.01	0	0
Delta-3-Carene	6.193	0.06±0.05	0.04±0.03	0.01±0.02	0.02±0.03	0.01±0.02	0	2.42±0.19	6.17±6.04	0.52±0.43	2.77±4.95	5.5±6.36	3.42±6.37
Delta-Amorphene	1482.4	0.3±0.6	0	0	0	0	0	0.67±0.07	3.25±0.11	2.84±0.74	1.37±1.59	3.28±2.89	2.32±1.55
Elemol acetate	1536.2	2±0.4	2.99±1.56	0.43±0.86	0.85±0.71	0.44±0.49	0.5±0.57	0	0	0	0	0	0
E-Nerolidol	1550.1	0	0.61±1.21	0.45±0.9	0	0	0	2.77±1.97	0	0	0.66±1.32	0	0
E-Nerolidyl acetate	1343.8	1.5±0.45	1.44±0.45	0.12±0.25	1.01±0.76	0.52±0.67	0.64±0.66	0	0	0	0	0	0
E-Phytol acetate	2099.1	0.01±0.02	0	0	0	0	0	0	0	0	0	0	0
Eugenol	1357.8	1.61±1.17	0.23±0.21	0.04±0.08	0.05±0.05	0.13±0.16	0.12±0.25	0.01±0.03	0.07±0.01	0	0	0	0.04±0.02
Formic aid	563.1	0.17±0.17	0.19±0.1	0	0.13±0.09	0.56±0.63	0.06±0.07	0	0	0	0	0	0

Compound	RI	1	2	3	4	5	6	7	8	9	10	11	12
Gamma-Costol	2533	1.12±0.06	1.39±0.6	0.15±0.29	0.97±0.65	0.33±0.52	0.6±0.69	0	0	0	0	0	0
Gamma-Muurolene	1473.0	0.08±0.09	0.1±0.08	0.05±0.1	0.08±0.09	0.36±0.41	0.05±0.05	0.01±0.03	1.03±0.68	0	0.03±0.04	0	0.03±0.04
Gamma-Terpinene	1050.3	0	0.18±0.36	0	0	0.27±0.55	0.8±0.76	3.61±1.44	5.49±0.3	1.01±1.99	2.07±2.4	1.08±1.28	1.13±0.82
Gamma-Terpineol	1148.1	0	0	0	0	0	0	0	0	5.48±5.12	0	4.95±6.18	0
Geranial	1247.1	0	0	0	0	0	0	0.02±0.01	0.03±0	0.01±0.01	0.02±0.03	0.01	0.01±0.01
Germacrene B	1535.1	1.45±2.52	1.87±3.28	0.21±0.42	0.1±0.2	0	0.07±0.14	0.73±0.19	0.97±0.44	0.58±0.27	0.23±0.27	0.22±0.25	0.47±0.33
Germacrene D	1475.9	0.03±0.07	0	0	0	0	0.02±0.04	2.03±1.35	2.52±0.19	1.49±1.8	0.89±1.03	1.49±1	1.7±1.17
Heneicosane	2100.0	2.13±4.26	0	0	0	0	0	0	0	0	0	0	0
Heptacosane	2700.0	0.98±0.8	1.15±0.5	0.02±0.05	1.32±0.89	0.55±0.43	0.29±0.31	0	0	0	0	0	0
Heptadecane	1883.1	0	4.61±3.45	3.1±3.07	0	3.11±2.34	1.96±1.68	0	0	0	0	0	0
Isoaromadendrene epoxide	1649.2	0.17±0.33	0.46±0.49	0	0	0	0.37±0.26	0	0	0	0	0	0
Isobazzanene	1442.0	0	0	0	0	0	0	0	1.75±2.03	3.54±0.45	0	0	0
Iso-sylvestrene	1009.0	0.03±0.06	0.03±0.06	0	0	0	0	1.51±0.62	1.27±0.63	0	0.7±0.89	1.06±0.83	0.55±0.64
Lavandulyl isovalerate	1273.2	7.62±5.28	8.89±6	2.33±3.99	7±4.81	3.37±5.68	7.33±4.95	0	0	0	0	0	0
Ledene oxide-(I)	1890.0	0.97±0.65	1.19±0.85	0	0.46±0.53	2.24±2.08	0.63±0.46	0	0	0	0	0	0
Limonene	1029.5	0.07±0.13	0	0	0	0	0	0.01	0	0.34±0.67	0	0	0
Linalool	1099.0	1.25±1.79	0.45±0.31	0.02±0.04	0.38±0.42	0.34±0.24	0	0	0	0.12±0.15	0.07±0.14	0	0.13±0.15
Linalool formate	1065.1	0.06±0.08	0	0	0.01±0.01	0	0	0	0	0	0	0	0
Linalool propanoate	1336.0	0.07±0.15	0.06±0.12	0.03±0.07	0.02±0.04	0	0	0.3±0.35	0.48±0.33	0	0.06±0.12	0.07±0.15	0
Myrcene	983.1	0	0	0	0	2.14±2.47	0	0.86±1	0	1.32±0.11	0	0.54±1.08	0.4±0.79
neo-Intermedeol	1636.1	1.17±0.34	0.74±0.51	0.19±0.38	0.66±0.76	0.5±0.34	0.15±0.1	0	0	0	0	0	0
Octadecane	2060.0	0.31±0.22	0.28±0.2	0	0.17±0.2	0	0	0	0	0	0	0	0
Para-Cymen-7-ol	1270.1	0.03±0.06	0	0	0	0	0	0	0.01	0.04±0.01	0.02±0.02	0	0.01±0.01
Para-Cymene	1015.1	0.17±0.32	0	0	0	0	0.18±0.14	0.02±0.02	0.05±0.01	0.03±0.04	0.03±0.03	0	0.04±0.03
Para-Mentha-1(7),8-diene	1202.5	0.13±0.15	0.18±0.13	0	0.23±0.15	0.04±0.06	0.05±0.06	0	0	0	0	0	0
Para-Mentha-3,8-diene	1072.0	0.25±0.09	0.16±0.1	0	0.25±0.25	0.04±0.03	0.05±0.1	0	0	0	0	0	0
Phytol	2099.1	0	0	0.55±1.1	0	0	0	0.17±0.1	0.13±0.04	0.48±0.14	0.04±0.09	0.06±0.07	0.14±0.1
Pinocarvone	1160.6	0.11±0.07	0.1±0.07	0.02±0.03	0.1±0.07	0.05±0.04	0.04±0.04	0	0	0.04±0.05	0.05±0.06	0.05±0.07	0.04±0.05
Premnaspirodiene	1510.0	0.28±0.56	0	0	0	0	0.05±0.04	0.16±0.14	0.1±0.1	0.09±0.06	0.09±0.1	0.08±0.13	0.21±0.16
Pyridine,	10003.0	1.77±0.28	1.74±0.5	0.44±0.87	1.15±0.78	0.42±0.31	0.66±0.62	0	0	0	0	0	0
Sabina ketone	1155.7	0	0	0	0	0	0	0	0	0	0.01±0.02	0	0
Spathulenol	1576.4	0.01±0.01	0	0	0	0	0	4.98±0.58	5±0.24	0.28±0.56	3.76±4.47	3.97±2.91	3.83±2.75
Sulfurous acid, 2-ethylhexyl octadecyl ester	2262.0	0	0.11±0.16	0	0	0	5.24±4.39	0	0	0	0	0	0
Terpinen-4-ol	1601.2	0.04±0.09	0	0	0	0	0	1.25±0.24	1.13±0.18	0.5±0.45	0.79±0.92	0.6±0.7	0.77±0.52
Terpinolene	1079.3	0.13±0.16	0	0	0	0	0	0.17±0.29	0.43±0.84	0.24±0.3	0	0.18±0.33	0.41±0.28
Tetradecanal	1594.7	0	0	0	0	0	0	0	0.11±0.08	0	0	0	0
Tetradecane	1400.0	0.77±0.3	0.65±0.42	0.09±0.17	0.41±0.29	0.2±0.15	0.09±0.06	0	0	0	0.01±0.03	0.01±0.02	0

Compound	RI												
Trans-beta-Guaiene	1492.0	0	0	0	0	0	0.98±1.96	0.07±0.08	0.08±0.06	0.06±0.11	0.02±0.04	0.01±0.01	0.02±0.05
Trans-Cadina-1(6),4-diene	1531.0	2.11±2.99	2.73±2.93	4.71±4.75	1.46±2.29	3.53±3.62	11.03±9.3	0	0	0.06±0.12	0	0	0
Trans-meta-Mentha-2,8-diene	1008.0	0.14±0.28	0.41±0.28	0	0.12±0.23	0	0.26±0.36	0	0	0	0	0	0
Trans-Murrola-4(14),5-diene	1463.6	0	0.15±0.3	0.08±0.17	0.34±0.39	1.02±0.76	0.46±0.33	0.12±0.08	0.03±0.07	0.13±0.16	0	0	0
Trans-Sabinene hydrate (trans-4-Thujanol)	1253.4	0	0	0	0	0	0	0.03±0.05	0.03±0.06	0.04±0.04	0	0	0
Tridecanal	1511.6	0	0	0	0	0	0	0.09±0.06	0	0.08±0.07	0	0	0
Tritriacontane	3292.8	0.05±0.04	0.28±0.25	0.12±0.23	0.08±0.07	0.21±0.14	0.57±0.56	0	0	0	0	0	0
Verbanol acetate	1125.9	0.52±0.18	0.43±0.1	0.02±0.04	0.29±0.19	0.22±0.21	0.11±0.09	0	0	0	0	0	0
Verbenone	1184.4	0	0	0.03±0.06	0	0.06±0.07	0.07±0.08	0.06±0.07	0.09±0.06	0.01±0.02	0.01±0.02	0.02±0.04	0.01
Viridiflorene	1488.9	0.08±0.1	0.17±0.09	0	0.06±0.05	0.07±0.06	0.23±0.33	0.05±0.07	0	0.04±0.08	0.05±0.1	0	0
Z-alpha-Bisabolene	1496.2	0	0	0	0	0	0	0	0	0	0	4.39±3.75	0
Z-alpha-trans-Bergamotol	1431.1	0	0	0	0	0	0	0.34±0.04	0.18±0.21	0.11±0.21	0	0	0.07±0.15
Z-Caryophyllene	1588.2	0	0	0	0	0.06±0.13	0.07±0.1	0.21±0.04	0	0.02±0.04	0.07±0.14	0.05±0.1	0.19±0.22
Z-Nerolidol	2007.3	0	0	0	0	0.04±0.09	0	0.63±1.25	3.17±0.34	4.94±3.03	0.71±1.43	1.25±1.45	1.45±0.97
Zonarene	1526.0	0.03±0.06	0	0	0	0	0	0.03±0.02	0.04±0	0.01±0.02	0.01±0.01	0.01	0.01±0.02

RI, Retention Index.   [a] Compounds identification based on data obtained from the NIST 2017 library of the gas chromatography-mass spectrometry system. [b] Relative percentage '–' not-detected. UM1-KK-Upper Midland 1-Kakamega, LM-KI- Lower Midland-Kiboko, CL-MT- Coastal Lowland-Mtwapa, LH-NJ-Lower Highland-Njoro, UM3-EM- Upper Midland 3-Embu, UM2-KA- Upper Midland 2-Kandara.
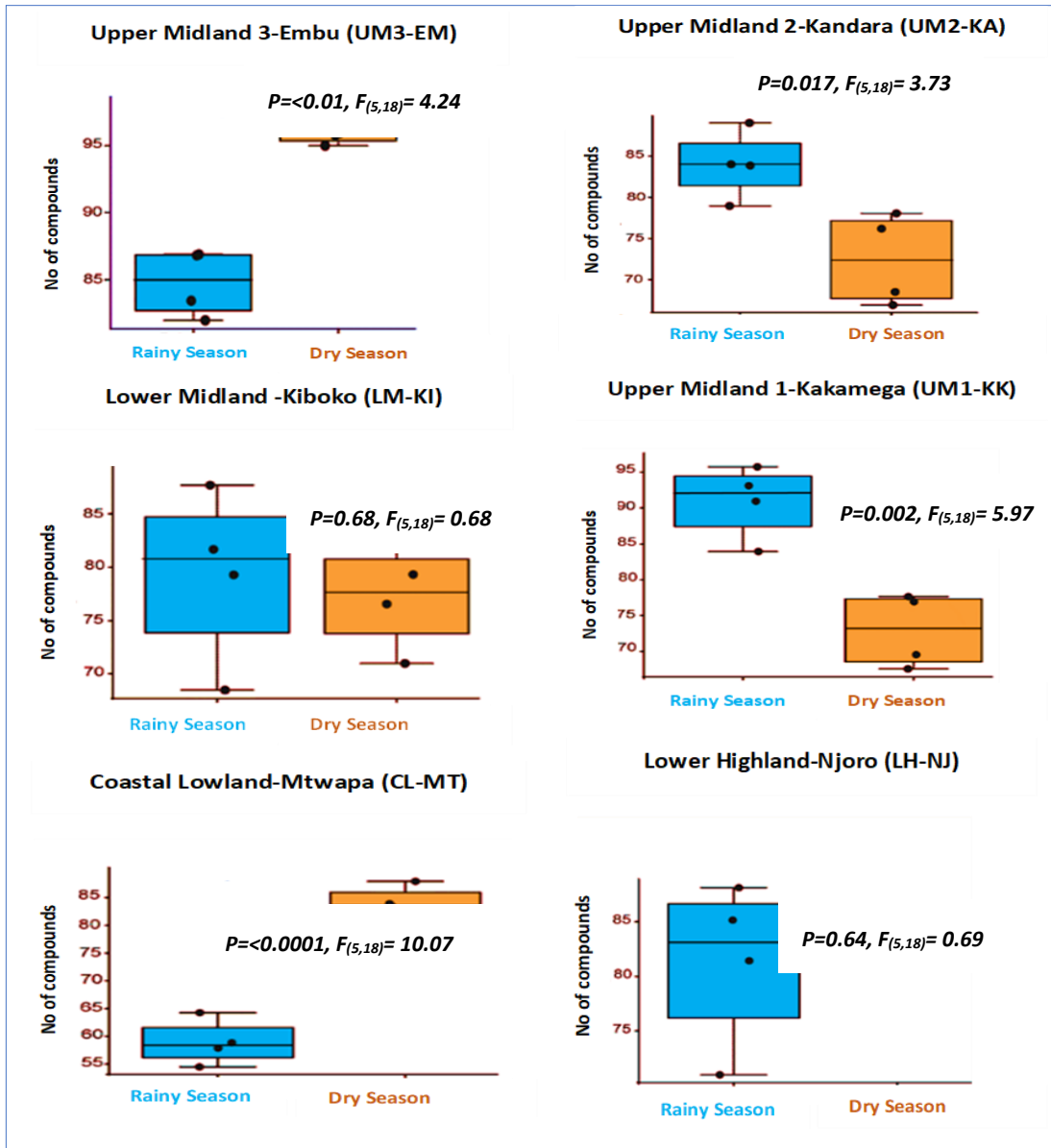
Figure 3.2: Box plots showing the quantitative variation in the number of secondary metabolites synthesised during the rainy (in blue) and dry (in brown) seasons across the different geographic zones (Locations) of plant collections. The ends of the boxplot whiskers represent the minimum and maximum of all the data.

The histograms depicted in Figure 3.3C elucidate the ten most significant compounds that contribute to the seasonal variation of essential oil of lantana plant. The Similarity Independently of the site, the analysis of similarity based on the Bray-Curtis distance matrix had a significant difference in the composition of secondary metabolites synthesized between the rainy and dry seasons. The secondary metabolites synthesised by lantana plant varied qualitatively and quantitatively between the rainy and dry seasons as depicted by the Non-metric multidimensional scaling (NMDS) plot based on Bray–Curtis dissimilarities index (ANOSIM: $p<0.0001$, R=0.465) (Figure 3.3A) with a clear separation of the distribution of compounds between the two seasons (Figure 3.3 B, stress: 0.199). The points show quite a dissimilar composition in the compound. Indicating that the samples of essential oil from the different Agroecological zone have diverse composition of secondary metabolites synthesized from each season. The dry season presented more compounds as compared to those synthesised during the rainy season among the most significant compounds contributing to the seasonal variability.

Percentage (SIMPER) analysis revealed that trans-cadina-1(6), 4-diene, (E-) Caryophyllene, and 1, 8-cineole are the three most significant compounds responsible for distinguishing lantana essential oil between the rainy and dry seasons (figure 3.3C). The three most identified compounds exhibit an increase or decrease in their concentration with the change in season. Specifically, (E-) Caryophyllene and trans-cadina-1(6), 4-diene is produced more during the dry season, while 1, 8-cineole are produced more in the rainy season (Table 3.2).

Figure 3.3: Secondary Metabolites synthesised *by Lantana camara* (A) A two dimensional plot of the essential oil samples investigated in Non-metric multi-dimensional scaling (NMDS) showing two clusters of samples of dry season (brown) and rainy season (blue). (B) Quality of the NMDS plot visualization showing dissimilarity distribution of compounds between the two seasons. (C) Similarity percentage (SIMPER) analysis showing compounds with the strongest influence on the two clusters based on seasonality.

3.1.1.1 Rainy season variation of *Lantana camara* Secondary Metabolites

The results show that the total number of compounds synthesised by lantana plant significantly differed across the different localities where this plant was collected Figure 3.5A). Total secondary metabolites synthesised by lantana plant showed a significant difference between locations (F $_{(5,18)}$ =2.93, P < 0.05). UM1-KK had the highest amount of secondary metabolites and was not significantly different from UM3-EM, UM2-KA, LM-KI and LH-NJ, but signifacantly different from CL-MT (Figure 3.4).

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Figure 3.4: Boxplots showing the variation of the amount of SMs synthesised by *L. camara* across the different geographic regions. Boxplot whiskers indicate ± 1.5 interquartile range limits. Boxplots with different letters show significant differences (P < 0.05).

The ANOSIM test and the NMDS plot revealed a significant difference in the composition of Lantana essential oil secondary metabolites, with compounds being grouped based on their origin. The NMDS ordination plot illustrated variations in the SMs synthesized by the Lantana plant across different localities during the dry season (Figure 3.5A). This dissimilarity was further supported by the Shepard plot (Figure 3.5B). Using SIMPER analysis, trans-cardina-1 (6), 4-diene, followed by Caryophyllene oxide and Lavandulyl isovalerate were identified as the most critical SMs contributing to the variations across regions Figure 3.5C). It is worth noting that the three most identified compounds exhibited very low to no concentration in the rainy season in all regions (Table 3.2).

The NMDS ordination plot also identified the influence of soil physicochemical and climatic variables on the synthesis of SMs in each region (Figure 3.5D). Specifically, in CL-MT, secondary metabolites synthesis was influenced by temperature, ultraviolet light (UV), and soil pH. Conversely, in UM3-EM, LM-KK, and UM1-KK, secondary metabolites synthesis was also influenced by soil parameters such as phosphorus,

potassium, and total organic carbon (TOC). In LH-NJ, the synthesis of SMs was significantly influenced by humidity.
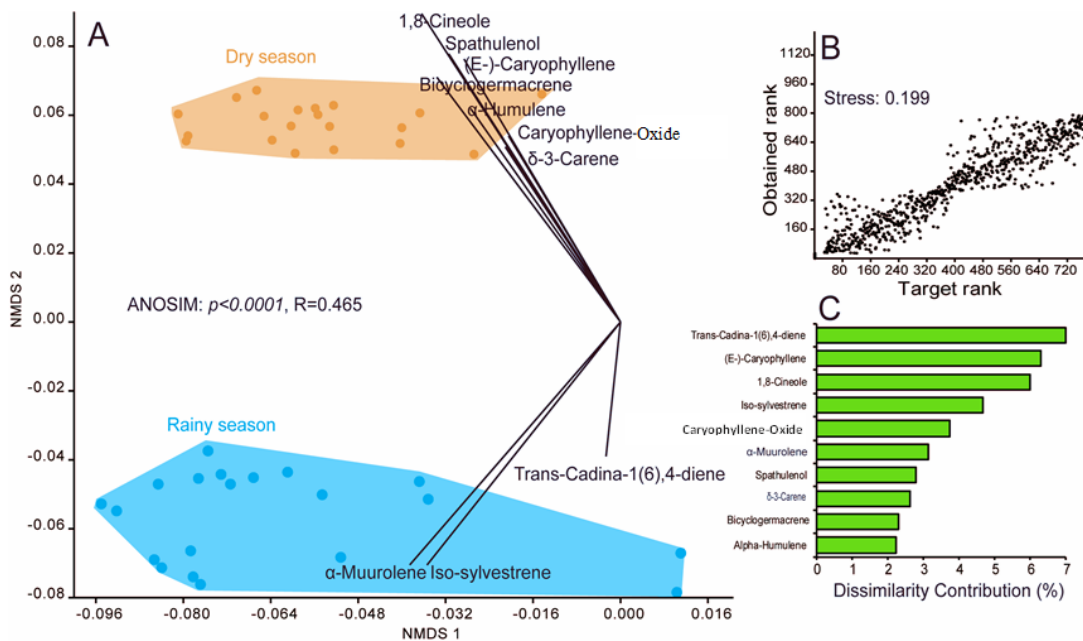

Figure 3.5: Secondary Metabolites synthesised by _L. camara_ in wet season. (A) A two-dimensional plot of the essential oil samples investigated in Non-metric multi-dimensional scaling (NMDS) showing the variation on significant SMs across regions. (B) Quality of the NMDS plot visualization showing dissimilarity distribution of compounds between the different regions. (C) Similarity percentage (SIMPER) analysis, showing the SMs contributing to the overall dissimilarity of the synthesised SMs across the different regions. (D) Non-metric multi-dimensional scaling (NMDS) ordination plot showing the soils physiochemical and the climatic variables that influence the SMs synthesis in each region.

3.1.1.2 Dry Season variation of *Lantana camara* Secondary Metabolites

The result for the dry season also shows quantitative and qualitative variations in lantana plant essential oil across the regions. The total number of secondary metabolites synthesised by lantana plant showed a significant difference between regions ($F_{(5, 18)}$ =6.11, $P < 0.01$). UM3-EM had the highest amount of SMs and was significantly different from LH-NJ and CL-MT. UM2-KA, LM-KI, UM1- KK (Figure 3.6) had significantly low amount of SNs when compared to UM3-EM but they were not different from CL-MT and LH-NJ (Figure 3.6).
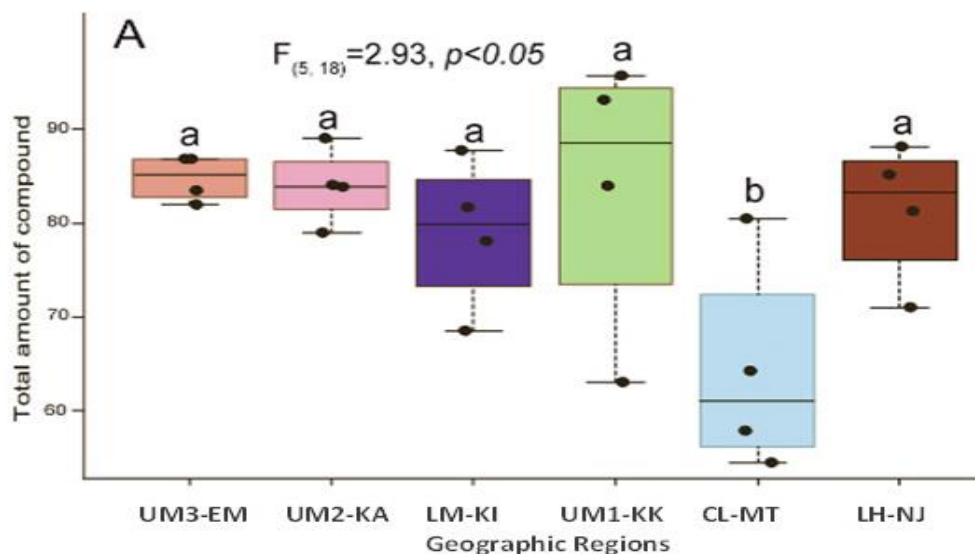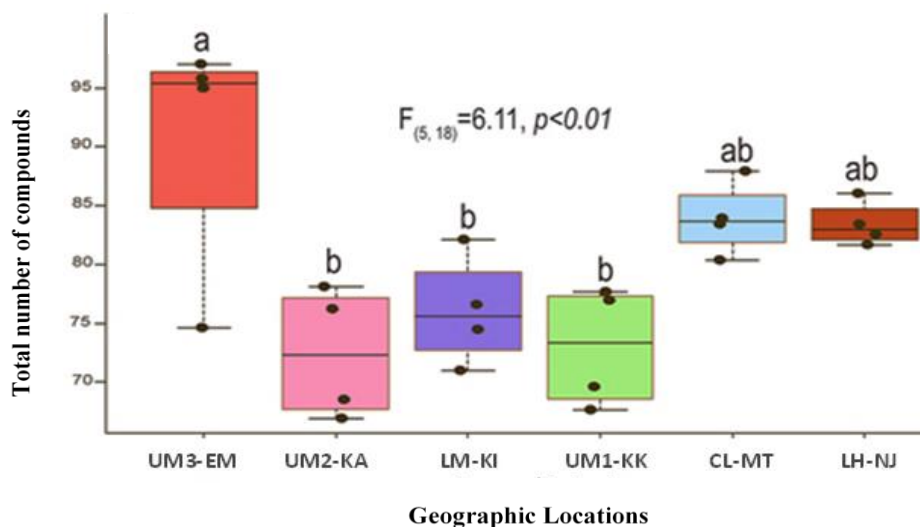

Figure 3.6: Boxplots showing the variation of the amount of SMs synthesised by *L. camara* across the different geographic regions. Boxplot whiskers indicate ± 1.5 interquartile range limits. Boxplots with different letters show significant differences (P < 0.05).

The non-metric multidimensional scaling (NMDS) ordination plot illustrates the variation in secondary metabolites synthesised by the lantana plant across the different regions. Samples clustered based on the area of origin on NMDS plot (Figure 3.7 A, Stress=0.1939, Figure 3.7 B), UM2-KA, UM1-KK and UM3-EM formed distinct groups, while LH-NJ and CL-MT overlapped. LM-KI samples showed a distinct cluster but with high variation within the samples. Based on the SIMPER analyses, the compounds (E)-caryophyllene, δ-3-Carene, and 1, 8-Cineole were the main compounds differentiating the

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clusters and regions in the dry season (Figure 3.7 C). Samples still clustered well based on the area of origin on NMDS plot (Figure 3.7 D Stress=0.1939, Figure 3.7 B) UM2-KA, UM3-EM, LH-NJ and UM1-KK formed distinct clusters. LM-KI still showed high variation and overlapped with CL-MT which showed low variation.

The NMDS ordination plot also identified the influence of soil physicochemical and climatic variables on the synthesis of SMs in each region Figure 3.7D). Secondary metabolites synthesis was influenced by soil parameters such as phosphorus, nitrogen, and soil pH and the climatic factors weresunshine, rainfall, temperature and humidity (Figure 3.7D)
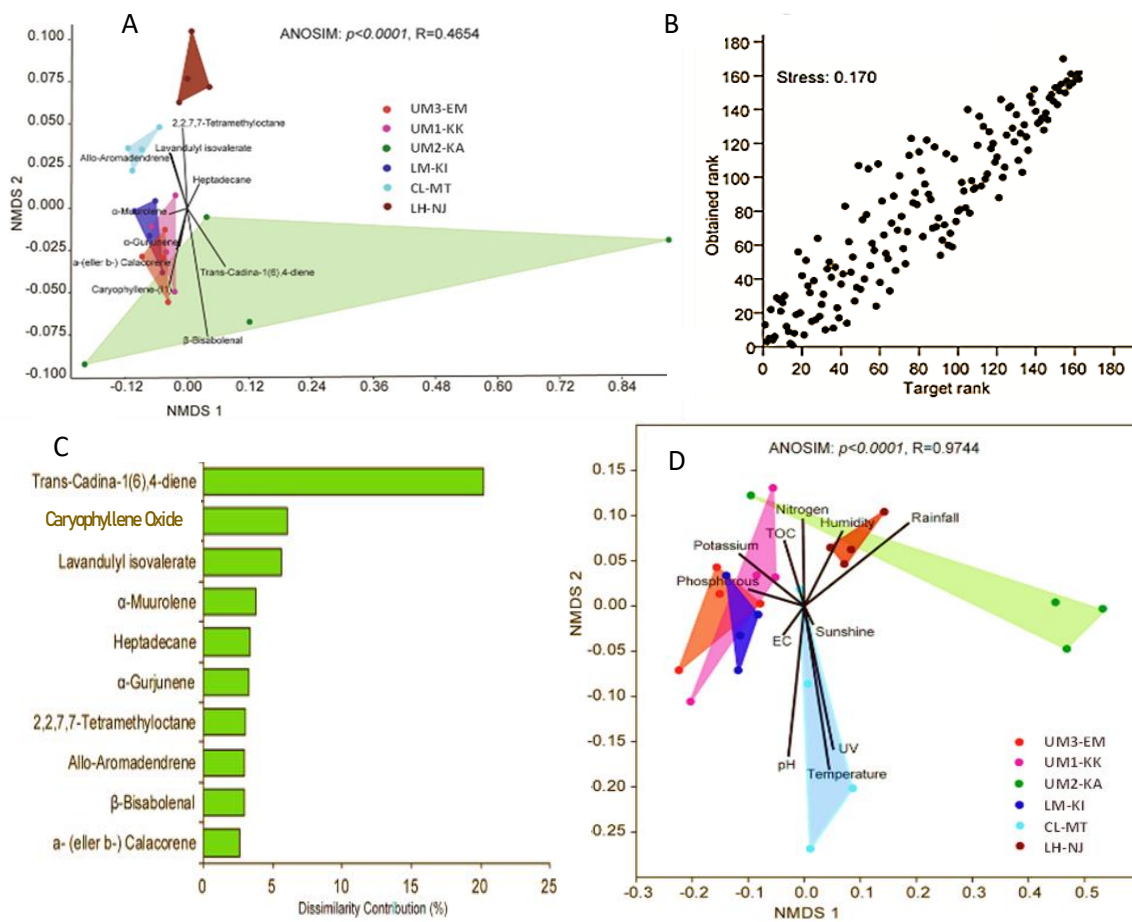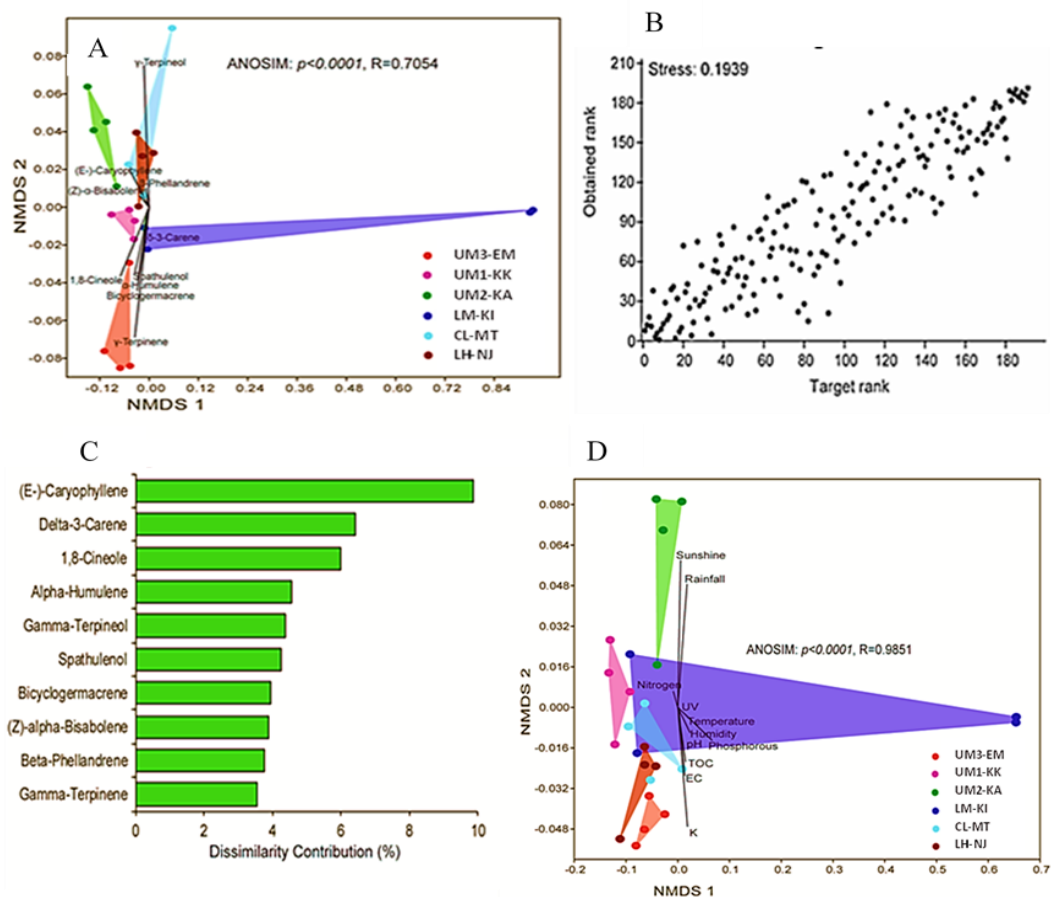

Figure 3.7: Secondary Metabolites synthesised by Lantana camara in dry season. (A) A two-dimensional plot of the essential oil samples investigated in Non-metric multi-dimensional scaling (NMDS) showing the variation on significant SMs across the

regions. (B) Quality of the NMDS plot visualization showing dissimmilarity distribution of compounds between the different regions. (C) Similarity percentage (SIMPER) analysis, showing the SMs contributing to the overall dissimilarity of the synthesised SMs across the different regions. (D) Non-metric multi-dimensional scaling (NMDS) ordination plot showing the soils physiochemical and the climatic variables that influence the SMs synthesis in each region.

3.4 Discussion

Essential oils from plants produce a significant amount of valuable biological compounds Compound accumulation and variations in essential oil composition are rather typical even within the same species growing in different geographic regions and seasons. It depends on the genotype, plant organ, harvest, geographical region, season, plant nutritional status, climatic conditions-temperature, humidity, and light intensity (Isah, 2019; Pereira et al., 2019). The oils of lantana showed considerable variability in the chemical composition, from the same species growing in diverse agro-ecological conditions of Kenya. Our results corroborate with Bendera (2007) and Syombua (2015), who also found variability in the chemical profile of Lantana essential oils harvested from Maseno-western Kenya and the eastern part (Kitui and Machakos), respectively. Similarly, reports from different parts of the world also show remarkable differences in the chemical composition (Moustafa et al., 2016; Murugesan et al., 2016; Nea et al., 2020)

This study established that the essential oil of the lantana wild population in Kenya is rich in sesquiterpene and monoterpene compounds, mainly the hydrocarbons and oxygenated sesquiterpene. Overal the sesquiterpenes were discriminated by the dominant presence of (E)-caryophyllene at 13.07- 0%, which agrees with previous studies, such as Dos Santos et al. (2019). They found (E)-caryophyllene content at 13-8.9% in Brazil, while Khalid (2019) and Dougnon and Ito (2019) found 17.9% and 16.7-8.9% in Egypt and Benin, respectively. On the other hand, 1,8 Cineole (11.01-0%) dominated the monoterpenes, corroborating the findings of Nea et al. (2017) with up to 9.0 % relative abundance.

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These results are consistent with our study; however, there are some differences in the percentages of detected compounds related to environmental factors (climate, seasons, geography and geology) variability. Several versatile standard components are present in all the essential oil analysed in this work, including; bicylogermacrene, spathulenol, eucalyptol, (E)-nerolidol, and caryophyllene oxide, which are valuable biological compounds (Olayemi, 2017).

The natural environment strongly affects the medicinal plants' chemo diversity by influencing the biosynthesis of SMs in a plant population founded on the environmental conditions where they grow (Allevato et al., 2019; Pereira et al., 2019). Most plants can control the secondary metabolites according to the growing environment (Isah, 2019; Li et al., 2020), causing changes in the plant's essential oils. This study has shown that the lantana plants growing in the wild significantly differed in their essential oil composition with geographical location and seasons. These differences were found to be likely driven by soil parameters and climatic conditions.

The variation in the essential oil of lantana leaf metabolic profile was observed from the samples collected from six different agroecological zones in Kenya, in the rainy and dry seasons. The results observed seasonal specificity, with variability in the number of secondary metabolites observed for each zone's rainy and dry seasons. UM1-KK, LM-KI and UM2-KA had an increased metabolite number than the other regions during the rainy season. Similarly, UM3-EM had an increased number of metabolites during the dry season. This increase in metabolic variation can be attributed to the plant's interaction with the prevailing conditions at that particular season (Li et al., 2020). Therefore, compounds that are influenced by rainy/dry seasons were produced in abundance. This is attributed to the amount of rainfall and maximum UV index and sunshine hours during both rainy and dry seasons (Khare et al., 2020). The results agree with other researchers that the metabolite synthesis of essential oil-producing plants is strongly influenced by the slightest changes and how they interact with the natural environment without detrimental effects resulting in newly formed compounds that could be valuable (Anjali et al., 2023; Divekar et al., 2022; Jan et al., 2021).

The environmental factors are the mechanism involved in influencing the Lantana plant secondary metabolite accumulation and biosynthesis. It is linked to seasonal induction of the plant's stress factors, such as humidity, soil conditions, changes in temperature and rainfall (Kleinwächter & Selmar, 2014; L. Yang et al., 2018). For instance, plants collected in the dry season may have encountered high temperatures affecting the production of Caryophyllene oxide, 1,8 cineole, trans-cadina-1(6), 4-diene, lavandulyl isovalerate and (E)-caryophyllene. Elevated temperatures induced the production of (E)-Caryophyllene, trans-cadina-1(6), 4-diene and lavandulyl isovalerate, while low temperatures during the dry season influenced a rise in 1,8 cineole, spathulenol and caryophyllene oxide. It has been shown that heat stress can affect the production of sesquiterpenes and monoterpenes. For example, heat stress exposed *C. nankingense* have elevated emissions of (E)-Caryophyllene (Wen et al., 2022). De Almeida et al., (2016), reported an increase in (E)-Caryophyllene in the essential oil composition of *Copaifera langsdorffii* in the dry season as compared to the wet season. While Botrel et al. (2010) found that monoterpenes and sesquiterpenes content of *H. marrubioides*, in spring, showed very similar values, however, in winter, the monoterpenes content doubled in relation to the sesquiterpenes. This variability can be attributed to the relationship between plants and environmental conditions during plant productivity and growth at that specific season, changing the biosynthetic path of secondary metabolites toward metabolites' production (Wen et al., 2022). Hence, different dominant environmental pressures between the two seasons played a significant role in the diversity of the secondary metabolites of *Lantana camara* leaf essential oil. These results agree with (Nea et al., 2020; Pereira et al., 2019), and (Dos Santos et al., 2019), who reported fluctuations in patterns of secondary metabolites produced by lantana plant to correlate to seasonal changes. Similarly, this results are also agree with the works of (Da et al., 2015) and (de Sá et al., 2016) who confirmed significant variability in secondary metabolites composition of the essential oils of *Platonia insignis* Mart and *Hyptis carpinifolia* plants to be influenced by seasons. Overall, three significant compounds, trans-cadina-1(6), 4-diene, (E-)-Caryophyllene, and 1, 8-cineole, contributed significantly to the seasonal variability in the secondary metabolites profile of the wild lantana essential oil.

The concentrations of various secondary plant products are strongly dependent on the growing conditions and have impact on the metabolic pathways responsible for the accumulation of the related metabolites (Ramakrishna & Ravishankar, 2011). This study observed significant variability in Lantana plant essential oils concentration across the various regions where samples were collected during the rainy and dry seasons. Three main compounds, caryophyllene oxide, lavandulyl isovalerate and trans-cadina-1(6), 4-diene were identified that significantly contribute to the regional difference during the rainy season, while δ-3-carene, (E)-Caryophyllene and 1, 8-cineole contributed to the variations during the dry seasons across the regions. These compounds were found to be reduced in concentration. Considerable quantitative variation was noted when compared to the secondary metabolites on dry and rainy seasons. These results are consistent with earlier reports (Murugesan et al., 2016; Pereira et al., 2019). Lantana plant samples collected from collected from 21 municipalities representing three regions in Brazil showed significant varaibility in the secondary metabolites (Pereira et al., 2019). Geographical location is a significant factor influencing plant growth's prevailing conditions, having significant effects on secondary metabolite processes in a plant species (Liu et al. 2015).

In this study, the number of compounds synthesised varied with season and region depending on the prevailing climatic condition. The drier season provided a condition for increased stress factors such as water stress and pests infestation, leading to cellular dehydration, which causes osmotic stress and removal of water from the cytoplasm to vacuoles (Ramakrishna & Ravishankar, 2011). Plants naturally produces terpenes to increase the efficacy of herbivore natural enemies (Bergman et al., 2019). For examples Caryophyllene oxide play an active role in plant defense (Mahmoud et al., 2021). The significant difference in secondary metabolite content were climatic factors of temperature, rainfall, UV index, and pH property. These environmental conditions changes may explain the variability of secondary metabolites in the CL-MT region, situated in the coastal areas and dominated by a more substantial climatic seasonal variability, especially temperature, compared to LM-KI and LH-NJ, which had a

reasonably stable climatic condition. (Allevato et al., 2019) reported that temperature change can substantially affect secondary metabolites synthesis since areas with more considerable climatic changes are faced with more variation and could result in a more significant difference in their secondary metabolites profile. Furthermore, (Molina-Montenegro & Naya, 2012) argue that locations with slight seasonal variations and constant warm temperatures have low environmental plasticity capacity. Subsequently, stability in the environment would reduce the plant's overall pressures, therefore reducing metabolite variation.

The significant differences in soil characteristics and climatic conditions (Table 3.1) among the six regions and genetic factors are the determinant factors for the inconsistency in the secondary metabolites profile observed in lantana plant essential oil of the same species growing in Kenya's different regions. Factors such as soil properties, temperature, humidity, rainfall, and sunshine duration directly respond to these variations. These factors jointly influence the biosynthesis and accumulation of secondary metabolites (Gobbo-Neto & Lopes, 2007). Among the environmental factors, (Muscolo et al., 2019; Ramakrishna & Ravishankar, 2011) explained that soil characteristics represent a complex biological system that strongly affects the plant's ability to produce secondary metabolites. The soil's nutritional components (such as N, P, K) are required to grow medicinal plants and are actively involved in the plant's metabolic activities (Chrysargyris, Xylia, et al., 2017; Muscolo et al., 2019). Acceptably, soil characteristics play a vital role in diverse soil conditions that cause major differences in SM accumulation and biosynthesis in plants of similar species. The secondary metabolites profile variation response of lantana plant to the soils with various characteristics agreed with (Ormeño & Fernandez, 2012) and (Muscolo et al., 2019) findings, showing that soil has its intrinsic characteristics are directly accountable for the production of plant metabolite.

This study established a relationship between metabolic profile and soil properties to be regionally specific. Extrapolating these results at the seasonal and regional level suggests that any alteration of the soil properties changes secondary metabolites accumulation in

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the Lantana plant population, affecting its quantity and quality. The plant material analysed in this study was collected from plants growing under different natural conditions. Hence, it was not easy to separate the effects of individual factors from the environment's multifactorial influence on soil and climatic variables). Therefore, we conclude that different climatic factors (humidity, rainfall, UV, temperature) have different intensities and effects on the accumulation of secondary metabolites in the EO of lantana plant. At the same time, different secondary metabolites are affected by different kinds of soil properties in the soil to a different extent, where the physicochemical properties including N, P, K, TOC, EC and pH in the soil all have a relatively significant strong effect on plant secondary metabolism. Thus, the correlation differs significantly with secondary metabolites composition from region to region and season to season.

3.5 Conclusion

From this study, it was evident that the secondary metabolites variability in the *Lantana camara* exists in plants of the same species growing in different regions and also the effects of seasons due to the prevailing environmental conditions at the time of growth and development. The study characterised the secondary metabolites present in the leaves of the wild population lantana grown in six different AEZ in Kenya and the chemical variability in the function of the place of collection and the influence of the seasonal period was evident. This difference could result in enhanced targeted analysis and understanding of biological pathways. Although determining the plasticity of a species is difficult without a guided experiment. This work contributed for the knowledge on the chemical composition of essential oils of *Lantana camara*. The Major compounds found in the essential oils from leaves were Caryophyllene oxide, 1,8-cineole, trans-1-cadina(6),4-diene, and (E)- caryophyllene. The results presented herein strongly suggest that there is a seasonal variability of chemical compounds related to season and region.

CHAPTER FOUR

EVALUATION OF THE EFFICACY OF LEAF ESSENTIAL OILS OF LANTANA CAMARA FROM VARIOUS AGROECOLOGICAL ZONES OF KENYA AGAINST TOMATO LEAF MINER

Abstract

In recent years, Essential Oils (EOs) as alternatives to synthetic pesticides in managing pests have been proposed or advocated. The use of bio-insecticide/pesticide in pest management is encouraged in agroecology for a sustainable agricultural system. In this study, the essential oils of *Lantana camara* L. leaves from different agro ecological zones of Kenya were extracted by steam distillation and analyzed through GC-MS to identify the compounds. The contact toxicity and repellent activity of EOs against the invasive tomato pest, *Tuta absoluta,* were tested. The toxicological assays were performed following the leaf-dip bioassay protocol, while the repellency activity was performed using the repellent response method for phytophagous pests and the data was analysed using the ANOVA test. The results of that showed that *L. camara* EO has good insecticidal activity with higher mortality (89%) on the 2nd instar larvae with a higher concentration (0.01µl/µl) of the essential oils. The repellence test also showed a higher average repellence (93.44%) effect with a higher concentration rate (0.01µl/µl) of the Eos tratments. According to these results, the EO of *L. camara* can be used as an eco-friendly alternative for synthetic insecticide in the *T. absoluta* management program.

4.1 Introduction

The leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is a pest of vital global economic importance. It limits tomato production (*Lycopersicum esculentum* L.) worldwide (Campos et al., 2017; Guedes et al., 2019). As a result, this pest has gained notoriety as a pest species that can cause destruction and losses of up to 100% when there is no intervention (Rwomushana et al., 2019).

Current management of *Tuta absoluta* typically relies solely on synthetic insecticides (Silva et al., 2019). However, this management strategy has not provided a total solution to the problem due to insecticide resistance and pest resurgence (Guedes et al., 2019). Furthermore, these products are already proving to be harmful to the environment (Damalas & Koutroubas, 2018), causing the development and progression of several health issues in humans (Özkara et al., 2016). To cut back the excessive use of synthetic insecticides, exploring environmentally friendly and sustainable alternative strategies, such as natural products from wild plants, remains a viable option. Natural products directly utilized as pest control agents offer a more sustainable pest management solution than synthetic pesticides (Nuruzzaman et al., 2019).

Essential oils (EOs) from pesticidal plants used as pesticides play a crucial role in controlling pests sustainably (Campolo et al., 2017) by reducing the pest population while minimizing the environmental effect. *L. camara* is a wild plant that produces essential oils and extensively studied for its bioactive properties and reported having insecticidal (Javier et al., 2017; Murugesan et al., 2016), larvicidal (Zandi-Sohani et al., 2012), acetylcholine inhibition, repellent (Zhonglin. Yuan & Hu, 2013), and antifeedant (Chau et al., 2019) action among other features against a wide range of pests.

These bioactive properties exhibited by Lantan essential oil make it a novel candidate for use as a pesticide with multiple actions. For example, Murugesan et al. (2016) tested the lantana essential oil on *Hyblaea puera* and *Ahevidae fabriciella* (Lepidoptera) at a concentration of 10000 ppm and reported a 62% larval mortality and concluded that the essential oil expressed insecticidal and antifeedant properties. Besides, Javier *et al.* 2017 tested the essential oil of lantana for bioactivity against *Spodoptera litura* (Lepidoptera), showing remarkable insect growth regulatory activities and direct Toxicity. Corroborating with (Deshmukhe et al., 2011) that it has the potential to be exploited as a botanical insecticide for cutworm management. In their study, Costa *et al*. (2010) tested larvicidal activity against *A. aegypti* larvae using the essential oils from the leaves of lantana and showed that it has larvicidal potential. However, all these studies show the

potential this plant has in the management of pests. There is no scientific investigation of its essential oil bioactivity on *Tuta absoluta*.

4.2 Materials and Methods

4.2.1 Establishment of Tomato Plants

Tomato (Rio Grande VF) plants (*L. esculentum*) used for rearing *Tuta absoluta* and for performing the bioassays experiments were grown in the greenhouse conditions in 2-litre pots, inside large insect-proof cages (plate 4.1B) and maintained pest-free under (30±3°C) temperature, (75-80%) relative humidity and (12:12) light: dark conditions. The plants screened for the presence of pests every second day; in the rare event of infestations detected, these were manually removed by cutting the infested leaves or removing and destroying the plant (Roditakis et al., 2013). Therefore, no insecticides were used during the plant development phase.

4.2.2 Rearing of test insects

To establish an insect colony for experimental purposes, tomato leaves that were infested with *Tuta absoluta* were obtained from the International Centre of Insect Physiology and Ecology (ICIPE) insectary laboratory in Nairobi, Kenya (01°13.140'; 036°53.440') (plate 4.4A). The leaves were obtained from tomato plants and were collected within a two-hour timeframe before being transported to the laboratory. Upon arrival at the laboratory, the insects were released into insect-proof rearing cages, with dimensions measuring 50 cm in width, 60 cm in length, and 80 cm in height. The cages were equipped with four insect-free potted tomato plants, each measuring 25 cm in height (plate 4.1 B), which were intended to serve as a food source for the insects until they produced the first filial (F1) generation (Roditakis et al., 2013).

Newly-emerged tomato leaf miner adults, belonging to the F1 generation, were subsequently released inside a separate net cage to obtain homogeneous larvae that were of the same age, nutritional status, and general health, as prescribed by the IRAC method

no 022 (Porter, 2012). Tomato leaf miner adults were provided with water and an energy source in the form of commercial honey that was diluted 1:1. The insects were then permitted to oviposit for a period of 24-48 hours, following which the oviposition level was visually assessed. If an adequate number of eggs were observed, the plant material was carefully removed, and new plants were placed in the oviposition area to facilitate the continuation of the oviposition, in accordance with the IRAC method no 022 (Porter, 2012). The plant material that was infested with tomato leaf miner eggs was then placed in an insect-proof rearing cage to allow larval development to the second instar.
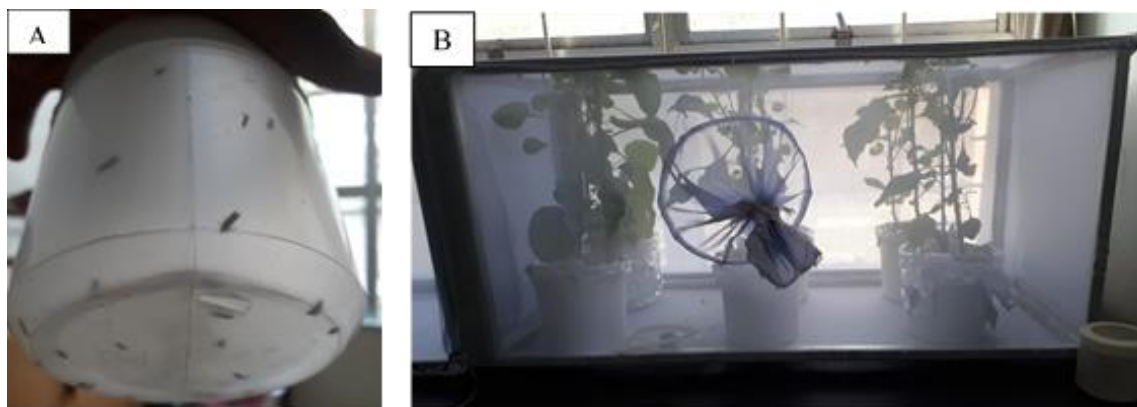
Plate 4.1: (A) *Tuta absoluta* **insects collected at ICIPE. (B) Rearing of the** *Tuta absoluta*

4.2.3 Bioassay Experiments

The experiments were conducted at the Department of Horticulture and Food Security at Jomo Kenyatta University of Agriculture and Technology (Kenya) under controlled environmental conditions in growth chambers maintaining a temperature of 25 ±2°C, 65-70% relative humidity, and 12:12 light: dark photoperiod regime.

Larvicidal Activity. The second instar larvae were collected from the rearing cages, and accordingly, the toxicological assays (plate 4.2 A) were performed following the leaf-dip bioassay protocol of the 199 Insecticide Resistance Action Committee (IRAC) test method 022 (Porter, 2012), with minor modifications. The study involved the preparation of essential oils extracted from lantana plants from different Agro-Ecological Zones

(AEZs) in three different concentrations, specifically 0.01, 0.001, and 0.0001 µl/µl. These concentrations were diluted with 0.1% Tween® 20 (Sigma-Aldrich, Germany), using a ten-fold serial dilution (1:10). Serial dilution of the 6 essential oils from the different agro ecological zones were prepared by first making a mother stock of 0.1% concentation EO by adding 100 µl of the 0.1% Tween 20 to a 1ml tube each and 10 µl of each EO was added to the tubes. Then 90 µl of the the Tween 20 was dipensed into of 3 test tubes for each zone EO with 100 µl pipettes. Label tubes 3-1 to 3-3 indicating dilution factor for each EO area o origin, then 10 µl was added of te mother stock to the first tube (3-1) and mixed gently. 10 µl of this dilution taken and added to the next tube (3-2), and mixed gently. The procedure repeated for the remaining tube (3-3). The resultant solutions were then utilized as treatments (18) in the experiment. A commercial formulation of Flubendiamide (BELT® 480 SC, Bayer AG, Germany) insecticide was used as a positive control, whereas sterile distilled water containing 0.1% Tween® 20 (Sigma-Aldrich, Germany) as a non-ionic wetting agent used as a negative control with three replications. The insecticide was used as per the manufacturer's recommendations (0.1µl/µl). Sufficient non-infested, untreated tender young whole tomato leaflets of uniform size were collected and kept in sealed plastic bags to prevent them from wilting.

Complete tomato leaflets were dipped for 5 seconds in the essential oil concentrations with gentle agitation to ensure the entire surface is covered equally. The treated leaflets dried on a wire net with an upper leaf surface (adaxial surface) facing skywards and placed in a labelled petri dish (Ø- 90mm) with slightly moistened filter paper covering the bottom. Around 0.2 ml of distilled water was used; this was sufficient to wet the filter paper keeping the leaf material turgid throughout the bioassay period. Second-instar larvae were carefully removed from the galleries in infested tomato leaves under a light-bed (transparent bench with fluorescent illumination underneath). In each petri dish, ten larvae (2nd instar) (4-5mm) (plate 4.2B) were released carefully using a subtle soft brush to avoid damaging the very fragile larvae. Subsequently, all the Petri dishes were sealed using a ventilated muslin cloth.

Larval mortality was assessed after 24, 48, 72, and 96 hours of exposure. Death larvae were identified under a magnifying glass (Osho® 10× magnification, Kenya) and recorded. The larvae scored as dead if they could not make coordinated movement from a gentle stimulus with a fine brush to the posterior body segment. The experiment was repeated three times, and the average mortality was obtained.
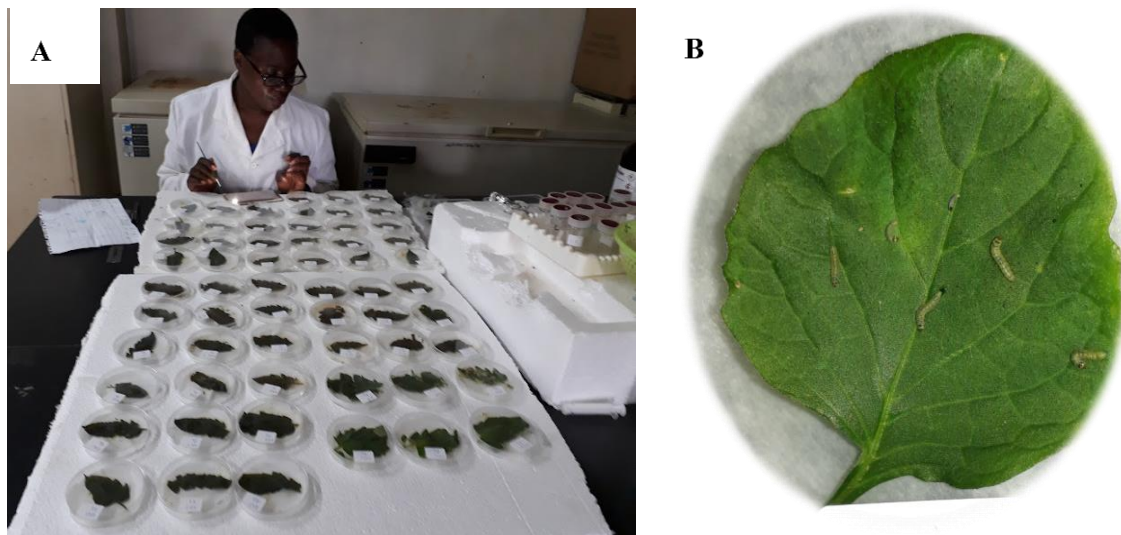
Plate 4.2: (A) Bioassay experiment (B) Morphology of the 2nd instar *Tuta absoluta* Larvae.

Repellent activity. Testing the repellent activity of the essential oils, Whatman No. 1 filter paper was cut to fit the size of the plastic dish and divided into two equal parts. We treated half of the filter papers with 0.1% Tween® 20 as control and the other half with EO concentrations diluted into 3 concentration with 0.1% Tween® 20 at a 10-fold dilution (0.01, 0.001, and 0.0001µl/µl). Twenty larvae (2nd instar) were placed in the middle of each dish. The plastic dishes were closed and tightened with Parafilm. The experiment was carried out in five replications and under the same environmental conditions as insect rearing. After four hours of exposure, the number of insects in each half of the filter paper was recorded and the percentage repellency (PR) was calculated using the following formulae:

$$PR = \frac{NC - NT}{NC + NT} \times 100 -(1)$$

PR = percentage repellency, NC = the number of larvae in the control area, and NT = the number of larvae in the treatment area.

The mean repellency value of each extract calculated and assigned to repellency classes from 0 to V: class 0 (PR ≤ 0.1%), class I (PR = 0.1–20%), class II (PR = 20.1–40%), class III (40.1–60%), class IV (60.1–80%), and class V (80.1–100%).

4.2.4 Statistical Analysis

The per cent mortalities were corrected for control (i.e., natural) mortality using Abbott's formula (Abbott, 1925). The two-way analysis was conducted with the essential oils concentration rate from different AEZ as the main effect, the zones as the covariate and larvae mortality registered at different time intervals (24, 48, 72, and 96 hours) as the response variable. In addition, the toxicity effect of the different essential oil concentrations on the second instar larvae was compared using the analysis of variance (two-way ANOVA) and the means compared by LSD test at 5% level (SAS®, On-Demand for Academics).

Concentration–mortality data (Data obtained from the bioassay) computed using the Probit procedure (PROC PROBIT LOG10; SAS®, On-Demand for Academics.) to estimate the median lethal concentrations (LC_{50} values) and their 95% fiducial limits (FL) and their respective standard errors, as well as slopes of the curves. The LC_{50} values were significantly different when their 95% fiducial limits did not show similarity.

Per cent of larval repellency analyzed using ANOVA (Analysis of variance) (SAS®, On-Demand for Academics.). The negative values were treated as zero. The larval repellency was calculated using the simplified contact repellency test. The mean percentage of larva that repelled into the untreated side and corrected by a control test. The square root of the per cent of repellency in each test was arcsine converted, and ANOVA and the multiple

comparisons of the repellency by LSD were performed using SAS®, On-Demand for Academics.

4.3 Results

4.3.1 Insecticidal activity of *L. camara* essential oils on *Tuta absoluta*.

4.3.1.1 Larvicidal Activity

The insecticidal activity of lantana essential oil was investigated. The essential oil had a insecticidal activity. The larva mortality were observed against the tomato leaf miner after an exposure period of 24 -96 h. The analysis of variance suggests that both the concentration rate ($F_{(7,2)}$= 13.11; p < 0.0001) and the essential oil from the area off origin ($F_{(7,2)}$ = 6.44; p < 0.0001), registered at 24 h, 48 h, 72 h, and 96 h after the treatment influenced the larva mortality. There was a significant interaction between concentration rate, essential oil from area of origin, and observation time (F $_{(7,2)}$ = ; p < 0.0001). However, there was no interaction between the two variables; the concentration and essential oils from the areas of origin ($F_{(10,2)}$ = 0.01; p =1.00)

The mean average larval mortality in the positive control insecticide -Flubendiamide) (PC-INS) was 16%, 30%, 76%, and 93% after 24, 48, 72, and 96 hours of exposure, respectively. Whereas in the negative control-water (NC-WA) treatment, no larvae died during the 96 hours of the experiment. The positive control (PC-INS) showed the highest larval mortality compared to the essential oil treatments (Figure 4.1).

In the first sampling (24 hours after the treatment), LM-KI (Kiboko) and UM1-KK (Kakamega) Eos were the most effective in killing the larvae, with 8% and 6% mortality, respectively. Overall, the positive control (PC-INS), on average, was most effective than the essential oil treatments (Figure 4.1). In the second sampling (48 hours), the mortality of *T. absoluta* larvae increased significantly in all the treatments compared to the first sampling. In the third, and fourth sampling, the mortality rate increased for all the treatments, each attaining above 40% larval mortality except UM-KA (Kandara),

treatment which was 39%. In the fourth sampling, all the treatments reached a mortality rate above 60%.

Throughout the trial, the essential oil from LM-KI (Kiboko) was the most effective against the larvae maximum average mortality of 77% at the 96 h, whereas the LH-NJ (Njoro) and UM3-EM (Embu) essential oils could only kill a maximum of 69% of the exposed larvae at 96 h. Overall, there was no significant difference in the mortality induction by the essential oils from the different area of origin.

The concentration rate of the essential oil influenced the larval mortality rate. In all the time points, the positive control (0.1µl/µl-insecticide recommended concentration rate), on average, was most effective in killing the larvae (average mortality= 93%) than the essential oils concentrations of 0.01, 0.001, and 0.0001µl/µl (average mortality= 89, 71 and 58% respectively (Figure 4.1). The longer the larvae's exposure to the treatment, the more the mortality rate increased in all the concentrations rates. The highest concentration of the essential oils (0.01µl/µl) showed the highest mortality at all the time points of sampling, which was reasonably comparable to the positive control (0.1µl/µl).
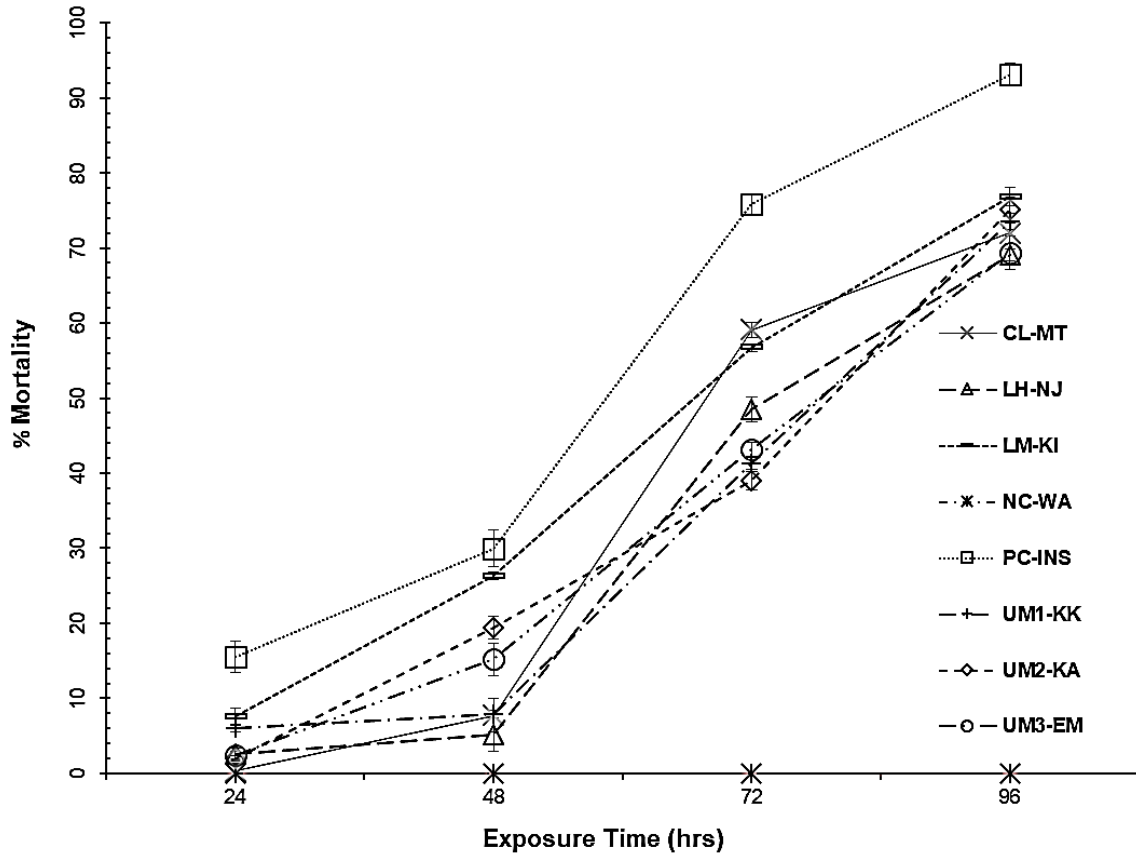
Figure 4.1: Mean mortality of *Tuta absoluta* larvae exposed to different EOs treatments and the two controls (± control) in the toxicological bioassay.

Lantana oil treatments sampled from different climatic zones- UM1-KK-Upper Midland 1-Kakamega, LM-KI- Lower Midland-Kiboko, CL-MT- Coastal Lowland-Mtwapa, LH-NJ- Lower Highland-Njoro, UM3-EM- Upper Midland 3-Embu, UM2-KA- Upper Midland 2-Kandara, and the control- PC-INS- positive control-insecticide–Flubendiamide, NC-WA- negative control-water.

The essential oil concentrations rate of 0.1µl/µl were the most effective in killing the larvae at 16% mortality, followed by 0.01µl/µl at 7% in the first sampling (24 hours after the treatment). The mortality rate of *T. absoluta* larvae increased in all the treatments in the second sampling (48 hours), and in the 0.01µl/µl concentration rate, the mortality rates increased three times, compared to the first sampling. None of the concentrations

70

rate, including the positive control, attained 50% larval mortality within the early 48 hours of larval exposure. However, by 72 hours, the positive and 0.01 µl/µl concentration rate had achieved above 50% mortality (figure 4.2).
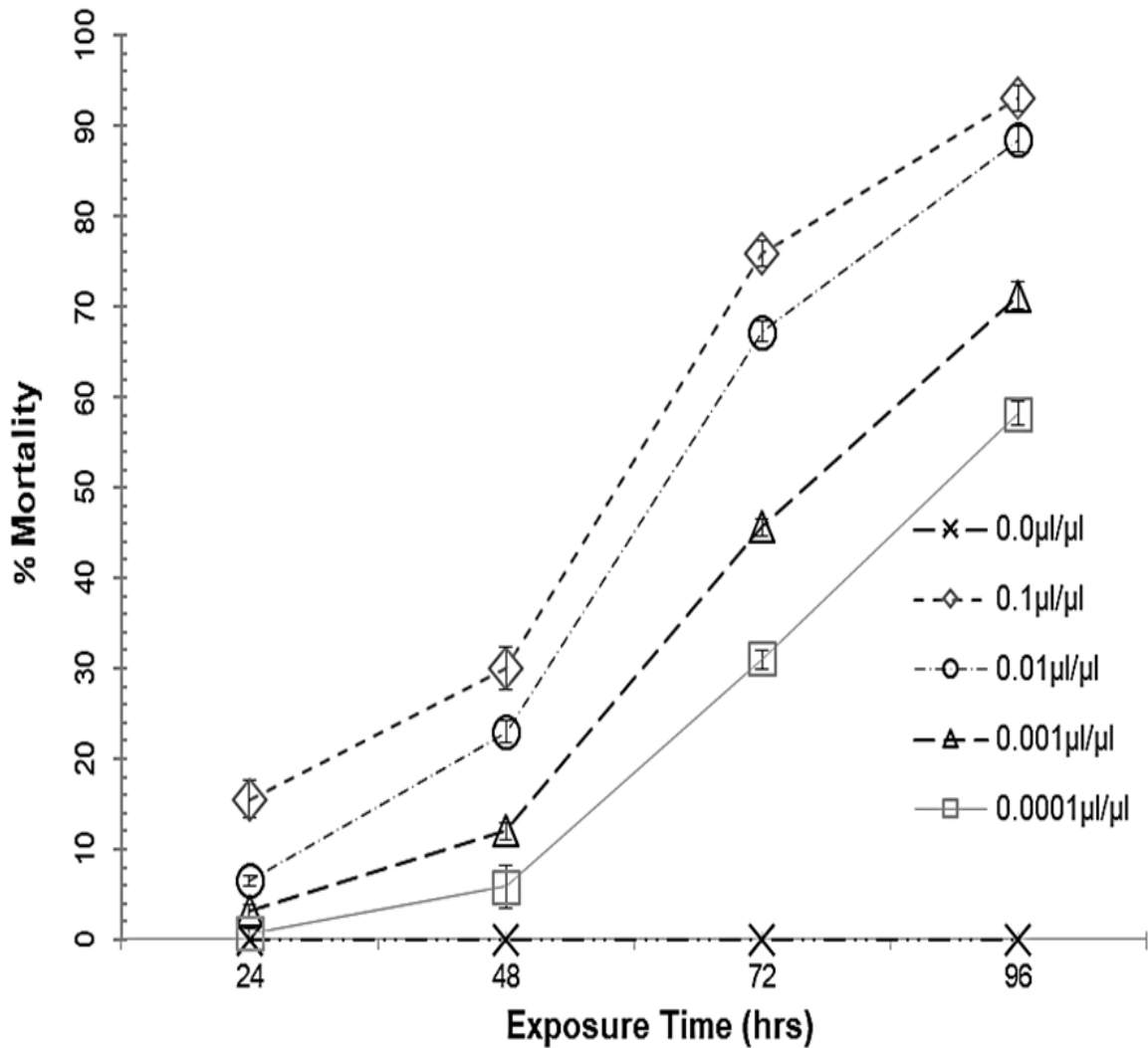
Figure 4.2: Mean mortality of Tuta absoluta larvae exposed to different essential oil concentration rates and the controls in the toxicological bioassay.

0.1µl/µl-positive control-insecticide recommended rate; 0.0µl/µl- negative control- water; 0.01, 0.001, and 0.0001µl/µl - essential oils concentrations.

4.3.1.2 The median lethal concentrations (LC$_{50}$) for the larvae.

The LC$_{50}$ values (Table 4.1) were calculated for 72 hours because the maximum mortality registered at 24 and 48 hours was less than 50%, while at 96 hours, it was over 50%. Therefore, this was the best time since the longer the exposure time, the lower the LC$_{50}$ values. The concentration-response mortality data exposed to the essential oil treatments presented χ^2 values < 34.33. This parameter shows the appropriateness of the model to estimate the LC$_{50}$. Evaluation of toxicity of the essential oils, based on LC$_{50}$ values and Fiducial limits, showed that there was a significant difference among- essential oil toxicity for CL-MT(Mtwapa), LH-NJ (Njoro), LM-KI (Kiboko), UM1-KK (Kakamega), UM2-KA (Kandara), and UM3-EM (Embu) were 0.00025, 0.00123, 0.00044, 0.00246, 0.00542 and 0.00242μl/μl, respectively (Table 4.1). The essential oil extracted from CL-MT (Mtwapa) (LC$_{50}$ = 0.00025μl/μl). and LM-KI (Kiboko) (LC$_{50}$ = 0.00044 μl/μl). showed the highest capacity to kill the exposed larvae. The essential oil from the UM2-KA (Kandara) treatment required the highest concentration (LC$_{50}$ = 0.00542μl/μl) to kill 50% of the exposed larvae (Table 4.1). The mortality rate of the larvae depended on the concentration of essential oil and increased with increasing oil concentration rates. For all the six oil treatments, the highest average mortality (93.44%) was observed at the highest concentration rate (0.01μl/μl) (Figure 4.2).

Table 4.1: Estimated median lethal concentrations (LC$_{50}$) of the different essential oils of *Lantana camara* on *Tuta absoluta* larvae at 72 hours in the toxicity test on larvae bioassays.

Different letters within the same column of each trial indicate statistical differences (p < 0.05); s = significant (α > 0.05), df –degree of freedom, SE- standard error.

Essential oil	LC$_{50}$ (µl/µl)	95% of Fiducial limits	Slope	Intercept ± SE	χ^2 (df = 1)
CL-MT	0.00025a	0.000059-0.00059	2.45	1.47±0.292	25.32s
LH-NJ	0.00123b	0.00056-0.0029	2.24	1.30±0.285	20.83s
LM-KI	0.00044a	0.00018-0.00085	1.94	1.73±0.295	34.33s
UM1-KK	0.00246c	0.00144-0.00478	0.94	2.79±0.492	11.97s
UM2-KA	0.00542d	0.00222-0.0296	2.49	0.91±0.281	10.31s
UM3-EM	0.00242c	0.001021-0.00621	1.26	2.07±0.468	19.61s

CL-MT- Coastal Lowland-Mtwapa, LH-NJ- Lower Highland-Njoro, LM-KI- Lower Midland-Kiboko, UM1-KK-Upper Midland 1-Kakamega, UM2-KA- Upper Midland 2-Kandara, UM3-EM- Upper Midland 3-Embu.

4.3.1.3 Repellent activity

Results in Table 4.2 describe the repellent activity of different essential oil treatments and concentrations rates of *L. camara* leaf. The results revealed that there was no significant difference between the essential oil treatments ($F = 1.68$; df = 5; $p = 0.1505$) and the interaction between the variables, concentration rate and essential oils treatments interaction ($F = 0.77$; df = 10; $p = 0.6539$) after 4 hours of exposure. The essential oil of *L. camara* treatmens showed significant repellent activity against the second instar larvae of *T. absoluta* at all concentrations ($F = 156.28$; df = 2; $p < 0.0001$) within the 4 hours of exposure. The repellency activity of *L. camara* essential oil was concentration-dependent.

The repellency increased when concentration rates increased. Complete repellency was observed when the highest concentration rate (0.01μl/μl) was applied. Although, there was a slight variation in the repellent effects within essential oil treatments. LM-KI (Kiboko) essential oil showed the most repellency activity with 99.97% of repellency induced by 0.01μl/μl whereas the treatment of 0.0001μl /μl concentration rate induced only 30.24% of repellency of *T. absoluta* larvae, with average rate of 60.93%. UM3-EM essential oil showed the lowest repellency activity within the 4 hours with 86.66% repellency induced by 0.01μl/μl whereas the concentration rate of 0.0001μl/μl induced only 20.40% of repellency of *T. absoluta* larvae, with an average rate of 55.70%. The essential oil treatments of all the *L. camara* leaves from diverse AEZs exhibited repellency classes II, III, and V based on the mean repellency rate, with an increasing concentration rate (Table 4.2).

Table 4.2: Repellent activity of different concentration rates of essential oils from *L. camara* leaves against *T. absoluta* larvae.

EOs	CL-MT	LH-NJ	LM-KI	UM1-KK	UM2-KA	UM3-EM	
Concentration (01µl/µl)	Repellency (%) ± SE (4 h)						Repellency Class
0.0001	27 ± 6.18Cc	29.15± 8.37Cc	30.24±4.17Cc	22.99±4.76Cc	22.99±4.77Cc	20.40±5.77Cc	II
0.001	52.60± 5.20Bb	39.78±5.47Bb	52.60±5.20Bb	55.16±5.94Bb	40.04±4.13Bb	50.06±5.19Bb	III
0.01	90.89 ± 6.12Aa	90.89±6.12Aa	99.98±9.19Aa	96.39±4.90Aa	93.29±7.14Aa	86.66±6.95Aa	V

Values followed by the same small letters within a column and capital letters within a row are not significantly different ($p < 0.05$) according to the LSD test. Lantana oil extracted from; UM1-KK -Upper Midland 1 Kakamega, LM-KI- Lower Midland-Kiboko, CL-MT- Coastal Lowland-Mtwapa, LH-NJ- Lower Highland-Njoro, UM3-EM- Upper Midland 3-Embu, UM2-KA- Upper Midland 2-Kandara.

4.4 Discussion

This study shows the bioactivity studies of *Lantana camara* essential oil as a potential bioinsecticide to manage the tomato leaf miner, *Tuta absoluta*. While previous reports showed the potential of *Lantana camara* oil to be effective in the control of lepidopteran pests (Chau et al., 2019; Kasmara et al., 2018), our study provides the observed bioactivity of the essential oils of *L. camara* from various AEZs against the second instar larvae of the leaf miner, *T. absoluta*, together with their repellence activity. Therefore, contributing to a thorough understanding of the potential use of essential oils in the management of agricultural pests.

Lantana camara essential oils have undergone bioassay tests on several pests. Most bioassays reported focus on trials lasting for less than 48 hours, therefore, the lasting effects of these compounds are lacking (Chegini et al., 2018; Javier et al., 2017; Soares et al., 2019; Yarou et al., 2018). To the best of our knowledge, no study on *L. camara* essential oil bioactivity against *T. absoluta,* together with their toxicity differences from oils sourced from the same plant but differing geographic zones, and their repellency activity recorded. However, previously several researchers have tested the essential oils from other plants against *T. absoluta* larvae for toxicity (Abdel-Baky & Al-Soqeer, 2017; Chegini et al., 2018; Javier et al., 2017; Soares et al., 2019; Yarou et al., 2018) and their repellence activity (Allam Tarik, 2015). The essential oils showed considerable toxicity and repellency activity on the *T. absoluta* larvae.

In our study, the insecticidal activity varied with the concentration rate of the essential oil treatments. In the larcicidal activity, the larval mortality was concentration rate-dependent, and larval susceptibility increased with increasing rate of concentration. Among the *L. camara* essential oil treatments, the larval mortality recorded did not significantly differ. Thus, the exposure concentration of the essential oil is proportional to the level of toxin and toxicity. Besides, the longer the exposure time, the higher the mortality rate recorded.

The present study shows that leaf application of *Lantana camara* essential oil is equally practical as a stomach action toxicant against the leaf miner, *Tuta absoluta*.

Theis agrees with reports by (Javier et al., 2017) and (Kasmara et al., 2018), who found that *L. camara* essential oil caused over 50% larval mortality of *Spodoptera litura* (Lepidoptera). Exposure of larvae to botanical compounds occurs through contact and systemic actions (Rwomushana et al., 2019). In particular, exposure to essential oil affected the larvae by weakening their development (Javier et al., 2017) and icreasing mortality. Larval mortality increased to over 80%, at the highest concentration , showing that the oil has larvicidal properties. The work echoes an experiment by (Elshiekh et al., 2014) using the neem extract at different concentrations on *Tuta absoluta* larvae, which resulted in 86.7 to 100% larval mortality of *Spodoptera litura* (Lepidoptera).

The study found out that *Tuta absoluta* second instar larval stage is relatively a critical stage to target the leaf miners. Results from this study coorroborates with those of (Kasmara et al., 2018) and (Javier et al., 2017), who found that *Lantana camara* essential oil caused over 50% larval mortality of *Spodoptera litura* (Lepidoptera). The results also show that the oil has an outstanding repellency action on the second instar larvae of leaf miner, *Tuta absoluta*.

The secondary metabolites such as β-caryophyllene, Sabinene, bicylogermacrene, spathulenol, eucalyptol, (E)-nerolidol, thujene, caryophyllene oxide, β-copaene, davana ether, trans-chrysanth enol, α-humulene epoxide, linalool, limonene, terpineol, terpene-4-ol, α-pinene and camphor recorded in Chapter 3 in the essential oils from the difere AEZs could be responsible for their observed insecticidal and repellent activity. (Zandi-Sohani et al., 2012) also concluded that the same compounds show repellent and insecticidal activities against *C. maculatus*. A trial against *T. absoluta* larval stages using pure α-pinene moderated toxicity effects was reported (Chegini et al., 2018). Limonene, 3-carene, terpinolene, β-myrcene, and γ-terpinene have larvicidal activities in *Aedes aegypti* and *Aedes albopictus* larvae (Cheng et al., 2009). However, the role of a single compound in essential oil is not definite, but the minor constituents act as synergists, enhancing the effectiveness of the EO (Akhtar & Isman, 2013).

4.5 Conclusion

The essential oils of *Lantana camara* were effective in controlling *Tuta absoluta* and thus can be a potential biopesticide to control this pest. Further studies to assess efficacy under field conditions are needed. Furthermore, there is need to increase efficiency by developing methods that will allow for long-lasting effectiveness. Information on the potentially lethal and phytotoxic effects of oil on non-target organisms and target crops. Therefore, this serves as a source of hypotheses for further research on *Lantana camara* essential oil as a potential bioinsecticide.

CHAPTER FIVE

ASSESSMENT OF THE INFLUENCE OF POTASSIUM AND WATER APPLICATION RATES ON THE OIL YIELD AND SECONDARY METABOLITE PROFILES OF THE LANTANA CAMARA PLANT UNDER CONTROLLED ENVIRONMENT.

The essential oil produced by aromatic plants is affected by the nutritional content and watering frequency. *Lantana camara* plant secondary metabolites are affected differently based on the prevailing conditions during growth and development. The effects ofpotassium and irrigation frequency on essential oil content and composition of *Lantana camara* were studied, in a greenhouse experiment. A complete randomised design with a two-factorial arrangement and 5 replications was set up. Potassium was supplied at five rates as muriate of potash (MOP) (0.00g (F1), 0.25g (F2), 0.51g (F3), 0.76g (F4) and 0.81g (F5)) and three watering level (10% (W1), 40% (W2) and 80% (W3)) volumetric water content. The plants harvested and oil extracted and the secondary metabolites were determined using the GC-MS. The results showed that the highest essential oil content (0.76%) was obtained in the lowest moisture levels (10% (W1)) and K-supplement levels (0.00g (F1)). Whereas, the highest moisture levels (80% (W3)) and K-supplement levels (0.81g (F5)), produced the lowest essential oil content (0.34%). The study revealed that the essential oil contained 68 different compounds that were identified succesfully. The dorminant compounds found in this study are (E)-caryophyllene (7.72-14.03 %) and Geranial (4.74-13.40 %). Other compounds that showed dominance included 1, 8 Cineole (2.94-6.10 %), Bicyclogermacrene (0.89-5.81%), β-Phellandrene (0.94-5.13%), Lavandulyl isovalerate (1.08-5.04%), α- Calacorene (0.52-4.50%), α- Pinene (0.46-3.49%) and β-Bisabolenal (1.03-2.97%) exhibiting variable levels in the lantana leaf essential oil. The study also found that the production of secondary metabolites was significantly influenced by the interaction of water and K-supply treatments with varying effects on different metabolites. The study suggests that supplementing water and nutrients will help to increase the amount of monoterpene compounds and not sesquiterpenes.

5.1 Introduction

Meeting the growing demand for medicinal plants requires a continuous and uniform supply, but with the depletionof resources, it's important to increase the number of medicinal plant species in cultivation (Schippmann et al., 2006). However, only a few species are cultivated, and it's worth asking why. One explanation is that some cultivated plants are considered inferior in quality when compared to their wild counterparts (Disciglio et al., 2017).

Variation in plant secondary metabolites between the same species occurs in nature (Gorelick & Bernstein, 2017), affecting the quality and quantity of metabolites produced (Disciglio et al., 2017), thereby, hindering its usage. This is certainly the case with *Lantana camara*, where large quantitative and qualitative differences in the composition of bioactive secondary metabolites between plants of the same species (Passos et al., 2012; Pereira et al., 2019). This variation can be in part eliminated through the selection of uniform plant material by the use of vegetative propagation, as opposed to harvesting wild plants, to ensure consistent levels of secondary metabolites. To utilise lantana as a biopesticide in the modern agricultural industry, the composition and concentrations of compounds need standardisation and increased oil yield productivity.

Lantana is gaining value for its essential oil, though it is not commercially cultivated and only sourced from the wild. The composition of secondary metabolites from wild-collected and field-grown plants exhibits considerable variation depending on the growth environment (Liambila et al., 2021; Nea et al., 2020; Pereira et al., 2019). These facts must be considered while developing quality parameters standards for medicinal plants and their finished products . However, the critical factor that determines the quality is the relative proportions of the major components in the essential oil (Morsy, 2017). The oil yield, content and composition of secondary metabolites of lantana can be significantly influenced by agronomic practices. However, there is no information reported so far on the effects of agronomic factors on the synthesis and degradation of the secondary metabolites of lantana. While genetics determines the production potential, and environmental conditions induce variations in quantity, quality, and distribution of the active compounds in the plant

(Bailey-Serres et al., 2019), agronomic practices increase plant biomass, quality and quantity of the oil (Cui et al., 2022; Schiffner et al., 2020).

Previous evidence suggests that water and mineral nutrition supplementation, according to the requirements of a plant, is one of the essential factors of field techniques affecting plant development, function and metabolism (Garcia-Mier et al., 2019). Advances in understanding the influences of agronomic practices in regulating compounds may lead to their advantageous manipulation in plants. The stresses that alter the secondary metabolite accumulation include water deficit and the nutritional deficiency (Irankhah et al., 2020; Muscolo et al., 2019).

Water is crucial for plants' productivity and quality; it regulates plants' morphological growth and development and alters their biochemical properties. However, water requirements differ according to the plant's species and growth media type (Brendel, 2021; Mráz et al., 2014). Limited availability of water has been considered to reduce plant growth and development. However, a moderate water deficit is beneficial for accumulating biologically-active compounds in medicinal plants (Miao et al., 2020). According to (Mahdavi et al., 2020), terpenes tend to increase under stress in herbaceous plants and shrubs, particularly under moderate water deficit.

On the other hand, nutrition plays a pivotal role in the growth and development of all crop plants. Supplemental nutrients can effectively increase the oil yield and quality of medicinal plants that synthesise essential oils (A. Hassan, 2012) Many studies have focused on increasing medicinal plants' oil yield and secondary metabolite accumulation through different approaches, particularly fertilisation. Nitrogen (N), phosphorus (P), and potassium (K) are the most abundantly acquired mineral elements by plants, and their use is considered a critical agronomic practice associated with conventional agriculture playing vital roles in many aspects of plant metabolism (Bernstein et al., 2019).

Water and nutritional supplement influence all primary physiological process and affects secondary metabolism (Li et al., 2020). In the case of secondary metabolite production and oil yield content in *Lantana camara*, little to no work has been performed to the best of our knowledge documenting the effects of mineral nutrition

and water regimen on the lantana secondary metabolite profile. The production of the secondary metabolites in lantana may be influenced by the nutritional elements available in the soil as well as the water availability. Agronomic approaches, as well as genetic and environmental factors, influence the secondary metabolite composition and yield content of lantana essential oils (Cui et al., 2022). Furthermore, because secondary metabolite composition varies in wild-collected plants, controlling influence on the history of the plant material and post-harvest handling will assist in the standardisation of the quality of the essential oil (K. A. Khalid, 2019).

The study focused on the chemical responses of lantana to inorganic fertiliser and water regimens. The study aimed to establish the effects of the supplemented K nutrients under the optimal range of these nutrients supplied for medicinal shrubs. We aimed to determine if alteration of the K supply affects the biosynthesis of secondary metabolites accumulation without harming the plant by imposing deficiencies or toxicities. The study was undertaken to evaluate the following hypotheses: (1) nutritional supplementations of N, P and different concentrations of K and water regimens under conditions of optimal fertilisation elicit changes in the Secondary metabolite profile and oil yield of the lantana plant; (2) the elicited changes are compound dependent. To test these hypotheses, the effects of the N, P, and K nutritional supplementations on (1) Secondary metabolite profile composition and concentration and (2) essential oil yield content of lantana was studied.

5.2 Materials and methods

5.2.1 Experimental Location and Soil Characteristics

The experiment was conducted at Jomo Kenyatta University of Agriculture and Technology (JKUAT), Faculty of Agriculture Experimental Greenhouse Complex, Juja, Kenya, from 2018–2020. The crop was grown three times from March to July each year. Before crop establishment, forest soil was sourced, and soil analysis was performed to establish the nutritional levels and the physical properties. The soil parameters were analysed following the methodology described in Section 3.2.2. The experimental pots were filled with 5.0 kg of forest soil. Table 5.1 presents the

physicochemical properties of the experimental soil used to grow the Lantana plants. The soil samples were tested at the Horticulture Department Soil Testing Laboratory (JKUAT).

Table 5.1: Physico-chemical properties of the soil.

Property	Value
Soil texture	Silty loam
Sand (%)	22.9
Silt (%)	70.2
Clay (%)	7
pH (1:2)	6.9
Electrical Conductivity (1:2) m mhos	0.51
Organic carbon (%)	1.11
Available nitrogen (kg ha^{-1})	363.47
Available phosphorus (kg ha^{-1})	13.28
Available potassium (kg ha^{-1})	592.30

5.2.2 Plant Material and Growing Conditions

The *Lantana camara* plant cuttings used as the model for this study were obtained from LM-KI (Kiboko) AEZ (Figure 3.1B). The cuttings were obtained from mLM-KI (kiboko) following the high number of secondary metabolites seen from the results obtained in in chapter 3. The cuttings were propagated from a single mother plant in sandy soil using a rooting powder (Rootex 6 ®) containing 0.6 % Indole butyric acid for three weeks (plate 5.1). Rooted cuttings were planted in 5L (17kg) black plastic pots in a potting mixture of loamy soil and cultivated under a 13/11-h light/dark photoperiod in a greenhouse for acclimatisation for a week. Cultivation was conducted under sunlight. The greenhouse's maximum and minimum temperatures were 26 and 18 °C day/night. Minimum day and maximum night

relative humidity were 60 and 90%, respectively. The density of plant per pot was one to allow for proper growth and development.
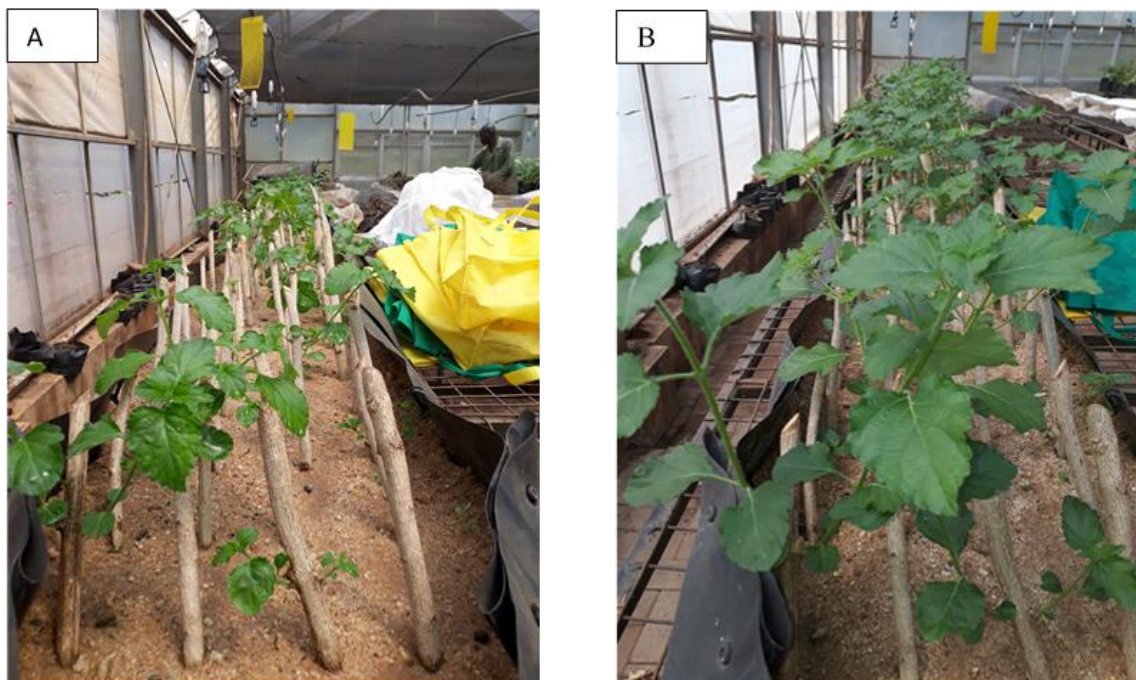
Plate 5.1: Lantana Camara plants grown in the sand under greenhouse conditions. Plant growth status at (A) 7 days and (B) 21 days of growth.

The experiment was set up with a completely randomised design (CRD) with a two-factor factorial arrangement and five replications. Fifteen treatment combinations consisting of three water levels (10 % (W1), 40% (W2) and 80% (W3) field capacity and five fertiliser treatments (a control (no fertiliser 0.0g) (F1), four different concentrations of K_2O_5 applied as muriate of potash (MOP), 0.25g (F2), 0.51g (F3), 0.76g (F4) and 0.81g (F5)) per plant were tested (Plate 5.2 A). The fertilisation was split into three phases (7, 30, and 60 days after transplanting), and each stage was about 33.3% of the total fertiliser nutrient. Every potassium treatment received urea (46% N; 0.72 g per plant) and Triple Super Phosphate, TSP (60% P; 0.51 g per plant) at 180 kg /ha rates. During the study, there was no indication of K deficiency in all the plants at 0.0g per plant. The water rates followed a recommendation for the prosuction of harbeceous plants depending on the objective under studyif maximizing growth is not the objective, substrate water content can be adjusted to

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manipulate plant growth, providing a non-chemical approach to growth regulation (Burnett et al., 2012).

A soil moisture measurement device (HydroSense II version CS659 (12 cm rod) (Campbell Scientific, Australia)) (plate 5.2B) was used to measure the soil moisture content daily in terms of the volumetric water content (in per cent). The Water Deficit Mode was used and the HydroSense measurements were taken at lower, middle and upper water contents that are 10% (W1), 40% (W2) and 80% (W3), respectively and stored in memory as reference values. The reference values were then applied to subsequent measurements.

The water deficit calculation was applied to a soil depth equal to the probe rod length of 12 cm. The sensor rod indicated the water deficit (the amount of water required to be added to each pot to maintain the set moisture content). Tap water was added to the required field capacity using a measuring cylinder. This approach ensured that the moisture levels set were maintained throughout the experiment. Other recommended agronomic practices, such as weeding, were adopted for better growth and development.
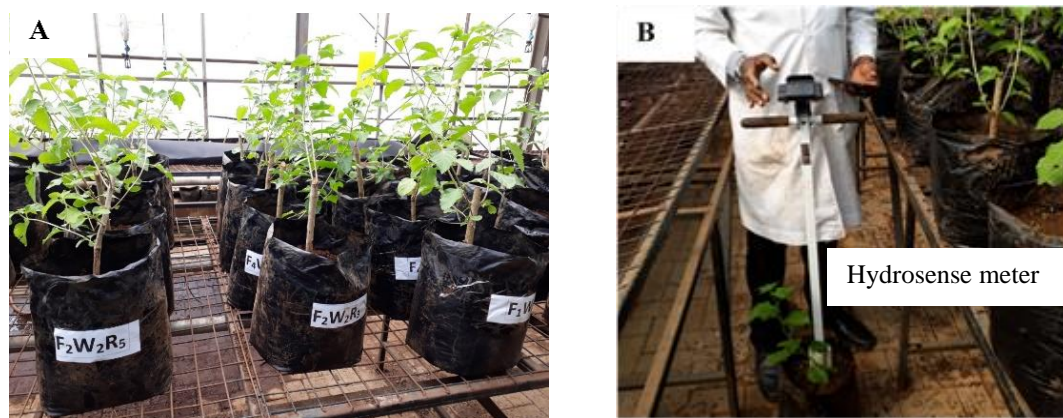
Plate 5.2: Lantana plant establishment (A) Experimental set-up design. (B) Soil moisture measuring device (HydroSense)

5.2.3 Sampling Plant Material, EO Extraction and GC-MS Analyses

Upon the first flowering of the plants (plate 5.3), harvesting of leaves was done separately from each treatment and processed as described in Section 3.2.1. The oil

extraction was done as described in Section 3.2.2, and the per cent oil yield was calculated using the following formlae (1) (Costa et al., 2014).

The essential oil yield was calculated using the following formula:

$$ROu = [M/Bm] \times 100 \tag{1}$$

Where: ROu = % oil Yield

          M   = Mass of extracted oil

          Bm = Initial plant Biomass

The Oil extraction and GC-MS analysis were done as described in Section 3.2.3 and 3.2.4 respectively.

Plate 5.3: Plants ready for harvesting

5.2.4 Statistical Analysis

The oil yield data obtained from *Lantana camara* for successive plantings were subjected to analysis of variance (ANOVA) to test the sole effect of water regime

and fertiliser application of K_2O_5 and water regime K_2O_5 and their interaction. In this experiment, the five replications were used in a one-way analysis of variance and multiple comparison methods to measure significant differences in the oil yield. Differences among the treatment means were assessed by the least significant difference (LSD) value at $P>0.05$.

The metabolite data for the lantana leaf essential oil plant samples were obtained with five replicates and are presented as means ± standard deviation. Raw GC-MS data were pre-treated using excel and then uploaded to MetaboAnalyst, and no filtering was applied. The metabolomics profile analysis was performed using MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/MetaboAnalyst/home.xhtml), a comprehensive web-based application for metabolic data analysis and interpretation combined with R-based MetaboAnalyst package (Chong et al., 2019). Successively, data were normalised with a 3 steps procedure: Sample normalisation to the sum of all the acquired values as a general-purpose adjustment for the differences among samples, Data transformation through a generalised logarithm transformation and Scaling procedure through Pareto scaling procedure.

Interactive Principal Components Analysis Visualization (iPCA), an unsupervised pattern recognition technique, was performed to detect overall patterns within the data to acquire a general insight and visualise any relation (trends, outliers) among the observations (samples). The GC-MS data were mean-centred, and the iPCA model was obtained at a confidence level of 95%. Pearson's correlation analysis and hierarchical clustering analysis (HCA), with Euclidean distance and Ward's linkage between the three moisture regimes and five K treatments, were performed using MetaboAnalyst 5.0 (www.metaboanalyst.ca) to reveal correlations among metabolites. The results were then plotted as a heat map showing the clusters. ANOVA-simultaneous component analysis (ASCA), a two-way ANOVA, was used to identify significant metabolite patterns associated with the different experimental factors (water regime conditions and K-treatments) and their interactions.

5.3 Results

5.3.1 Essential Oil Yield of *Lantana camara* Plant

Upon analyzing the results, it can be concluded that the yield of essential oil was influenced by the water and potassium (K) treatment, as well as their interaction (Table 5.2). The essential oil yield varied significantly ($p < 0.001$) across different samples, ranging from 0.34% to 0.76% (Table xx). The highest yield of essential oil (0.76%) was observed in plants from sample populations that were not treated with fertilizers (0.0g) and exposed to lower field moisture capacity (VMC 10%), in contrast to the optimum field moisture capacity (VMC 80%) coupled with high Ktreatment (0.81g), which resulted in a yield of only 0.34% of essential oil. It was observed that the water regimen of VMC 80% along with 0.81g K reduced the oil yield content by 55.26%; however, the reduction in oil yield content was only 46% when the K application rate was 0.00g. The smallest reduction in oil yield content in leaves, 1.3%, was observed with water regimen of VMC 10% together with 0.25g K application rate.

Table 5.2: Essential Oil yiel of L. camara plants in response to water regimen and potassium treatment.

	Treatments	Essential oil yield
Water_Regime	Potassium-Treatment	% (w/w)
10%	F1_0.0g	0.76+/-0.13 [de]
	F2_0.25g	0.75+/-0.15 [d]
	F3_0.51g	0.71+/-0.14 [d]
	F4_0.76g	0.69+/-0.08 [cd]
	F5_0.81g	0.64+/-0.04 [c]
40%	F1_0.0g	0.63+/-0.06 [c]
	F2_0.25g	0.63+/-0.08 [c]
	F3_0.51g	0.62+/-0.05 [c]
	F4_0.76g	0.61+/-0.04 [c]
	F5_0.81g	0.45+/-0.04 [b]
80%	F1_0.0g	0.41+/-0.04 [ab]
	F2_0.25g	0.40+/-0.04 [ab]
	F3_0.51g	0.39+/-0.05 [ab]
	F4_0.76g	0.38+/-0.05 [a]
	F5_0.81g	0.34+/-0.05 [a]
P-value	W	<0.001
	K	<0.001
	W × K	<0.001

The treatments were initiated 7, 30 and 60 days after transplanting and lasted 120 days. Essential oil yield values in this table are the mean of five determinations (two distillations for each sample) on a percentage dry wt basis.Means followed by the same letter in a column are not significantly different at P = 0.05 by Tukey's test. W, water regimen; K, potassium treatment.

5.3.2 Metabolite profile of the *Lantana camara E*ssential oil

A total of 76 compounds were quantified across all samples, of which sixty-eight (68) were putatively identified by comparison of mass spectra to NIST (2017) library

and retention index data (Table 5.3). Metabolites synthesised by the plant samples treated with low moisture content comprised most of the metabolites detected in the essential leaf oil at five different K- treatments (94.46%) compared to those treated with optimum moisture level and K- treatment (59.35%) (Table 5.3).

The dominant compounds from this study were (E)-caryophyllene (7.72-14.03 %) and Geranial (4.74-13.40 %). Other compounds that showed dominance included 1, 8 Cineole (2.94-6.10 %), Bicyclogermacrene (0.89-5.81%), β-Phellandrene (0.94-5.13%), Lavandulyl isovalerate (1.08-5.04%), α- Calacorene (0.52-4.50%), α- Pinene (0.46-3.49%) and β-Bisabolenal (1.03-2.97%) exhibiting variable levels in the lantana leaf essential oil. Among the dorminant compounds the sesquiterpenes were dominated by (E)-caryophyllene, Bicyclogermacrene, α- Calacorene, β-Bisabolenal and Lavandulyl isovalerate. On the other hand, the monoterpenes were dorminated by Geranial, β-Phellandrene, 1,8-cineole and α-Pinene.

The dominant metabolites, Caryophyllene and Geranial, their concentrations were reduced significantly in the leaf essential oil, with an increase in moisture content and K treatment. On the contrary, metabolite such as Decane was only synthesised in samples treated with high moisture levels and increased K-treatment. In comparison, β-Chemigrene was only synthesised in samples with low moisture levels and reduced with an increase in K-treatment. Therefore, the soil moisture conditions and K-nutrient played a significant role in metabolite production, with a significant difference between the metabolic profiles of leaf essential oil of lantana from the 15 plant samples grown with different treatments under the same environmental condition. These results suggest that the production of metabolites in the leaf of lantana is metabolite-specific.

Table 5.3: Effect of irrigation and K-treatment rates on chemical profile of the essential oils from *L. camara* plants

Name of compound[a]	Essential oil (%)[b]														
	1_W_1_F_1	2_W_1_F_2	3_W_1_F_3	4_W_1_F_4	5_W_1_F_5	6_W_2_F_1	7_W_2_F_2	8_W_2_F_3	9_W_2_F_4	10_W_2_F_5	11_W_3_F_1	12_W_3_F_2	13_W_3_F_3	14_W_3_F_4	15_W_3_F_5
(2E)-Hexenal	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.01	0.01	0.02	0.02	0.02
(E)-alpha-Damascone	0.04	0.04	0.03	0.03	0.02	0.04	0.03	0.02	0.02	0.02	0.04	0.02	0.02	0.02	0.02
(E)-beta-Ocimene	0.57	0.51	0.52	0.38	0.28	0.46	0.47	0.54	0.33	0.33	0.36	0.47	0.58	0.26	0.33
(E)-Isovalencenol	0.01	0.01	0.02	0.02	0.02	0.03	0.03	0.03	0.02	0.02	0.07	0.07	0.07	0.03	0.03
(Z)-alpha-Bisabolene	0.94	1.00	1.05	1.11	1.17	1.96	2.26	2.31	1.75	1.70	2.06	2.14	2.22	2.16	2.14
(Z)-beta-Ocimene	0.05	0.06	0.06	0.06	0.07	0.08	0.08	0.09	0.09	0.10	0.15	0.14	0.14	0.13	0.12
1,8-Cineole	6.10	5.34	5.27	4.99	4.71	4.25	3.96	3.82	3.82	3.95	3.96	3.59	3.33	3.10	2.94
14-hydroxy-(Z)-Caryophyllene	0.35	0.46	0.63	0.51	0.50	0.08	0.08	0.09	0.10	0.11	0.12	0.13	0.13	0.14	0.15
3-Octanol	0.28	0.28	0.21	0.18	0.19	0.28	0.27	0.25	0.24	0.16	0.32	0.32	0.36	0.36	0.29
Allo-Aromadendrene	1.31	1.77	2.16	2.17	1.91	1.64	1.95	2.11	2.11	1.91	1.92	1.94	2.09	2.12	2.12
Allo-Aromadendrene epoxide	0.48	0.69	0.66	0.58	0.56	0.09	0.25	0.29	0.35	0.39	0.11	0.43	0.50	0.60	0.68
Alloaromadendrene oxide-(I)	0.07	0.05	0.04	0.04	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02

Name of compound[a]	Essential oil (%)[b]														
	1_W₁_F₁	2_W₁_F₂	3_W₁_F₃	4_W₁_F₄	5_W₁_F₅	6_W₂_F₁	7_W₂_F₂	8_W₂_F₃	9_W₂_F₄	10_W₂_F₅	11_W₃_F₁	12_W₃_F₂	13_W₃_F₃	14_W₃_F₄	15_W₃_F₅
Allo-Ocimene	0.08	0.08	0.07	0.05	0.06	0.04	0.03	0.03	0.02	0.01	0.01	0.01	0.01	0.01	0.01
Alpha-Bulnesene	0.12	0.12	0.13	0.14	0.15	0.17	0.18	0.19	0.20	0.21	0.27	0.28	0.30	0.32	0.33
Alpha-Calacorene	**4.50**	**4.32**	**2.78**	**1.99**	**1.14**	**0.52**	**0.56**	**0.61**	**0.64**	**0.69**	**0.75**	**0.80**	**0.85**	**0.91**	**0.95**
Alpha-Cubebene	0.13	0.18	0.24	0.28	0.34	0.24	0.27	0.29	0.32	0.34	0.30	0.32	0.35	0.37	0.40
Alpha-Farnesene	0.48	0.50	0.51	0.53	0.54	1.03	1.09	1.20	1.21	0.81	1.32	1.35	1.34	1.28	1.18
Alpha-Gurjunene	1.02	1.08	1.15	1.22	1.30	1.57	1.64	1.71	1.79	1.88	2.38	2.34	2.32	2.25	2.78
Alpha-Humulene	0.60	2.19	2.54	3.24	3.25	2.31	2.53	2.57	2.24	2.10	2.21	2.24	2.28	2.30	1.99
Alpha-Muurolene	0.62	2.21	2.22	2.21	2.21	2.16	2.18	2.17	2.17	2.16	2.13	2.13	2.12	2.12	2.12
Alpha-Neocallitropsene	0.47	0.49	0.52	0.54	0.57	0.83	1.00	1.06	1.15	1.24	1.06	1.14	1.23	1.33	1.42
Alpha-Phellandrene	0.64	0.47	0.40	0.26	0.21	0.15	0.14	0.14	0.12	0.09	0.07	0.06	0.06	0.06	0.07
Alpha-Pinene	**3.49**	**3.48**	**3.38**	**3.23**	**3.13**	**0.98**	**0.90**	**0.83**	**0.75**	**0.66**	**0.76**	**0.67**	**0.58**	**0.46**	**0.48**
Alpha-Terpinene	0.85	0.62	0.50	0.33	0.26	0.87	0.52	0.49	0.39	0.28	0.88	0.52	0.49	0.48	0.33
Alpha-Terpineol	1.93	1.93	1.79	1.73	1.71	1.44	1.18	1.02	0.86	0.68	0.76	0.65	0.54	0.44	0.30
Alpha-Thujene	0.83	0.69	0.50	0.34	0.31	0.74	0.50	0.41	0.28	0.24	0.53	0.26	0.23	0.18	0.16
Amorpha-4,7(11)-diene	0.51	0.45	0.39	0.29	0.17	0.48	0.38	0.34	0.26	0.19	0.49	0.40	0.32	0.25	0.22
Aromadendrene oxide-(2)	0.09	0.04	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

							Essential oil (%)[b]							
Name of compound[a]	1_W_1_F_1	2_W_1_F_2	3_W_1_F_3	4_W_1_F_4	5_W_1_F_5	6_W_2_F_1	7_W_2_F_2	8_W_2_F_3	9_W_2_F_4	10_W_2_F_5	11_W_3_F_1	12_W_3_F_2	13_W_3_F_3	14_W_3_F_4	15_W_3_F_5
Beta-Alaskene	0.12	0.13	0.14	0.14	0.15	0.18	0.19	0.20	0.21	0.22	0.24	0.26	0.34	0.33	0.32
Beta-Bisabolenal	2.97	2.89	2.74	1.65	1.34	2.83	2.63	2.39	1.58	1.03	2.79	2.59	2.19	1.97	1.29
Beta-Bourbonene	0.05	0.06	0.06	0.06	0.07	0.14	0.11	0.08	0.09	0.09	0.34	0.28	0.11	0.11	0.11
Beta-Calacorene	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.03	0.03	0.07	0.03	0.03	0.04	0.06	0.17
Beta-Chamigrene	1.60	1.08	0.76	0.67	0.23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Beta-copaen-4-alpha	0.90	0.90	0.81	0.66	0.57	0.91	0.78	0.61	0.43	0.29	0.87	0.63	0.39	0.24	0.26
Beta-Copaene	0.57	0.28	0.25	0.22	0.20	0.24	0.11	0.09	0.10	0.10	0.11	0.11	0.11	0.12	0.13
Beta-Cyclocitral	0.30	0.41	0.43	0.45	0.40	0.33	0.35	0.29	0.28	0.19	0.35	0.45	0.32	0.28	0.19
Beta-Elemene	1.70	1.60	1.43	1.40	1.35	0.66	0.50	0.32	0.34	0.36	0.40	0.42	0.41	0.43	0.45
Beta-Phellandrene	5.13	4.79	4.49	4.30	3.94	1.88	1.56	1.24	1.12	1.02	0.94	0.98	1.02	1.07	1.13
Beta-Pinene	0.13	0.14	0.15	0.15	0.16	0.17	0.18	0.20	0.21	0.23	0.21	0.22	0.27	0.36	0.48
Beta-Vetispirene	1.58	2.26	2.23	2.36	2.71	2.11	1.96	1.78	1.61	1.26	1.06	0.85	0.60	0.40	0.25
Bicyclogermacrene	5.81	2.78	2.63	2.27	1.31	0.89	0.95	1.01	1.08	1.09	1.13	1.18	1.25	1.31	1.37
Borneol	0.74	0.51	0.41	0.39	0.50	0.33	0.30	0.26	0.24	0.05	0.18	0.23	0.23	0.19	0.07
Camphene	1.70	1.54	1.51	0.90	1.10	0.50	0.46	0.40	0.41	0.44	0.45	0.48	0.49	0.51	0.54
Camphor	1.24	1.99	1.95	1.91	1.85	0.55	0.53	0.51	0.50	0.41	0.29	0.31	0.33	0.35	0.36
(E)-Caryophyllene	14.03	13.96	13.49	13.39	12.49	12.73	11.61	10.23	8.81	8.75	10.62	9.56	9.00	8.16	7.72
Cis-beta-Guaiene	0.21	0.15	0.14	0.14	0.16	0.06	0.06	0.05	0.05	0.05	0.05	0.05	0.06	0.06	0.06

Name of compound[a]	Essential oil (%)[b]														
	1_W_1_F_1	2_W_1_F_2	3_W_1_F_3	4_W_1_F_4	5_W_1_F_5	6_W_2_F_1	7_W_2_F_2	8_W_2_F_3	9_W_2_F_4	10_W_2_F_5	11_W_3_F_1	12_W_3_F_2	13_W_3_F_3	14_W_3_F_4	15_W_3_F_5
Cumin aldehyde	0.14	0.11	0.11	0.10	0.09	0.05	0.04	0.03	0.03	0.02	0.03	0.03	0.02	0.02	0.02
Davana ether	0.40	0.39	0.30	0.28	0.26	0.09	0.10	0.10	0.11	0.12	0.12	0.13	0.14	0.14	0.15
Davanone B	0.12	0.23	0.22	0.17	0.15	0.09	0.05	0.05	0.06	0.06	0.07	0.07	0.07	0.08	0.08
Decane	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.35	0.36	0.38	0.39
Delta-2-Carene	0.87	0.85	0.73	0.60	0.32	0.20	0.21	0.22	0.23	0.25	0.26	0.28	0.29	0.31	0.32
Delta-3-Carene	0.13	0.13	0.14	0.15	0.16	0.18	0.19	0.19	0.20	0.21	0.23	0.24	0.25	0.26	0.26
E-Nerolidol	1.83	1.53	1.36	1.21	1.07	0.60	0.45	0.31	0.32	0.34	0.37	0.38	0.40	0.42	0.44
Eugenol	0.14	0.15	0.16	0.17	0.18	0.20	0.21	0.22	0.23	0.25	0.24	0.25	0.26	0.27	0.32
Gamma-Terpineol	1.47	1.55	1.64	1.72	1.80	2.04	2.15	2.25	2.33	2.41	2.62	2.74	2.85	2.94	3.02
Geranial	13.40	11.28	10.92	9.43	8.55	13.25	10.56	9.75	8.62	6.39	8.71	7.65	7.05	6.10	4.74
Germacrene B	0.26	0.50	0.48	0.43	0.38	0.57	0.47	0.39	0.25	0.11	0.39	0.41	0.41	0.39	0.23
Germacrene D	0.76	0.98	1.22	1.20	0.95	0.96	0.95	0.99	0.88	0.82	0.88	0.88	0.81	0.80	0.75
Lavandulyl isovalerate	3.84	3.79	4.34	4.98	5.04	1.26	1.08	1.45	1.49	1.45	1.28	1.32	1.40	1.47	1.55
Linalool	0.94	1.01	1.13	1.19	1.23	1.16	1.32	1.60	2.16	2.38	1.39	1.52	1.73	2.77	3.19
Myrcene	1.05	1.09	1.13	1.18	1.23	1.39	1.44	1.50	1.56	1.67	1.96	1.94	1.91	2.08	2.10
Phytol	0.51	0.53	0.59	0.62	0.65	0.67	0.70	0.75	0.81	0.92	0.84	0.86	0.89	1.31	1.53
Spathulenol	1.06	1.10	1.15	1.20	1.23	1.44	1.50	1.58	1.84	1.86	1.71	1.77	1.85	2.17	2.14
Terpinen-4-ol	0.18	0.19	0.20	0.21	0.22	0.23	0.25	0.26	0.29	0.31	0.28	0.32	0.33	0.59	0.60

Name of compound[a]	1_W_1_F_1	2_W_1_F_2	3_W_1_F_3	4_W_1_F_4	5_W_1_F_5	6_W_2_F_1	7_W_2_F_2	8_W_2_F_3	9_W_2_F_4	10_W_2_F_5	11_W_3_F_1	12_W_3_F_2	13_W_3_F_3	14_W_3_F_4	15_W_3_F_5
							Essential oil (%)[b]								
Tetradecane	0.45	0.48	0.50	0.53	0.55	0.61	0.69	0.77	0.85	0.93	0.76	0.84	0.92	1.00	1.08
Trans-Cadina-1(6),4-diene	0.81	0.86	0.90	0.95	0.99	1.13	1.18	1.22	1.26	1.71	1.39	1.43	1.47	1.52	2.36
Verbenone	0.04	0.04	0.05	0.05	0.05	0.06	0.06	0.06	0.07	0.07	0.07	0.08	0.08	0.09	0.09
Z-Nerolidol	0.71	0.75	0.79	0.83	0.86	1.04	1.06	1.08	1.09	1.11	2.73	2.13	1.53	1.36	1.36
Total identified	**94.46**	**92.11**	**89.44**	**84.73**	**79.36**	**74.20**	**69.49**	**67.12**	**63.03**	**59.35**	**69.77**	**66.72**	**64.69**	**64.16**	**63.24**

[a] Compounds identification based on data obtained from the NIST 2017 library of the gas chromatography-mass spectrometry system. [b] Relative percentage '0' not-detected. Sample names; WI- 10%, W2-40%, and W3- 80% VMC; F1-0.0g, F2-0.25g. F3-0.51g, F4-0.76g and F5-0.81g of potassium (K) fertiliser. Essential oil yield values in this table are the mean of five determinations (two distillations for each sample) on a percentage dry wt basis.

The results showed that the SM profiles of lantana plant could be explained by the first two PCs: PC1 and PC2. The results showed a clear variation through threeThe cumulative proportions of PC1, PC2, and PC3 were 75.6%, 10%, and 5.6%, respectively, accounting for 91.2% of the total variance of lantana leaf essential oil (Figure). The plot patterns of the essential oil samples on K-treatment rates; 0.0g, 0.25g, 0.51g, 0.76g and 0.81g revealed a clear association between PC1 and SM synthesis. In particular, the samples VMC 10, VMC 40 and VMC 80 were were segregated from each other by PC1, with the intermediate VMC 40 samples in a transient state. The results showed a clear variation through three principal components (PC1, PC2 and PC3). An effect of the treatment factors is visible in the separation of the colours. In essence, iPCA showed excellent clustering of metabolomic changes for different water regime conditions and K- treatments, leading to distinct metabolite profiles and providing a high-level summary of the main patterns of data variance.
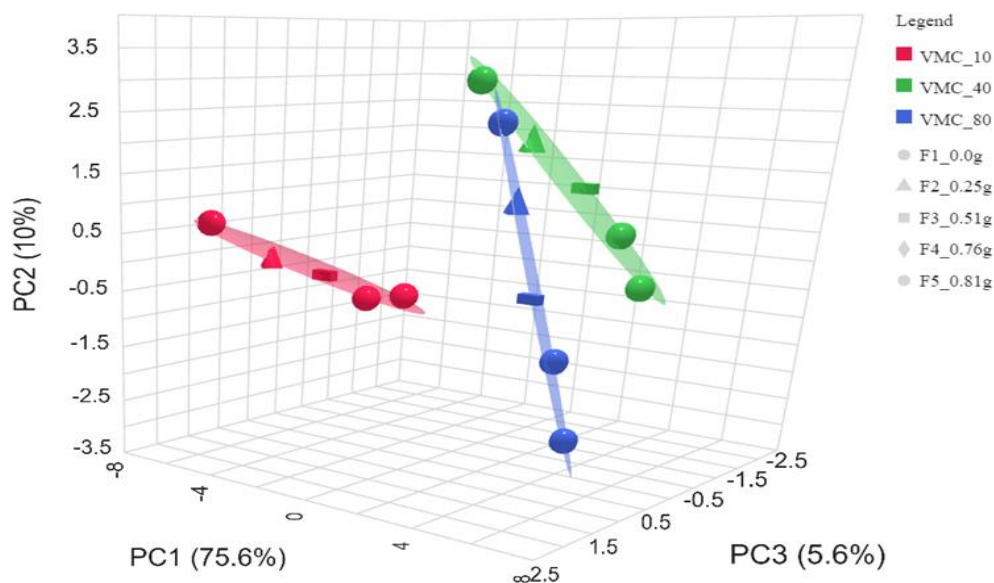

Figure 5.1: Interactive Principal Component Analysis (iPCA) 3D scatter plots showing the differences in SM profile between water regimen and potassium (K)- treament factors.

The primary experimental factor water regime is indicated in different colours (Red-VMC 10%, Blue-VMC 40% and Green-VMC 80%) and the secondary factor, K-treatment indicated in the different symbols, circle, square, triangle, rhombus and octagon-indicate FI_0.0g, F2_0.25g, F3_0.51g, F4_0.76g and F5_0.81g of K, repetively.

Clustered heatmapping separated the secondary metabolites into 3 general categories, 1) those elevated in water stressed and reduced K-tretment condition, 2) those elevated in highly watered and higher rates of K-treatment condition , and 3) those that did not change muchin a medium watered and K- treatment condition (Figure 5.2). Hierarchical clustering analysis (HCA) confirmed three distinct clusters that the metabolites synthesised from the 15 samples (vertical dendrogram), as shown in Figure 5.2, suggesting that the 15 samples of the essential leaf oil of *Lantana camara* might share certain metabolic features, although grown under different conditions. The three clusters represented by the metabolites express higher, medium and lower levels of the metabolites that compose the differential metabolic profile.

The metabolite pattern of each sample from three sample groups clustered, and individual clusters, were distinct, indicating the uniqueness of the chemical profiles of the 15 samples of lantana plant essential oil. Unlike score plots, heat-maps display the actual data values using carefully chosen colour gradients, as shown in Figure 5., where blue bars indicate a low concentration and red bar denotes a high concentration. Most metabolites change in intensity across all samples, indicating a change in the total concentration with different sample treatments. Based on the heat map β-Phellandrene, α-Pinnene and Cumin aldehyde show a decreasing concentration, and Phytol, β-Pinene and α-Neocallitropsene show an increasing concentration trend with increasing moisture levels (VMC 10% to VMC 80%), respectively
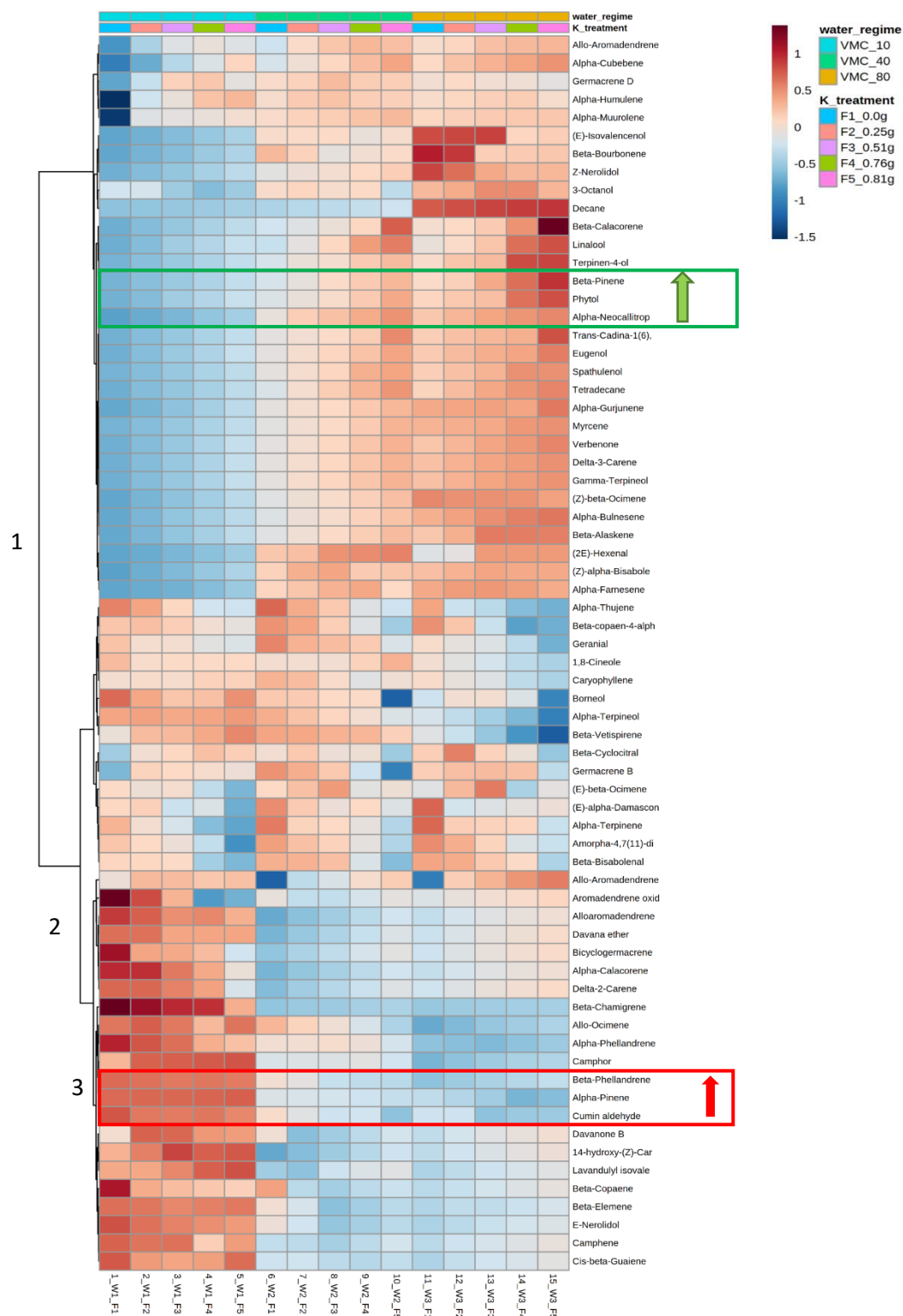
Figure 5.2: Hierarchical clustering analysis (HCA) of the fifteen treatment samples of essential oil of L. camara, showing discrimination between the sample types and differential abundances of 68 secondary metabilites. Each row represents the SM and each column represents the individual sample group. The scale bar represents the

normalised intensity of metabolites, where blue colour indicates low/decrease, and red colour indicates high/increase. Note: Heat-map was purposefully generated on all the 68 SM to show the overall pattern between the sample groups.

The metabolites in cluster 3 generally have a higher abundance in low moisture conditions (VMC 10) compared to high moisture conditions (VMC 80), which is opposite to the trend in cluster 1. However, in cluster 2, the metabolite concentration appears to vary with no specific pattern across different moisture regimens and K-treatment, but they do vary significantly with increasing/decreasing K-treatment. The metabolite intensities change between plant samples, and the change is more drastic in some cases than others (Figure 5.3).

Caryophyllene and Geranial, the dominant metabolites, had significantly reduced concentrations in the leaf essential oil with increased moisture content and K-treatment (Figure 5.4). In contrast, Decane was only synthesized in samples treated with high moisture levels and increased K-treatment, while β-Chemigrene was only synthesized in samples with low moisture levels and reduced with an increase in K-treatment.
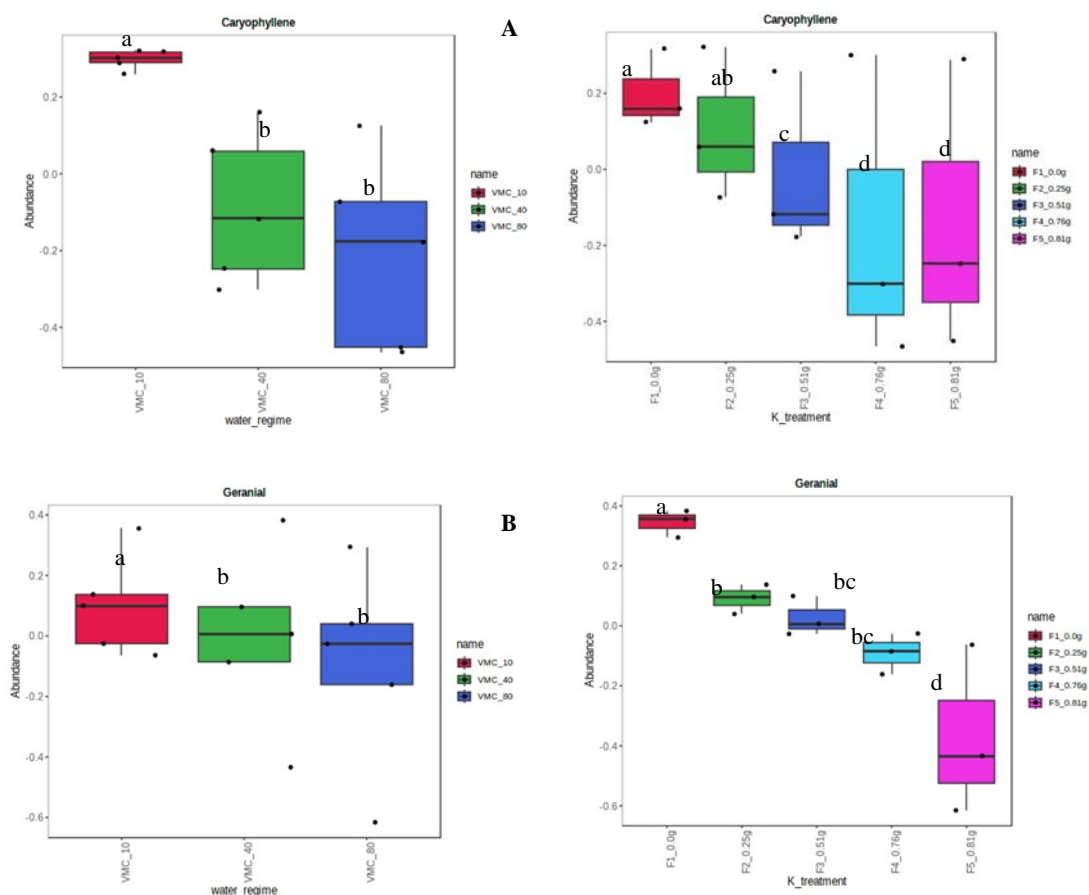
Figure 5.3: Boxplots showing the variation of the amount of (A) caryophyllene and (B) Geranial produced by *L. camara* with the different water regimen and K-treatment rates. Boxplot whiskers indicate ± 1.5 interquartile range limits. Boxplots with different letters show significant differences (P < 0.05).

We also performed a correlation analysis to understand the influence of these variables on metabolite intensities and generated a correlation matrix comparing metabolite intensities with water regimen and K-treatment. The compounds (Z)-β-Ocimene (r = 0.97) and Allo-Aromadendrene (0.56) were ranked as the most positively correlated, while Camphor (r = 0.96) and Amorpha-4,7(II)-diene (0.89) were ranked as the most negatively correlated with water regime and K-treatment, respectively (Figure 5.4Figure).
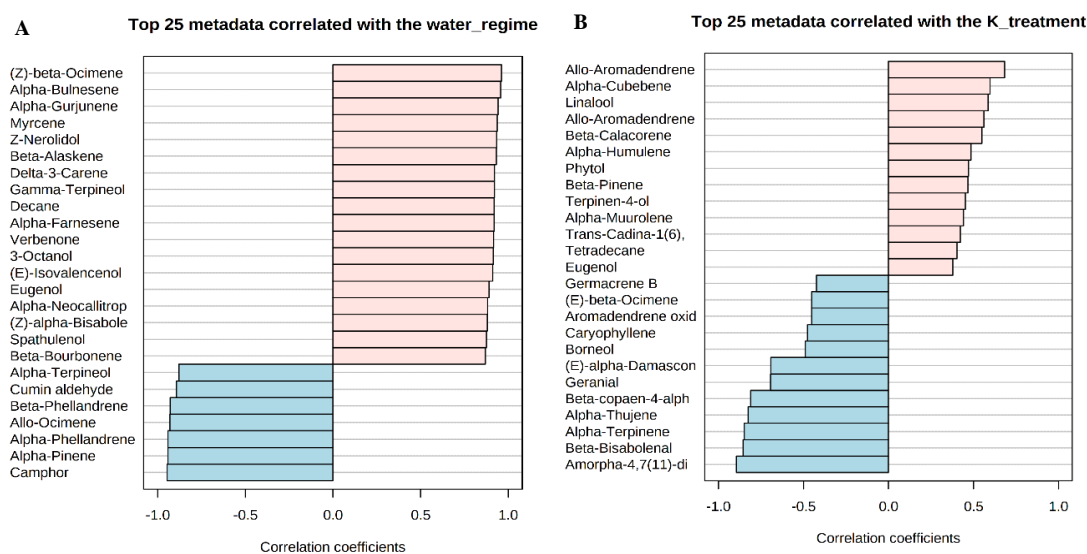
Figure 5.4: The top 25 metabolites that are significantly associated with (A) Water regime and (B) K-treatment using Pearson r rank correlation as a distance measure.

Both positively correlated (in light pink) and negatively correlated (in light blue) compounds are displayed in a vertical bar graph.

The ANOVA-Simultaneous Component Analysis (ASCA) was applied as a two-way ANOVA multivariate analysis to detect the significant temporal trends associated with the water regimen and K-treatment experimental factor and their interaction effects Figure 5.5). In the case of an interaction effect, the pattern water regime versus K-treatment was applied to show the main pattern change for the different water regime conditions with increasing K-treatment. The significant patterns associated with the water regime (Figure 5.5A), K- treatment (figure 5.5B) and their interaction (figure 5.5C) are shown in the scatter plots based on PC1 of the corresponding sub-model. The compound 1 interaction effect clearly shows the opposite trends with increasing K-treatments (Figure 5.5C), consistent with the heatmap visualisation (Figure 5.5). It is evident that, although the two factors had a dramatic impact on the leaf essential oil of *Lantana camara* metabolome, these changes were independent, as indicated by no interaction ($p > 0.925$). However, the water regimen was significant ($p < 0.01$).
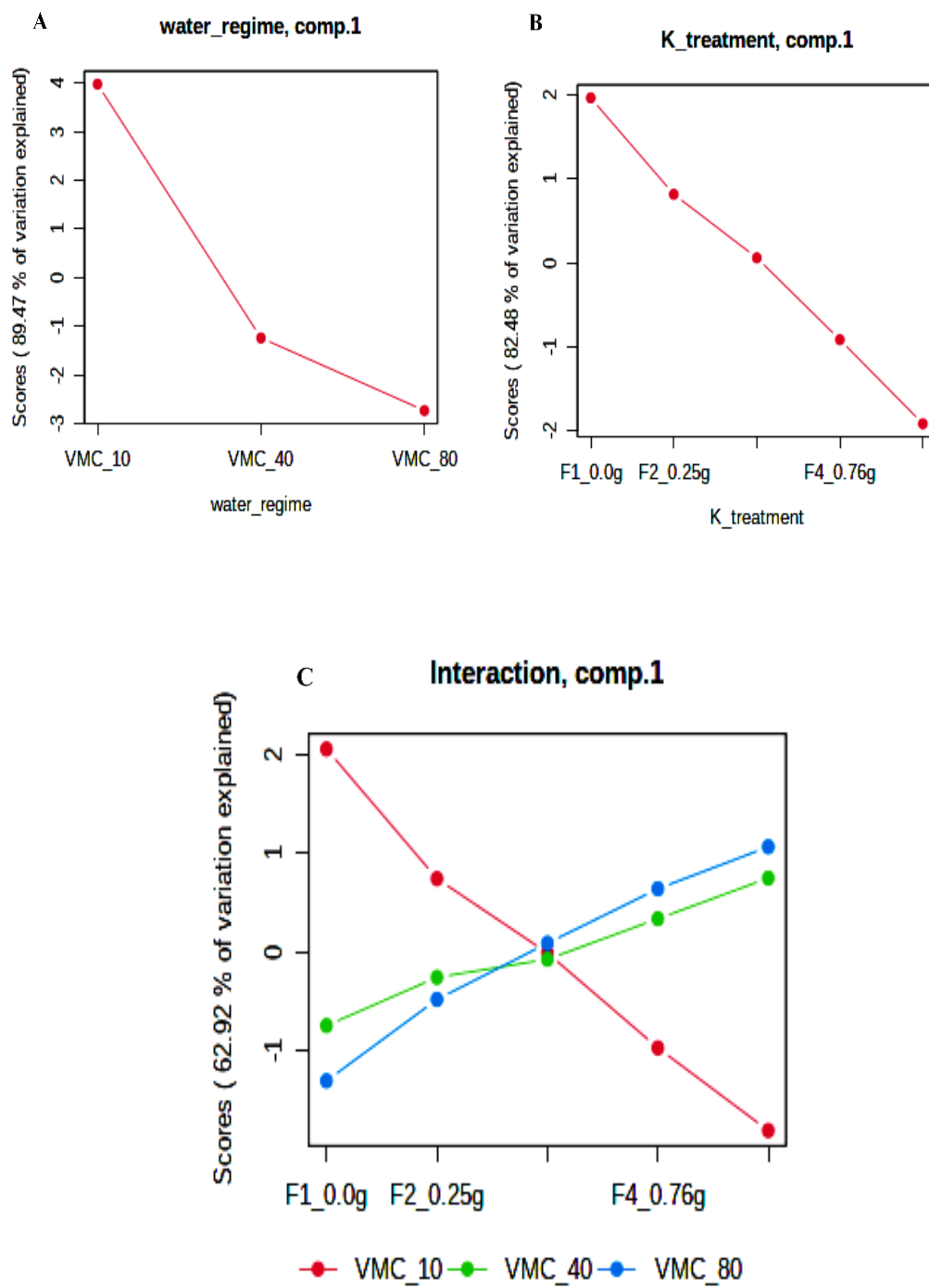
Figure 5.5: ANOVA-simultaneous component analysis (ASCA) of *Lantana camara* leaf essential oil synthesised during growth. Major pattern associated with (A) water regimen, (B) K-treatment and (C) their interaction.

Leverage/squared prediction error (SPE) plots show that the water regime (Figure 5.6Ai) the high-lighted bar is close to the right side of the distribution, meaning a statistically significant separation between the water regime groups (p=<0.05). while the K-treatment (Figure 5.6Aii), the high-lighted bar is close to the left side of the distribution showing a statistically non-significant separation between the K-treatment groups (p=0.415). The interaction between the water regime and K-treatment (Figure 5.6Aiii), with the high-lighted bar, the far left side of the distribution shows a statistically non-significant separation between the Water regime and K- treatment groups (p=0.925).

Sixty-six metabolites were well-modelled, except Decane and Beta Vetispirene in the Water regime treatment (Figure 5.6 Bi). While five metabolites were not well modelled in the K- treatment, including (E)-beta-Ocimene, Allo-Aromadendrene epoxide, Germacrene B, Beta-Cyclocitral and Borneol (Figure 5.6Bii). Three metabolites, Borneol, Germacrene B and Davanone B, were not well-modelled based on the interaction effect (Figure 5.6Biii).
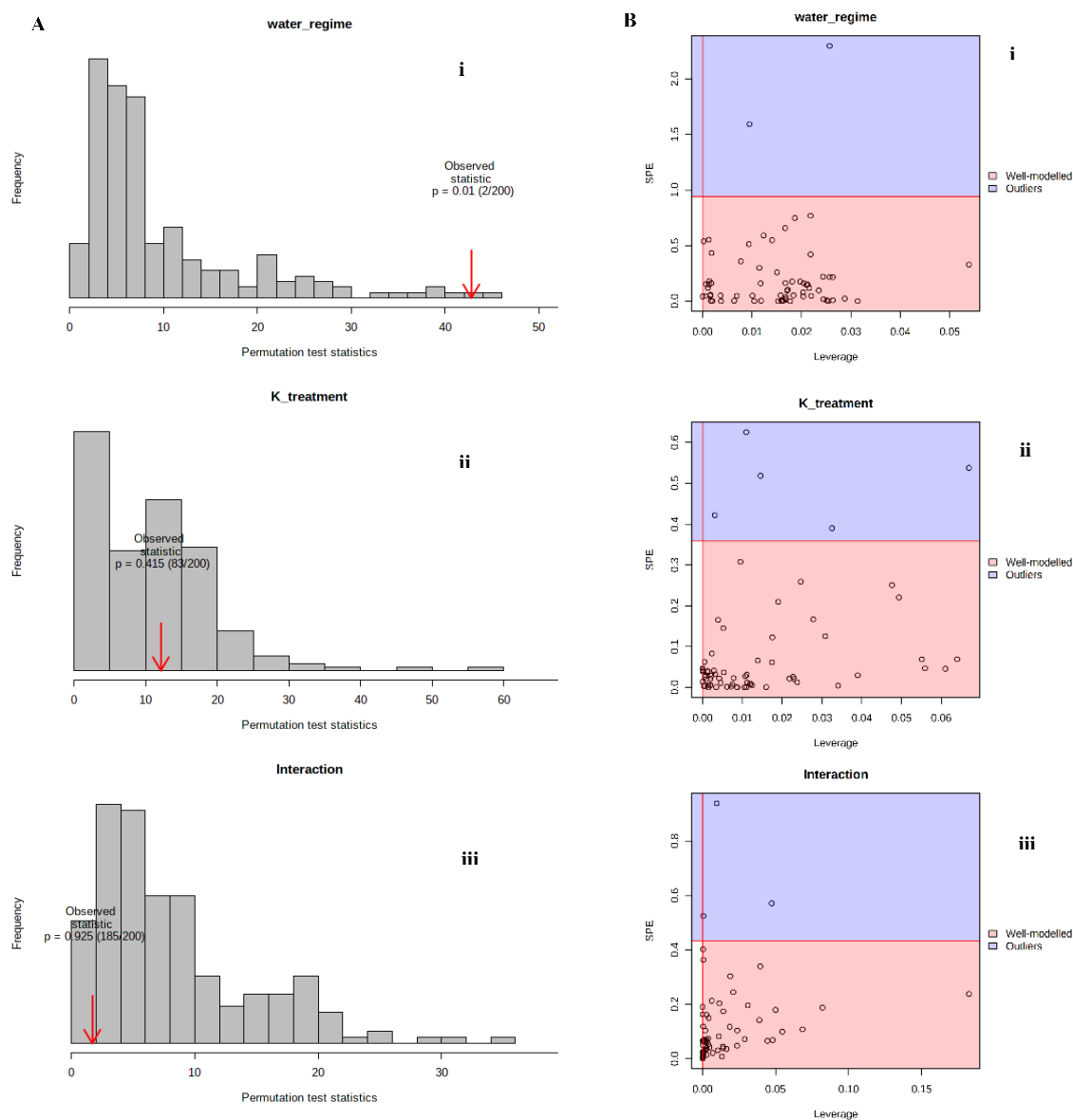
Figure 5.6: Leverage/SPE scatter plots of the ASCA variables submodels for water regimen, K- nutrition and their interactions.: Metabolites in red have high loadings that follow the expression patterns of the submodels. Metabolites in blue have expression patterns that are different from the major patterns. (A) Histogram showing the distribution of the permuted samples. (B) ASCA selection of important variables (metabolites) associated with water regimen, K-treatment and their interaction by leverage/SPE analysis.

5.4 Discussion

The present study aimed to evaluate the oil yield and the sensitivity of the metabolic profile of *Lantana camara* to moderate changes in water and K supplementation under a controlled environment. The results showed that the response of lantana leaf essential oil to water and K supplementation is metabolite-dependent.

In this study, the composition and contents of essential oils were compared among fifteen samples subjected to different treatments. The essential oil yields of the samples ranged from 0.34- 0.76% (w/w). The oil yield was exceptionally high for samples exposed to a lower moisture content level, with decreasing K-supplementation ranging from 0.64-0.76% (w/w). In contrast, samples treated with optimum moisture content (80% VMC), with decreasing K- supplementation gave a lower yield ranging from 0.34-0.41% (w/w). Our results are similar to the oil yield percentage reported by (Pereira et al., 2019) from plants collected in the wild under different environmental conditions in east Brazil, ranging between 0.1-0.5% (w/w). For instance, in our previous study, the oil yield content of *Lantana camara* collected from different environmental conditions gave an average yield range of 0.25% and 0.37 % w/w: highlands and coastal lowlands, (Liambila et al., 2021). The two regions are characterised by different conditions, presenting the plants with different developmental environments. Moreover, (Al-mansour & Adra, 2021) agree that essential oil's total oil yield content in most plants is minimal and rarely exceeds 1% w/w. The study results show that oil yield could be increased by carefully manipulating the fertiliser and water management regimens.

Low moisture treatment to the plant samples led to a higher concentration of the total metabolites. Our results corroborate with (Ibrahim et al., 2012) results in *Labisia pumila*, where they found out that the total phenolics and flavonoids per plant increased in drought-stressed plants. The information suggests that the rate of SM was enhanced in drought-stressed plants compared to the well-watered ones. This is attributed to the fact that potassium provides assistance in plants against abiotic stress conditions in the environment. The metabolites that prefered stress conditions for their synthesis were seen to reduce with an increase in K-supplement agreeing

with (Hasanuzzaman et al., 2018) that K- nutrition in plants alleviates drought stress conditions, thereby reducing the favourable condition for the synthesis of the specific metabolites. Hence, the rate of biosynthesis must be affected. Similarly, some metabolites increased with increasing moisture levels. These results corroborate with (Chen et al., 2011). They found out that the overall content of furoquinones in *Salvia miltiorrhiza* was increased slightly under well-watered plants compared to water-deficient plants. Although the change in concentration of the metabolite compounds did not unequivocally result from the enhancement of the plant metabolite biosynthesis, but could also be due to a drought-related condition in growth while biosynthesis remains constant.

This study also showed variation in the metabolic profile due to the influence of K supplementation. Some metabolites increased with increasing K. The study showed that the changes in metabolic occurred quantitatively, for example, the compounds (Z)-β-Ocimene (r =0.97) and Allo-Aromadendrene (0.56) increased with an increase in water and K-treatment, respectively compared to Camphor (r =0.96) and Amorpha-4,7(II)-diene (0.89) which increased with a decrease in the water regime and K- treatment, respectively. Similarly, (Troufflard et al., 2010) and (Lubbe et al., 2010) also found that the production of oxylipins and galanthamine in *Arabidopsis thaliana* and Narcissus bulbs were reduced in K-deficient plants, respectively. These results all suggest the importance of K in regulating the production of secondary metabolites in plants. The identified impact of the metabolic profile under these conditions points to the potential of slight variation in nutritional status for the regulation of secondary metabolism in *Lantana camara*.

Studies involving the variation of the entire metabolome of individual lantana plant species grown under greenhouse conditions and the effects on metabolic fingerprints are lacking. Therefore, GC-MS combined with suitable chemometric methods, such as the iPCA, CA and ASCA analysis, were used to identify the differences between the characteristic metabolites of the plant samples investigated. Additionally, HCA was performed to confirm sample differences or similarities; therefore, three distinct clusters were confirmed that are defined by the metabolite distribution. The complementary value of the cluster heatmaps allowed us to identify the three clusters

of metabolites with similar metabolic patterns and groups of discriminating metabolites that drive sample clustering. The expected class separation of the metabolites was observed with a high clustering coefficient.

Altogether, the production of EO in plants differs in quantity and composition, influenced by many prevailing factors (Mahdavi et al., 2020; Sun et al., 2022). In this sense, the agricultural management of medicinal plants can also influence the chemical profile of EO. However, knowledge about specific practices is crucial to achieving the desired compound synthesis or EO production in the lantana plant with an adequate and desired secondary metabolite composition for use as a biopesticide.

5.5 Conclusion

The present study has shown that the content of secondary metabolites in *Lantana camara* can be influenced by the water regimen and nutrient supplementation. However, the relationship between the metabolites, water levels, and K-nutrition is not yet clear. This apparent connection is likely a complex interplay of various factors such as water and nutrient availability, plant biosynthetic conditions, and physiological signals. The role of nutrition in lantana has not been fully characterized, and the effect of nutritional supplementation on growth and secondary metabolite concentration is not yet well understood. Further research is necessary to identify the optimal methods for each desired metabolite profile and to characterize the plants' response to a broader and more detailed range of individual nutrient applications.

The interaction of water and nutritional supply significantly affected the variability of secondary metabolite biosynthesis, highlighting the importance of developing agro techniques to standardize the chemical profile in lantana leaf essential oil. The changes in oil yield in response to different water and K-treatments suggest that strategies can be developed to increase the productivity of lantana leaf essential oil under different conditions. Domestic cultivation is a viable option for producing secondary metabolites of choice, but successful cultivation and use of lantana plants require reproducibility of bioactive compounds. Cultivated lantana plants can provide stable and controlled-manner bioactivity; however, suitable cultivation

practices are needed, and controlled water and K-mineral uptake is one effective way for favorable plant growth and appropriate EO biosynthesis.

CHAPTER SIX

GENERAL CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Summarizing, the findings presented in the three chapters corroborate the feasibility of using the *Lantana camara* essential oil as an alternative control biopesticident and to manipulate the accumulation of secondary metabolites by modulation of the growing conditions. Several points can be concluded from this study. The essential oils derived from lantana have shown larvicidal activities against the 2nd instar tomato leaf miner larvae.

The research findings suggest that there is significant variability in the chemical composition of lantana's essential oils, even within the same agro-ecological zone. By investigating the impact of environmental factors on the synthesis of metabolites, the study has shed light on which compounds are likely to be abundant depending on prevailing conditions. Specifically, water-stressed conditions tend to promote the production of (E)-caryophyllene, lavanduly isovalerate and Trans-Cadina-1(6),4-diene whereas high moisture levels lead to higher levels of 1,8 cineole, and caryophyllene oxide. The dominant compounds with pesticidal properties found in lantana, as reported in literature and confirmed in this study, are (E)-caryophyllene, α-pinene, α-humulene, 1,8 cineole, α-phellandrene, and limonene. As such, the larvicidal activities of the plant can be attributed to these major compounds and their synergistic interactions with minor compounds. Therefore, when exploring the use of essential oils from wild lantana plants for pest management, it is crucial to consider these metabolites.

This study has shown that providing lantana plants with water and additional potassium (K) nutrition can greatly impact the accumulation of secondary metabolites (SM) in their leaves. While increasing nutrients and water usually results in enhanced plant growth, the outcome of fertilisation and watering practices can differ significantly when the goal is to extract secondary metabolites with high quality and quantity. Consequently, it was concluded that the synthesis of secondary metabolites in lantana essential oil can be affected differently by water and K

supplementation, and the impact can vary depending on the metabolite. Therefore, it is crucial to implement precise and customised fertilisation and watering practices, carefully planning and managing the form, amount, and timing of fertilisers and watering based on the specific chemical compound being targeted.

The potential for biosynthetic regulation of metabolites in a controlled environment presents new possibilities for exploring chemical standardisation. Advanced techniques and improved cultivation practices that provide optimal water, nutrients, and environmental conditions such as temperature, light, and humidity can achieve this. These practices can help tackle issues encountered during the production of medicinal plants, such as toxic components, contamination, and low levels of active ingredients. Moreover, they can enhance the yields of essential oil and the concentration of targeted active compounds, ensuring production stability and decreasing prices to a more reasonable range.

6.2 Recommendation

1. The form of application of the essential oil studied in this work presents the contact and ingestion modes with second-stage larvae. However, different forms of application of essential oil have been investigated, such as contact, immersion, fumigation, ingestion and different insect phases (adult, first, second and third-stage larvae). However, from the findings, it is important to note that several authors have evidence that the most significant challenge in resistance is when applied to mature larvae. Therefore, the biological assays testing insecticidal activity against *Tuta absoluta* (tomato leaf miner) should also be directed to the mature larvae and pupae which have higher resistance to make control realistic, particularly in field conditions, where all the stages exist.

2. Further studies concerning the activity of essential oil candidates, as biopesticides in tomato leaf miner control, are still needed to focus on the sub-lethal concentration effects on their reproduction and longevity. Another area of great promise is the information on the mechanism of action with the research of morphological biomarkers of structure damage assessment to investigate the intoxication of target cells, enzymatic biomarkers, and

synergistic interactions between individual compounds. These data would support the use of blend compounds, increasing insecticide activity and reducing the products' volume.

3. Considering the differences in plant secondary metabolism in lantana is a function of genetic factors, environmental conditions and agronomic practices, the more applied research in this area, the closer we will find optimal conditions to develop the correct product. As we aim to produce quality raw material for biopesticide production, the standardization of cultivation practices is one of the most important factors to be observed, as significant changes occur in the essential oils' chemical profile. Adequate fertilisation, plant spacing, harvesting times, drying and extraction methods are some of the main factors that deserve attention. It is necessary to study the best practices to produce high vegetative growth, high essential oil yields, and reasonable contents of the compounds of interest. Thus, a well-directed cultivation of this species, and analogously the domestication of wild lantana plants, requires a carefully optimized and controlled fertilization as well as adequate K- nutrient availability to steer the biosynthesis of pesticidal valuable compounds to move toward eco-friendly pesticide products.

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