EVALUATION OF ROSEMARY (*ROSMARINUS OFFICINALIS*) AS A POTENTIAL PUSH PLANT FOR THE MANAGEMENT OF CABBAGE APHID (*BREVICORYNE BRASSICAE),* AND IT'S IMPACT ON NATURAL ENEMIES IN SMALLHOLDER KALE (*BRASSICA OLERACEA)* PRODUCTION SYSTEMS

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DECLARATION

I understand that plagiarism is an offense and I therefore declare that this research thesis is my original work and has not been presented to any other institution for any other award.

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DEDICATION

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ABBREVIATIONS AND ACRONYMS

ICIPE	**:**	International Centre of Insect Physiology and Ecology
GDP	**:**	Gross Domestic Product
HCD	**:**	Horticultural Crop Directorate
SAICM	**:**	Strategic Approach to International Chemical Management
WHO	**:**	World Health Organization
FAO	**:**	Food and Agricultural Organization
PPEs	**:**	Personal Protective Equipment
VOCs	**:**	Volatile Organic Compounds
DBM	**:**	Diamond Back Moth
TP	**:**	Trap Plant
PP	**:**	Push Plant
EU	**:**	European Union
RCBD	**:**	Randomized Complete Block Design
ANOVA	**:**	Analysis of Variance
FID	**:**	Flame Ionization Detection

DEFINITION OF TERMS

Push plant:	A plant producing volatile organic compounds that keep insect pests away from the primary crop by repelling them.
Pull plant:	A plant that draws pests and keeps them away from the primary crop.
Herbivore-induced plant volatile:	Volatiles produced by a plant in communication with the pests or natural enemies as a result of damage through either infestation or feeding.
Rosemary:	A perennial bushy shrub that is used as a culinary herb and a medicine.
Predators:	An organism that feeds on another.
Parasitoids:	An organism whose young one develops within another organism, eventually killing it.
Integrated pest management:	A combination of common-sense practices including biological, cultural, mechanical, and chemical practices, combined to provide an efficient and environmentally friendly way of controlling pests.
Push-pull technology:	A pest management strategy that uses an intercrop that repels pests and an attractant trap plant as the border crop.

ABSTRACT

Kale (*Brassica oleracea* var *acephala* (L.) production holds global significance as a result of its exceptional nutritional and economic value. However, the cabbage aphid (*Brevicoryne brassicae*), poses an important challenge to *B. oleracea* var. acephala production, with substantial direct and indirect yield losses. Due to its rapid reproduction and resistance development, farmers overly rely on synthetic pesticides to eliminate the pest. Integrated pest management (IPM) strategies, including cropping systems and the use of natural enemies that minimize reliance on synthetic pesticides, are essential for sustainable and environmentally friendly pest control in vegetable production systems. This study hypothesized that *R. officinalis* plant odour disorients *B. brassica*e through specific volatile organic compounds (VOCs). It tested this by assessing *B. brassicae* and its parasitoid (*Aphidius colemani*) responses to *R. officinalis* and *B. oleracea* var. acephala volatiles using a four-arm olfactometer. Gas chromatography-mass spectrometry (GC-MS) identified compounds triggering these behaviors, while gas chromatography-electroantennographic detection (GC-EAD) pinpointed the responsible VOCs. Finally, the impact of intercropping *B. oleracea* var. acephala with *R. officinalis* on the population dynamics of *B. brassicae, A. colemani*, and spiders was evaluated. The collected data was analyzed using R statistical software version 4.2.2. *Brevicoryne brassicae* preferred the olfactometer arm with *B. oleracea* var. acephala plant and its volatiles over those with *R.officinalis* and its volatiles. *Aphidius colemani* preferred the arms with *B. oleracea* var. acephala plant and its volatiles, over the arm containing *R. officinalis* and its headspace volatiles. Gas Chromatography-Mass Spectrometry analysis revealed that *R. officinalis* emitted more and diverse compounds such as camphene, *α*-Phellandrene, δ-2- Carene, (Z)-Sabinene hydrate, linalool, borneol, *α*-Terpineol, verbenone, citronellol, geraniol, bornyl acetate, *β*-Caryophyllene, *α*-humulene, and caryophyllene oxide. GC-EAD analysis indicated that *B. brassicae* antenna responded to Linalool, α-Terpineol, Verbenone, Geraniol, Camphor, and Borneol from *R. officinalis*, and Sabinene, γ-Terpinene, and β-Caryophyllene from *B. oleracea* var. acephala. Intercropping *R. officinalis* with *B. oleracea* var. acephala significantly reduced *B. brassicae* populations, while increasing the numbers of *A. colemani*, highlighting *R. officinalis* potential as a push plant in sustainable *B. brassicae* management systems.

Keywords**:** Push plants, kale, rosemary, cabbage aphids, biological control

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the study

Kenya's economy is mostly driven by the agricultural sector, which contributes approximately 33% of the total Gross Domestic Product (GDP) (RSA, 2015). It also indirectly contributes 27% to the GDP through linkages to sub-sectors like manufacturing and distribution (Gebregergis, 2016). The agricultural sector is a major source of employment, providing jobs for about 40% of the nation's population and 70% of the rural populace (The Government of Kenya, 2019). Within the agricultural sector, the horticultural sub-sector was ranked third as a result of its contribution to GDP (HCD, 2019). Additionally, the agricultural sector has been instrumental in contributing to food security through the provision of key nutritional elements such as vitamins and minerals to the Kenyan population (Lans *et al*., 2012), which is a critical mandate of the Kenyan government (The Government of Kenya, 2019).

Among the top foreign exchange earners, the horticultural sector generated approximately $ 1.5 billion, with fruits and vegetables contributing $ 0.4 billion in total (HCD, 2019)In 2020, the vegetable sub-sector contributed Ksh 24 billion to the GDP (Lengai *et al*., 2022). Since 2015, the horticultural sub-sector of Kenya has been experiencing an increase in the total size of land, estimated at 719,158 ha. The total area under vegetable production in the same year was 326,837 ha contributing 36% to the domestic value of horticulture. Over the years, Kenyan vegetable exports have increased by 20%, meaning that horticulture is a key foreign exchange earner (Muendo and Tschirley, 2004). About ninety percent of the grown vegetables in Kenya are consumed locally, while ten percent are exported (RSA, 2015). The presence of exotic vegetables in Kenyan markets has been increasing over the years due to factors such as globalization and changing consumer preferences (RSA, 2015). The vegetable subsector is of importance in improving food security as well as the livelihoods of the smallholder farmers who produce 70% of exotic vegetables and 100% of the African vegetables (RSA, 2015, HCD, 2019). Some of the cruciferous vegetables grown by smallholder farmers mainly for consumption in local and export markets in Kenya include

B. oleracea var. acephala, spinach, collards, lettuce, and cabbage among others (The Government of Kenya, 2019).*Brassica oleracea* var. acephala is a leafy vegetable of global importance, primarily cultivated by small-scale farmers for both subsistence and income generation, particularly in tropical and subtropical regions (Mutiga *et al.*, 2011; Peris and Kiptoo, 2017; Šamec *et al*., 2019). According to the Center for Disease Control (CDC), *B. oleracea* var. acephala was ranked 15th of the 47 powerhouse fruits and vegetables, producing more than 17 essential nutrients (CDC, 2014). *Brassica oleracea* var. acephala has garnered significant attention recently owing to its notable health advantages (Šamec *et al*., 2019). It contains phytochemicals that have been linked to reduced risk of cancer and other chronic diseases, due to antioxidant properties and high dietary fiber content (Šamec *et al*., 2019). Additionally, *B. oleracea* var. acephala is known for its resilience to adverse effects of climate change, rendering it adaptable to extreme climatic conditions (Lagerkvist *et al.*, 2012). In Kenya, *B. oleracea* var. acephala has become increasingly popular due to its ability to maximize land use and address food security and nutrition concerns amidst challenges such as land degradation and population pressure (HCD, 2019; Mutiga *et al*., 2011; Olwande *et al*., 2015). Due to the low input and labour requirements for *B. oleracea* var. acephala production, the crop stands out as one of the most accessible vegetables to cultivate (Canwat *et al*., 2021; Lans *et al*., 2012). Its cost-effective production methods contribute to relatively low market prices, ensuring affordability for consumers. Consequently, it is widely consumed in households and extensively sold in urban areas (Otieno, 2019).

Despite these benefits, the successful production and productivity of *B. oleracea* var. acephala face various constraints such as pests and disease pressures, poor soils, limited market access, climate change, and inadequate production techniques (Canwat *et al*., 2021). *Brevicoryne brassicae* is one of the most destructive insect pests that affects production of *B. oleracea* var. acephala and other Brassica sp. crops worldwide (Cole, 1994; Gill *et al*., 2013). The pest is native to Europe but has been reported in many parts of the world (Gill *et al*., 2013; Munthali and Tshegofatso, 2014). The adults feed on the sap of plant tissues using their piercing-sucking mouthparts, causing direct crop damage through wilting, stunted growth and deformation, and transmission of diseases such as mosaic virus and ring necrosis, which eventually result in plant death (Powell *et al*., 2006; Mutiga *et al*., 2010; Chalise and Dawadi, 2019). *Brevicoryne brassicae* has a wide host range of crops belonging to Brassicaceae family such as kale (*Brassica oleracea* var. acephala), cabbage (*Brassica oleracea* var. capitata), Brussels sprout (*Brassica oleracea* var. gemmifera) and Broccoli (*Brassica oleracea* var. italica) (Döring, 2014; Douloumpaka and Van Emden, 2003; Van Emden and Harrington, 2007).

Smallholder farmers with limited resources have resorted to indiscriminate use of synthetic insecticides to control the pest (Shelton and Badenes-Perez, 2006; Otieno, 2019). The repeated use of these chemical insecticides has resulted in additional economic costs to farmers, insecticide resistance, and pest resurgence, and has proven detrimental to agrobiodiversity, human and environmental health (Kianmatee and Ranamukhaarachchi, 2007; Macharia and Afr, 2009; Ngolo *et al*., 2019; Ricupero *et al*., 2020). There is therefore an urgent need to develop alternative control options that will be ecologically friendly, cost-effective, sustainable, and suitable for resource-limited vegetable farmers. Therefore, this study aimed to evaluate *R. officinalis* as a potential push plant that could be integrated into *B. oleracea* production systems to reduce *B. brassicae* populations while attracting natural enemies, in order to reduce overreliance on synthetic pesticides.

1.2 Statement of the problem

Brassica oleracea var. acephala is a widely consumed indigenous vegetable in Kenya (HCD, 2019). On the other hand, *B. brassicae* causes detrimental *B. oleracea* var. acephala destruction, lowering its economic value. *B. brassicae* also transmit pathogens such as viruses that affect *B. oleracea* var. acephala eventually resulting in plant death.

The honeydew and sooty moulds secreted by aphids attract other insect pests adding on to the damaged plants (Byamungu *et al*., 2019). The farmer's solution has always been indiscriminate use of insecticides which bring serious health risks to consumers. Additionally, the costly nature of pesticides results in financial strain on farmers (Ngolo, 2019). This has always led to food insecurity within the population as there is little to save, and the profit obtained is not far more than the incurred costs (Chepchirchir *et al*., 2018).

Pesticide use affects both the target and the non-target organisms, interfering with the ecosystem's functioning (Ricupero *et al*., 2020). Additionally, the use of pesticides is ineffective due to the overlapping generations of the *B. brassicae* and the ability to hide under the leaves makes them inaccessible to pesticides thus multiplying constantly. Continuous spraying with pesticides leads to the risk of pesticide resistance and pest resurgence (Vilcinskas *et al.*, 2016; Mayanglambam *et al*., 2021).

Additionally, Kenya as a member of environmental conventions and treaties like the Strategic Approach to International Chemical Management (SAICM) and the Stockholm Conventions, is mandated to minimize pesticide usage within recommended levels, particularly on fruits, flowers, and vegetables (Aktar *et al*., 2009). Despite these obligations, the lack of implementation programs has led to the excessive application of pesticides, neglecting concerns for both environmental and human health. For instance, around ten percent of exported *B. oleracea* var. acephala must comply with pesticide residue standards set by the WHO and the FAO (Macharia and Afr, 2009; FAO, 2018). Failure to meet these standards results in exclusion from the export market (Ambrus and Yang, 2016).

1.3 Objectives of the study

1.3.1 General objective

The main objective of this study was to evaluate *R. officinalis* as a potential push plant for the management of *B. brassicae* and its impact on the natural enemies in smallholder *B. oleracea* var. acephala production systems.

1.3.2 Specific objectives

The specific objectives of this study were to:

i. To determine the behavioral response of *B. brassicae* and its natural enemy to constitutive volatiles of *R. officinalis*.
ii. To characterize the VOCs emitted by *R. officinalis* responsible for the observed behavior in both *B. brassicae* and its parasitoid.

iii. To determine the electroantennographic responses of *B. brassicae* to *R. officinalis* and *B. oleracea* var. acephala constitutive volatiles.
iv. To assess the impact of intercropping *B. oleracea* var. acephala with *R. officinalis* on *B. brassicae* and its natural enemies' population.

1.4 Hypothesis of the study

i. H_0 *1*: The constitutive volatiles of *R. officinalis* have no significant repellence to *B. brassicae* or attraction properties to its natural enemy.
ii. H_0 *2*: *Rosmarinus officinalis* does not contain VOCs which can repel *B. brassicae* or attract its natural enemy.
iii. H_0 *3*: *Rosmarinus officinalis* does not contain VOCs that can be detected by the *B. brassicae* antenna.
iv. H_0 *4*: Intercropping *B. oleracea* var. acephala with *R. officinalis* does not significantly reduce *B. brassicae* populations in *B. oleracea* var. acephala while increasing the population of natural enemies.

1.5 Justification of the study

Vegetables, especially *B. oleracea* var. acephala, are highly grown in Kenya due to their fast growth and minimum input requirements (HCD, 2019). *Brassica oleracea* var acephala play a crucial role in enhancing livelihoods of smallholder farmers, particularly in rural areas, and in driving the horticulture industry's contribution to Kenya's GDP (HCD, 2019). *Brevicoryne brassicae* causes substantial yield losses, reducing both the quality and quantity of *B. oleracea* var. acephala produced, which is a critical staple and cash crop for many households (Muendo and Tschirley, 2004). Conventional chemical insecticides have been widely used to manage aphid infestations; however, they present several drawbacks, including environmental pollution, pest resistance development, and adverse effects on non-target organisms, such as pollinators and natural enemies of pests (Kumela *et al.*, 2019). Moreover, the high cost of chemical control methods is often prohibitive for smallholder farmers, contributing to limited access and over-reliance on unsustainable practices (RSA, 2015). This necessitates the search for a sustainable solution to this pest which is driving smallholder farmers out of *B. oleracea* var. acephala production, negatively affecting their livelihoods and food security (Sharma, 2014). Various alternative management approaches have been utilized especially for pre-harvest management such as cultural control methods (field sanitation), the use of greenhouses, and insecticidal oils and soaps obtained from plants (Mutiga *et al*., 2010). However, the success of these approaches is limited due to some challenges such as the associated costs, access to some of the control methods by smallholder *B. oleracea* var. acephala farmers, and the behavior and nature of the pest (Nampeera *et al*., 2019). *Rosmarinus officinalis* has been recognized for its pest-repellent properties due to the production of volatile compounds that deter aphids and other pests (Dardouri *et al*., 2019). As a readily available and drought-tolerant perennial plant (Sasikumar, 2012), *R. officinalis* could serve as an accessible and cost-effective push plant for smallholder farmers. By evaluating its potential to repel *B. brassicae*, this study aims to contribute to the development of a more sustainable integrated pest management (IPM) strategy for *B. oleracea* var. acephala production.

The study is essential because it aligns with the broader goals of promoting environmentally sustainable agricultural practices and enhancing the livelihoods of smallholder farmers. By exploring the efficacy of *R. officinalis* in reducing *B. brassicae* infestations, the study aims to contribute to sustainable pest management practices that support smallholder farmers in maintaining high-quality *B. oleracea* var acephala yields without reliance on synthetic pesticides. The findings from this research could offer practical insights into pest control methods that are affordable, accessible, and aligned with the agroecological conditions of smallholder farming systems, ultimately increasing the cost effectiveness in *B. oleracea* var. acephala production.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *Brassica oleracea* (var acephala)

Brassica oleracea var acephala, belongs to the Brassicaceae family, and its native to eastern Mediterranean countries (Šamec *et al*., 2019). It is relatively hardy, has finely divided leaves and doesn't form head. *Brassica oleracea* var acephala is particularly valued for their rich nutritional profile, containing high levels of vitamins (A, C, and K), essential minerals, dietary fiber, and antioxidant properties, which promote overall health and help prevent chronic diseases (Migliozzi *et al*., 2015). Although *B. oleracea* var acephala has been cultivated for several centuries and it has been included in many traditional meals, especially in Mediterranean area, it has become very popular in the United States after 2010, China and Asia (Šamec *et al*., 2019). It was introduced as a fodder crop by the Europeans in the Kenyan highlands, and it has become a valued vegetable in most parts of the country (Park *et al*., 2018). It is currently grown as a source of income and nutritional security amongst the smallholder farmers in the country (Qureshi *et al.*, 2014). It can tolerate a range of climatic conditions, including cool weather, which often enhances the flavor and quality of the leaves (Ashenafi and Tewodros, 2018). For smallholder farmers, *B. oleracea* var acephala is a fast-growing crop that can be harvested multiple times during the growing season, providing a continuous supply of fresh produce for home consumption and local markets (Olwande *et al*., 2015). Its resilience and adaptability make it an ideal crop for improving food security, particularly in regions vulnerable to climate variability (Lagerkvist *et al*., 2012).

In Kenya, *B. oleracea* var acephala is a staple vegetable, often cultivated in small plots for household use and as a source of income (Mutua *et al*., 2024). It thrives under a variety of farming systems, including organic, agroforestry, and other integrated farming practices (Omwenga *et al*., 2021). *Brassica oleracea* var acephala, is frequently intercropped with legumes, tubers, or other vegetables to optimize land use, improve soil fertility, and reduce pest pressure through diversified cropping systems (Mutiga *et al*., 2010).

Despite its many benefits, *B. oleracea* var acephala production faces several challenges, with pest infestations being a primary concern. *Brevicoryne brassicae*, diamondback moths (*Plutella xylostella*), and whiteflies are common pests that attack *B. oleracea* var acephala ,leading to significant yield losses (Migliozzi *et al*., 2015).

2.2 Biology of *Brevicoryne brassicae*

Brevicorye brassicae (L.) (Hemiptera: Aphididae) belongs to the Aphididae family, with approximately, 5,000 species (Van Emden and Harrington, 2007). They are typically 1.5-2.5mm long soft-bodied and oval, with a pair of cornicles projecting backward and appear in both winged and wingless form (Gill *et al*., 2013). They have long antennae and two distinct tube-like structures called cornicles on the posterior end, which secrete defensive substances (Webster, 2009; Döring, 2014). They are usually grey-green but appear white or grey because of the waxy substance covering their bodies, and they usually appear in clustered colonies on various parts of the plant such as the base of the plant leaves, buds, branches, fruit, and soft stem when it is tender and succulent (Van Emden and Harrington, 2007). They have piercing mouthparts usually called the stylets, adapted for extracting the sap from the plant (Van Emden and Harrington, 2007). *Brevicoryne brassicae* increase rapidly in numbers and establish a high population due to their sexual and asexual reproduction nature (Vilcinskas, 2016). The asexual mode of reproduction gives birth to immature-wingless live nymphs in warm climates, which molt multiple times typically passing four to five instar stages before they become adults (Döring, 2014). Many of the aphids develop from nymphs to reproducing adults between 16 and 50 days depending on the temperature, and that's why their population increases rapidly (Douloumpaka and Van Emden, 2003). One *B. brassicae* can give birth to up to 100 nymphs during her lifespan (Gill *et al*., 2013).

2.3 Ecology of *Brevicoryne brassicae*

Brevicoryne brassicae are native pests to Europe and distributed in all warm and temperate regions globally (Gill *et al*., 2013). They have different host plants, particularly those of the Brassicaceae family (Döring, 2014). They also attack other wild and cultivated cruciferous crops (Sapkota *et al*., 2022). Additionally, they transmit viruses in crops such as cucumber, melon, pumpkin bean, lettuce, and potatoes, which cause stunted crop growth, mottled leaves, and curled or yellow leaves (Döring, 2014). *Brevicoryne brassicae* attract many other predators, which may carry with them disease-causing vectors (Kumar *et al*., 2011).

Brevicoryne brassicae feed on the plant vascular tissues by the use of sieve elements, maxillae, and elongated mandibles (Byamungu *et al*., 2019). They produce dense and jellying substances around the plant areas, which helps in penetration of their piercing stylets into the phloem (Klingler *et al*., 1998). After they reach the phloem, they produce watery saliva that is injected into the plant tissues and contains digestive enzymes (Pontoppidan *et a*l., 2003). Their mode of feeding produces a gradual effect on the crop unlike other pests' damages (Klingler *et al*., 1998; Van Emden and Harrington, 2007; Gill *et al*., 2013). Most aphids attack during spring and summer and constitute females, and their dispersal is affected by the direction of the wind (Trionnaire *et al*., 2008). Although wings also allow them to migrate to other host plants after the quality of the initial host reduces (Singh and Kothari, 1997; Van Emden and Harrington, 2007). Their infestation starts when a few winged adults are blown into the area with a suitable host on which they deposit many nymphs before they fly to a new host plant, expanding their range (Döring, 2014). The nymphs mature within 7 to 10 days and start producing new ones (Döring, 2014). The winged fly to a new host plant and the process is repeated (Vilcinskas, 2016).

Brevicoryne brassicae have a mutual relationship with ants, which are attracted to the honeydew excreted by the aphids, and in turn, the ants offer protection against predators (Klingler *et al*., 1998). Their populations are controlled by predators such as ladybugs, hoverflies, lacewings and spiders, and parasitic wasps such as *Diaeretiella rapae, Aphidius ervi* and *Aphidius colemani,* which lay eggs inside the aphid, and the developing larvae consume the aphid from within eventually killing it and emerging as adult wasps (Kalule and Wright, 2002). Parasitized aphids form Mummified aphids, which are golden brown in color (Peris and Kiptoo, 2017).

2.4 Economic importance of *Brevicoryne brassicae*

Around 400 of *B. brassicae* species feed on fibre and food crops, which are a serious problem within the field of agriculture and act as a challenge to smallholder farmers, not only in Kenya but also the whole world (Liu and Sparks, 2001). *Brevicoryne brassicae* form colonies that attack the host's stems, leaves, or petioles whereby, they cause direct damage through the sucking of the sap, extracting nutrients and water (Klingler *et al*., 1998). Infestation particularly under the leaves and the soft parts of the plant where the colonies cluster results in leaf deformation, discoloration, and eventually plant death (Cole, 1994). *Brevicoryne brassicae* act as vectors for several plant viruses such as the Turnip mosaic virus and the cauliflower mosaic virus, which further lead to yield losses (Tatchell, 1989; Pontoppidan *et al*., 2003). These viruses are difficult to prevent because infestations occur even when the aphid population is very low (Allen-Perkins and Estrada, 2019). While they feed on the plant sap, they excrete honeydew, a sugary substance that promotes the growth of sooty mold fungi on the leaves and negatively affects the plants' photosynthetic activities (Kinyanjui *et al*., 2016). Some aphids inject toxic substances into the plant phloem causing leave curling and distorted growth and a few aphids' species cause the formation of galls (Tatchell, 1989; Pontoppidan *et al*., 2003). When the infestation and the indirect damages are severe, *B. brassicae* can reduce crop production from 80% to 70%, as it necessitates crop destruction to prevent further spread (Kumar, 2017).

The use of chemical control methods to manage *B. brassicae* increases the cost of production especially during the peak of their growing activity (Boivin *et al*., 2012). Additionally, repeated synthetic pesticide use requires extra labour costs for monitoring and scouting the *B. brassicae* (Mala *et al*., 2020). In the present era faced with the challenge of climate change and global warming, ecologists are unable to accurately predict variations that occur in the ectotherm organism's biology, which might affect crop production (Trionnaire *et al*., 2008).

2.5 Pest management practices against cruciferous pests

2.5.1 Chemical control

Chemical control remains one of the most widely used methods for managing pests in cruciferous crops, including kales, cabbages, and other brassicas (Van Emden and Harrington, 2007). Chemical pesticides provide quick and effective pest suppression, making them appealing to farmers who seek immediate results in large-scale commercial production (Otieno, 2019). Some of the commonly used chemical pesticides include emerald and Pymetrozine, which are both contact and systemic (Otieno, 2019). However, the overreliance on chemical control presents several challenges, one major issue being the development of pest resistance (Van Emden and Harrington, 2007). *Brevicoryne brassicae* in particular, is notorious for developing resistance to a wide range of insecticides due to its mode of feeding and high reproductive rate (Mayanglambam *et al*., 2021). This resistance reduces the effectiveness of many conventional pesticides, forcing farmers to use higher doses or switch to more toxic alternatives, which can be both costly and environmentally harmful (Bale *et al*., 2008).

Another concern is the impact of chemical pesticides on non-target organisms, including beneficial insects such as pollinators and natural enemies of pests (Mayanglambam *et al*., 2021). Broad-spectrum insecticides can disrupt the ecological balance in agricultural systems, leading to secondary pest outbreaks or a reduction in biodiversity (Mala *et al*., 2020). Additionally, the excessive use of chemical pesticides can lead to environmental contamination, particularly through runoff, which can affect soil health and water quality (Otieno, 2019).

Human health risks are also associated with the use of chemical pesticides, especially when proper safety precautions are not followed (Norris and Congreves, 2018). Residues on food crops may pose risks to consumers, while farm workers are exposed to these chemicals during handling and application (Ngolo *et al*., 2019). To mitigate these risks, there is a growing demand for the development and adoption of safer, more targeted pesticides, as well as sustainable pest management strategies that reduce the use chemical control and instead focus more on cultural and biological control.

2.5.2 Biological control

Natural enemies like parasitoids and predators have widely been used in the management of cruciferous pests (Acheampong and Stark, 2004; Naranjo *et al*., 2015). The ability of plants to emit herbivore-induced plant volatiles (HIPVs) when under attack makes it possible to communicate with the natural environment and the organisms in the whole food chain such as the predators and the parasitoid (Gols *et al*., 2011). Experienced female wasps can differentiate between a blend of volatiles emitted by a healthy and a damaged plant, and they prefer the latter as it hosts their target prey or host (Mutyambai *et al*., 2015). These volatiles do not reveal which particular pest is feeding on the emitting plat as they are produced in a blend and therefore the information is conveyed differently in the ecosystem (Gols *et al*., 2011). *Brevicoryne brassicae* parasitoids show different kinds of specialization as some specialize in a particular species while others specialize in the most abundant species in a certain habitat (Boivin *et al*., 2012). In vegetable production, using parasitoids for pest population management has been demonstrated as an Integrated Pest Management approach that could add to the reduction of pesticide use as pest resistance continues to be a problem (Bale *et al.*, 2008), although little has been done on *A. colemani* that parasitizes *B. brassicae*. Natural enemies tend to be prevalent in floral ecosystems (Boivin *et al*., 2012). Therefore, intercropping vegetables with other aromatic and flowery plants improves their landing (Li *et al.*, 2021).

2.5.3 Cultural control

2.5.3.1 Companion cropping

The use of ecologically sustainable methods of pest control such as companion cropping offers a harmonious approach to cultivating crops to maximize yield and act as an alternative to synthetic pesticides, although its effectiveness has not been put into much consideration (Hooks and Johnson, 2003; Chidawanyika *et al.*, 2023). Companion cropping is a form of growing diverse crop plants deriving benefits such as pest control, increasing the natural enemies, biotic stress management, field utilization, pollination, balanced nutrition, and overall ecosystem resilience (Brennan, 2016; Reddy, 2017). There are different types of companion cropping including intercropping, trap cropping, push-pull technology, and relay cropping among others (Sarkar *et al*., 2018). Trap crops act by attracting or reducing the target pest in the target crop (Badenes-Perez and Shelton, 2006). The primary advantage of companion cropping is its ability to promote resilience in the ecosystems and make the target crops less susceptible to pests and diseases (Peter *et al*., 2023). It utilizes allelopathic effects of some plants to deter, trap, or repel pests depending on host preference (Khan *et al*., 2000, 2010). When utilized correctly, it enables farmers to minimize the use of pesticides while increasing profits and improving their health as well as the reduction in the cost of production through the selection of suitable crops (Hasheela *et al*., 2010). Numerous studies have demonstrated the effectiveness of companion cropping with success in deterring pests (Hooks and Johnson, 2003; Mutiga *et al*., 2010; Kebede *et al*., 2018; Midega *et al*., 2018; Sarkar *et al*., 2018; Chidawanyika *et al*., 2023). However, the adoption of companion cropping in cruciferous vegetables is still limited despite its advantages. This is partly because of the high initial cost of setting up the system and the fear of economic losses in case the strategy fails (Kebede *et al*., 2018).

2.5.3.2 Intercropping

Intercropping is an ancient farming method involving planting more than one crop in the same land (Khan *et al*., 2001; Ben-Issa *et al*., 2017; HE *et al*., 2019)). When implemented practically by planting in rows or alternating crops in the same row, intercropping can significantly reduce pest populations (Smith and Liburd, 2012; Ben-Issa *et al*., 2017). Intercropping can combine the main crop with cover crops or any other beneficial non-food crops (Couëdel *et al*., 2019). It increases the efficiency of moisture conservation, and nutrient utilization as well as increases yields as compared to monoculture (Smith and Liburd, 2012). Intercropping is widely used in the whole world, especially for the management of crucifer pests (Singh and Kothari, 1997). The intercropped plants control pests by masking the odor of the host plant and disorienting the pest or by serving as a trap crop, therefore, diverting the insect pest from the primary crop (Sarker *et al*., 1970; Khan *et al*., 2001; Ben-Issa *et al*., 2017). These crops work best because the pests require a conducive environment for oviposition and with the neighboring intercrop being a non-host, its oviposition behaviors is altered, hence reducing its population (Yarou *et al*., 2017). Gravid females change their location to a new host or lay a fewer number of eggs (Hooks and Johnson, 2003). The production of VOCs by a non-host plant influences the behavior of many cruciferous pests (Agelopoulos *et al*., 1999; Mala *et al*., 2020).

Intercropping combined with other control options has been tried in different cruciferous vegetables to control major pests such as the *B. brassicae* and *P. xylostella* as part of the Integrated Pest Management (IPM) system (Mutiga *et al*., 2010). To reduce the *B. brassicae* population in broccoli, broccoli was intercropped with strips of alyssum, and a reduction in their population was reported compared to a monocrop of broccoli (Brennan, 2016). The population reduction was attributed to the ability of alyssum to attract hoverflies which are predators of *B. brassicae* (Brennan, 2016). Similarly, Ponti *et al*. (2007) reported reduced populations of *B. brassicae* in composted broccoli when intercropped with *Brassica carinata* or buckwheat compared to the synthetically fertilized plots during the summer season. The reduced populations were attributed to the ability of buckwheat to attract natural enemies that fed on *B. brassicae* (Ponti *et al*., 2007). To identify a suitable push crop, cabbage was intercropped with 54 different plant species. Among these, cabbage interplanted with garlic, barley, dill, oat, safflower, and tomatoes showed the lowest populations of diamondback moth (Talekar *et al*., 1986). The reduced populations were attributed to the fact that the intercropped plants grew tall and acted as a barrier against diamond back moth (Talekar *et al*., 1986).

Despite the shadowing effect, other studies have shown the effectiveness of tomato, dill, and garlic in reducing pest infestations in crucifers (Hooks and Johnson, 2003; HE *et al*., 2019). Intercropping mustard with spice crops such as onion, coriander, ajwain, chamomile, fennel artemisia, and garlic was done to determine its effect on the populations of mustard aphids (Noman *et al*., 2013). It was observed that the mustard aphid populations were suppressed in the mustard and coriander intercrop as compared to the other intercrops while the number of natural enemies increased (Noman *et al*., 2013). When mustard was intercropped with onions and garlic, the population of mustard aphids was significantly reduced compared to the mustard monocrop, with garlic intercrop having the highest significance of pest reduction (Sarker *et al*., 1970). Intercropping sacred basil with the Chinese kale *(Brassica oleracea* L.*)* reduced pest populations of both cabbage webworm

(*Hellula undalis)* and flea beetle, (*Phyllotreta sinuata*), and *Spodoptera litura* (Kianmatee and Ranamukhaarachchi, 2007). In a study conducted in 2010, the damage of *H.undalis* was found to be minimal cabbage which was intercropped with pepper or onions (Asare-Bediako *et al*., 2010). Similarly, intercropping cabbage with tomatoes and onions in Ghana effectively pushed away cabbage pests due to the ability of the VOCs to confuse the pests' host-finding behavior (Asare-Bediako *et al*., 2010). In Kenya, intercropping collards with spring onions and collards with chili were found to have a lower population of *B. brassicae* compared to collard monoculture (Mutiga *et al*., 2010). Therefore, intercropping *B. oleracea* var acephala with repellent plants is a valuable integrated Pest Management strategy that has not been adequately evaluated for sustainable farming practices. It requires careful consideration of plant compatibility, management demands, and potential trade-offs. Success depends on site-specific conditions, and further research is needed to optimize these systems for different environments and pest species.

2.5.3.3 Push-Pull Technology

Push-pull technology represents a form of intercropping employed for pest control by the use of repellent (push plants) and attractant plants (pull plants) (Khan *et al*., 2008). It was developed in the 1990s by ICIPE, Kenya, in partnership with the Rothamsted Research of the UK and national partners (Khan *et al*., 2000, 2001). It aimed to manage stemborer pests in often-infested cereal plants, such as sorghum and maize (Khan *et al*., 2014). Drought-tolerant *Desmodium intortum* was used as a 'push' plant due to its ability to emit VOCs that can repel stemborer moth as well as its ability to control a parasitic weed, Striga (Khan *et al*., 2010, 2014). Napier grass (*Pennisetum purpureum*) was used as the 'trap' plant (TP), whereby it was planted along the borders of the maize plots as the stemborers are pulled by the VOCs produced by the Napier grass more than those of maize (Khan *et al*., 2008, 2010).

Different trials have been conducted to control brassicas insect' pests (Singh and Kothari, 1997; Kianmatee and Ranamukhaarachchi, 2007; Noman *et al*., 2013; Ben-Issa *et al*., 2017; Couëdel *et al*., 2019). For instance, in a study conducted in Benin to evaluate the impact of planting tropical basil round cabbage plots, fewer pest damages were reported compared to the sole cabbage plots (Yarou *et al*., 2017). In another study, cabbage was planted with clover and it was observed that *Pieris brassicae L.* and *P. xylostella* showed low oviposition on cabbage plants due to the repellant compounds produced by the cloves (Finch and Kienegger, 1997).

Some VOCs can attract the natural enemies of the insects' pests, which also help reduce the pest populations. *Barbarea vulgaris* is an example of a trap plant, which was shown to attract the cabbage pest, *P. xylostella*, however, the plant is not suitable for growing in arable fields hence complications in field management practices (Parolin *et al*., 2012). Push-pull technology generally employs manipulation of the behavior of the insect pests, especially feeding and oviposition (Li *et al*., 2021). Most of the push-pull plants that have been used are the non-host plants, especially the aromantic ones (Finch *et al*., 2003). Due to their attractiveness, these plants increase the availability and effectiveness of other biological control agents such as predators and parasitoids (Shrestha *et al*., 2019). While the cereal push-pull system has been extensively researched and successfully implemented in cereal crops, its application in vegetables is still in the early stages. More research is needed to fully understand how well the system works for different vegetable crops, pest complexes, and agroecological zones. Without comprehensive data and trials, the scalability and reliability of the system for vegetable production remain uncertain.

2.5.3.4 Plant-based extracts and botanicals

Botanicals are natural products obtained from plants to advance the traditional knowledge of pest control (Peris and Kiptoo, 2017; Munyoki *et al*., 2024). They emerge as a promising strategy that uses nature-based products to manage pests while minimizing ecological harm due to their biodegradability and their ability to preserve beneficial insects compared to synthetic pesticides (Cloyd *et al*., 2009; Peris and Kiptoo, 2017). They provide insecticidal activity by repelling the pest by their smell and taste or acting as antifeedants which make the pest reduce food intake thereby starving to death (Baryakabonaa and Mwine, 2017). Other botanicals act as inhibiting the pest from laying eggs hence interfering with the life cycle (Baryakabonaa and Mwine, 2017).

Different plant extracts have been used in management of vegetable pests, for instance, neem extracts were tested against *Myzus persicae*, two spotted spider mites, and citrus mealybugs, whereby, the extract showed a high mortality rate (Cloyd *et al*., 2009). Additionally, extracts from *Mexican marigold*, garlic, ginger, and Sodom apple were tested against *B. brassicae*. Mexican marigold extracts were found to be more effective in controlling *B. brassicae* as compared to garlic extracts which had lethal effects on the parasitoids (Peris and Kiptoo, 2017). In a study conducted to investigate the impact of coriander and *R. officinalis* volatiles from their extracts on the citrus brown mite, *R. officinalis* oil extract was found to be effective in controlling the pest at different developmental stages, and its effectiveness increased with the increase in concentration, with the highest repellency being observed in coriander extracts (Elhalawany *et al*., 2019).

2.6 *Rosmarinus officinalis* as a potential push plant

Rosmarinus officinalis is an aromatic perennial herb from the Lamiaceae family, that is native to the Mediterranean regions and is cultivated worldwide due to its culinary uses (Sasikumar, 2012). Recently, it has been used in making insect repellents and bioinsecticides, cosmetics, for medicinal purposes, and ornamental uses (Li *et al*., 2021). Previous studies have demonstrated the potential of *R. officinalis* as a repellent, showcasing its effectiveness against a wide range of insect pests (Hori, 1998; Miresmailli and Isman, 2006; Cook *et al*., 2007; Cloyd *et al*., 2009; Zhang and Chen, 2015; Waithaka *et al*, 2017; Dardouri *et al.*, 2019; Elhalawany *et al*., 2019; Li *et al*., 2021). Hori, (1998) found out that *R. officinalis* and thyme oils strongly repelled *M. persicae* at different doses and that there were particular VOCs like linalool and camphor among others that were responsible for the repellence.

Intercropping *R. officinalis* with sweet pepper suppressed the population of *Frankliniella intonsa, Bemisia tabaci*, and *Myzus persicae* with no effect on the population dynamics of the natural enemies (Li *et al*., 2021). Its ability to reduce the populations of the aphids was associated with the production of VOCs such as (E)-β-farnesene, responsible for its repellence properties (Li *et al*., 2021). Despite repellence, intercropping *R. officinalis* with other crops increases utilization of resources like land and water hence increasing productivity per unit of land, weed and pest control, and ensures yield stability over seasons (Miresmailli and Isman, 2006; Genet *et al*., 2020). Similarly, laboratory experiments using different *R. officinalis* clones have shown their ability to produce VOCs that are repellent to *M. persicae* (Dardouri *et al*., 2019). No competition effects have been reported both below and above ground on *R. officinalis* intercrops. Intercropping *R. officinalis* with *B. oleracea* var acephala has not been extensively studied as *R. officinalis* is usually planted as part of kitchen gardening.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Plants

Seeds of *B. oleracea* var. acephala (var. simlaw select) used in this study were obtained from Simlaw Seeds Company, Nairobi, Kenya. The seeds were sown in a 2 by 1 m nursery bed and allowed to grow for three weeks before the seedlings were transplanted to individual plastic pots of a 5-litre capacity. The pots were filled with soil and organic manure mixed in a ratio of 2:1 and grown in an insect-free screen house at ICIPE, Nairobi, Kenya at geographical coordinates; 01º 13’ 25. 6” S 036º 53’ 49. 1” E, and at an altitude of 1616 m above sea level. Watering was done once a day using a watering can while no chemicals were applied to the plants or fertilizers added. *Rosmarinus officinalis* (var. Tuscan Blue) seedlings, known for its upright, vigorous growth and larger, intensely blue flowers, setting it apart from other *R. officinalis* varieties, and the commonly grown variety in Kenya were sourced from Kimplanter Seedlings and Nurseries, Thika, Kenya and as earlier indicated the plants received the same treatment as the *B. oleracea* var. acephala plants. The *B. oleracea* var. acephala plants used for experiments were six weeks old while the *R. officinalis* plants were eight weeks old.

3.2 Insects

The initial colony of *B. brassicae* was started using aphids collected from infested *B. oleracea* var. acephala leaves from small-holder farms in Limuru geographically located at 1° 10’ 9. 13” S; 36° 41’ 25. 18” E, Kiambu County, Kenya, and at 2500m above sea level. The adults were first identified using their morphological features provided in the taxonomic keys followed by verification at the national museums of Kenya. The adult *B. brassicae* was then cultured on *B. oleracea* var. acephala plants in simple 50×80×40 cm clear perplex cages in the laboratory and kept at 25 ± 1°C and 65 ± 5% RH for reproduction purposes (Webster, 2009). The insects were fed with fresh *B. oleracea* var. acephala leaves after every three days. The field-collected insects were introduced into this colony every 14 days to avoid loss of genetic vigor. After 10 days, the newly emerged generation was transferred to a different rearing cage, and *B. oleracea* var. acephala plants were provided for feeding. Insects were bred up to the 10th generation, which provided a sufficient number of adults without field traits to be used for bioassays (Dardouri *et al*., 2019). Twenty adult insects from the rearing cage were randomly collected using an aspirator for bioassays after which they were disposed of. For bioassay, all the used aphids were fourteen days old.

Mummified aphids, characterized by rounder, golden brown, and dead *B. brassicae* were collected from *B. oleracea* var. acephala farms in the same location and kept in separate cages. The emerging adults of *A. colemani* were identified by their light to dark brown appearance and other distinct physical features using taxonomic keys. Confirmation of the identity was done by taxonomists at the national museums of Kenya. Cotton wool dipped in diluted honey was provided in the cage for their feeding. *Brevicoryne brassicae*-infested plants were also kept in the cage containing the *A. colemani* for oviposition to ensure the continuity of the colony (Douloumpaka and Van Emden, 2003). For carrying out bioassays, mummified aphids were kept in a separate cage. After emergence, one-day-old gravid females without prior exposure to *B. brassicae* were used after which they were kept separately for continuity of the colony. Twelve female parasitoids were used.

3.3 Headspace sample collection

Headspace sampling technique was used to collect volatiles from the experimental plants (*B. oleracea* var. acephala and *R. officinalis*) and control (empty bag) for continuous 24 h starting from the last two hours of the photo phase as described by Mutyambai *et al*. (2015). The aerial parts of the plants were enclosed in polyethylene terephthalate (PET) bags with an average of 12. 5mm thickness and volume of 3. 2L, which were initially conditioned at 150°C for 30 min before use and were fitted with Swagelok inlet /outlet valves as described by Mutyambai *et al*. (2015). Air prefiltered with activated charcoal was drawn through the inlet port at a rate of 600 mL/min. Volatile organic compounds were condensed on Charcoal filters (0. 05 g, 60/80 mesh, Supelco, USA) mounted at the outlet where the air was drawn at a rate of 400 mL min-1. Following trapping, the collected volatiles were eluted in 250 µL dichloromethane (analytical grade, Sigma-Aldrich, USA) in 2 mL micro vials (Agilent Technologies, Warsaw, Poland) and kept in a -40°C freezer for further chemical analysis and bioassays. Entrainments from four different plants were used for data analysis.

3.4 Determination of behavioral responses of *Brevicoryne brassicae* and its parasitoid to *Rosmarinus officinalis* plant volatiles

Three separate experiments were conducted to assess the olfactory response of *B. brassicae* and their parasitoids using a modified Perspex four-arm olfactometer, based on Mutyambai *et al*. (2015).

In the first experiment, two opposite arms of the olfactometer were connected to *B. oleracea* var. acephala and *R. officinalis* plants, while the remaining arms were connected to empty bags as controls. Charcoal-filtered air was pumped at 300 mL/min into the headspace of the test plants, enclosed in heat-sterilized PET bags, and the controls. To prevent contamination, the pots were wrapped in aluminum foil, exposing only the aerial parts of the plants. Air was drawn from the plants to the olfactometer at 100 mL/min using a suction tube, distributing volatiles at 25 mL/min per arm, and then exhausted from the laboratory (Lohonyai *et al*., 2019). Fourteen-day-old *B. brassicae* were first separated in Petri dishes (φ90 x 20mm) and then acclimatized for 1 hour before being individually introduced at the olfactometer center. Similarly, one-day-old female parasitoids were introduced, and their movements were monitored.

In the second experiment, a choice test was conducted to determine the response to plant-derived volatiles and a solvent (dichloromethane) control. Two opposite olfactometer arms held 10 µl aliquots of plant headspace samples from the test plants, while the other two held 10 µl of solvent. Headspace samples were applied to filter papers (4×25mm) using a micropipette (Drummond Scientific, Broomall, USA) and placed at the olfactometer arm inlets. Fourteen-day-old *B. brassicae* and one-day-old gravid female parasitoids were introduced at the olfactometer center using a camel hairbrush. A suction pump connected to the olfactometer facilitated the movement of volatiles to the center at 300 mL/min. The time spent in each arm was recorded using Olfa software (F. Nazzi, Udine, Italy).

In the third experiment, *R. officinalis* and *B. oleracea* var. acephala plants were tied together in a heat-sterilized PET bag, with the opposing arm of the olfactometer holding *B. oleracea* var. acephala. The other arms contained clean air controls, and the procedure from experiment one was repeated.

In all experiments, twelve *B. brassicae* and twelve *A. colemani* (Mutyambai *et al*., 2015; Mutua *et al*., 2024) were observed, each used only once. To avoid positional bias, the olfactometer was rotated every 4 minutes, and each olfactometer was used only once to prevent contamination. Observations lasted 20 minutes for *B. brassicae* and 12 minutes for *A. colemani*. Insects remaining stationary for 2 minutes at any point of the olfactometer were deemed inactive, leading to the rejection of that replicate.

3.5 Characterization of *Brassica oleracea* var. acephala and *Rosmarinus officinalis* constitutive volatile organic compounds

The headspace volatiles from *R. officinalis* and *B. oleracea* var. acephala were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS; 7890A GC and MSD 5975C triple-axis; Agilent Technologies, Palo Alto, CA, USA). The GC-MS was operated in electron impact ionization mode at 70 eV. An HP5-MSI low-bleed capillary column with dimensions of 30 m length × 0.25 mm i.d. × 0.25 μm film thickness (J & W Scientific, Folsom, USA) was used. Helium gas, at a flow rate of 1.2 mL min^{-1}, served as the carrier gas. The oven temperature was initially set at 35°C for 5 minutes, then increased at a rate of 10°C min^{-1} to a final temperature of 280°C, which was maintained for 10 minutes and 30 seconds. The headspace samples were injected into the GC using an autosampler in measured aliquots of 1 µL.

3.6 Coupled gas chromatography-electroantennography

Adult *B. brassicae* were individually transferred from the Perspex rearing cage into a 100 mm x 15mm plastic petri dish with the help of a paint brush. Antennae were prepared by separating the head of ice-chilled *B. brassicae* from the rest of the body using a scalpel. Two silver-silver chloride (Ag-AgCl) borosilicate glass micro electrodes, 2 mm o.d. X 1.16 mm i.d. with an inner filament (INR-II, Syntech, Hilversum, the Netherlands) filled with

Ringer saline solution (7.5 gl-1 sodium chloride, 0.7 gl-1 potassium chloride, 0.2 gl-1 calcium chloride, 0.2 gl-1 magnesium chloride) but without glucose were used for electroantennogram recordings (Zhang *et al*., 2015; Fombong *et al*., 2016; Mutua *et al*., 2024). Using an electrode holder, the head was placed at the indifferent electrode so that the tip of the antenna would make contact with the recording electrode.

The glass tube featured a side hole through which the column effluent was introduced. The splitter applied to this configuration was the glass-lined stainless-steel tubing and the deactivated fused silica tubing. Gas chromatography was used to identify the VOCs to which *B.* b*rassicae's* antenna responded. One µl of the concentrated entrainment sample was injected onto a nonpolar column (HP5-MSI, 30 m × 0.25 mm i.d. × 0.25 µm film thickness), (Agilent Technologies, California, USA) in an HP7890 GC (Agilent Technologies, Palo Alto, USA) equipped with a cool on-column injector and a flame ionization detector (FID). Initially, the oven temperature was programmed at 35°C for 2 min and then at 10°C min^{-1} to 280°C. Hydrogen was used as the carrier gas. Simultaneous recordings of the EAG and FID responses were obtained with specialized software (EAD 2015 version 1.2.6, Syntech, Hilversum, The Netherlands). The EAD outlet contained an uninterrupted airflow filtered through charcoal at a rate of 200mL min^{-1} directed to the *B. brassicae* antenna as described by Fombong *et al*. (2016). A total of six coupled runs were completed. Only FID peaks that corresponded to an EAG peak in at least 3 replicates were considered electro-physiologically active.

3.7 Field trial determination of the effect of intercropping *Brassica oleracea* var. acephala with Rosmarinus officinalis on *Brevicoryne brassicaes'* populations in *Brassica oleracea* var. acephala

3.7.1 Field Study site

On-farm field trial was carried out at Kangari (0° 47' 44.5" S, 36° 52' 52.7" E, 2066.25m above sea level), Murang'a county. The area is located in the highlands of Murang'a and it is characterized by hills and valleys, and the soil type is mainly humic Nitisols (Climate and Assessment, 2023). The topography is also characterised by rivers which play a crucial role in agricultural activities. The area experiences two rainy seasons: the long rain season

(March to May) and the short rain season (October to December) (Government of Kenya, 2016). The average annual rainfall ranges between 1,600mm to 1,800mm per year (Climate and Assessment, 2023). Average daily temperature ranges from 20°C to 25°C and the humidity levels can be as high as 77.43%, especially during the rainy season. The area has ample sunshine during the dry seasons, which is ideal for agricultural activities. It is majorly covered by forests, grass, and shrubs. The major economic activity in Kangari is agriculture, with farmers cultivating a variety of crops such as tea, maize, coffee, beans, and vegetables (Climate & Assessment, 2023).

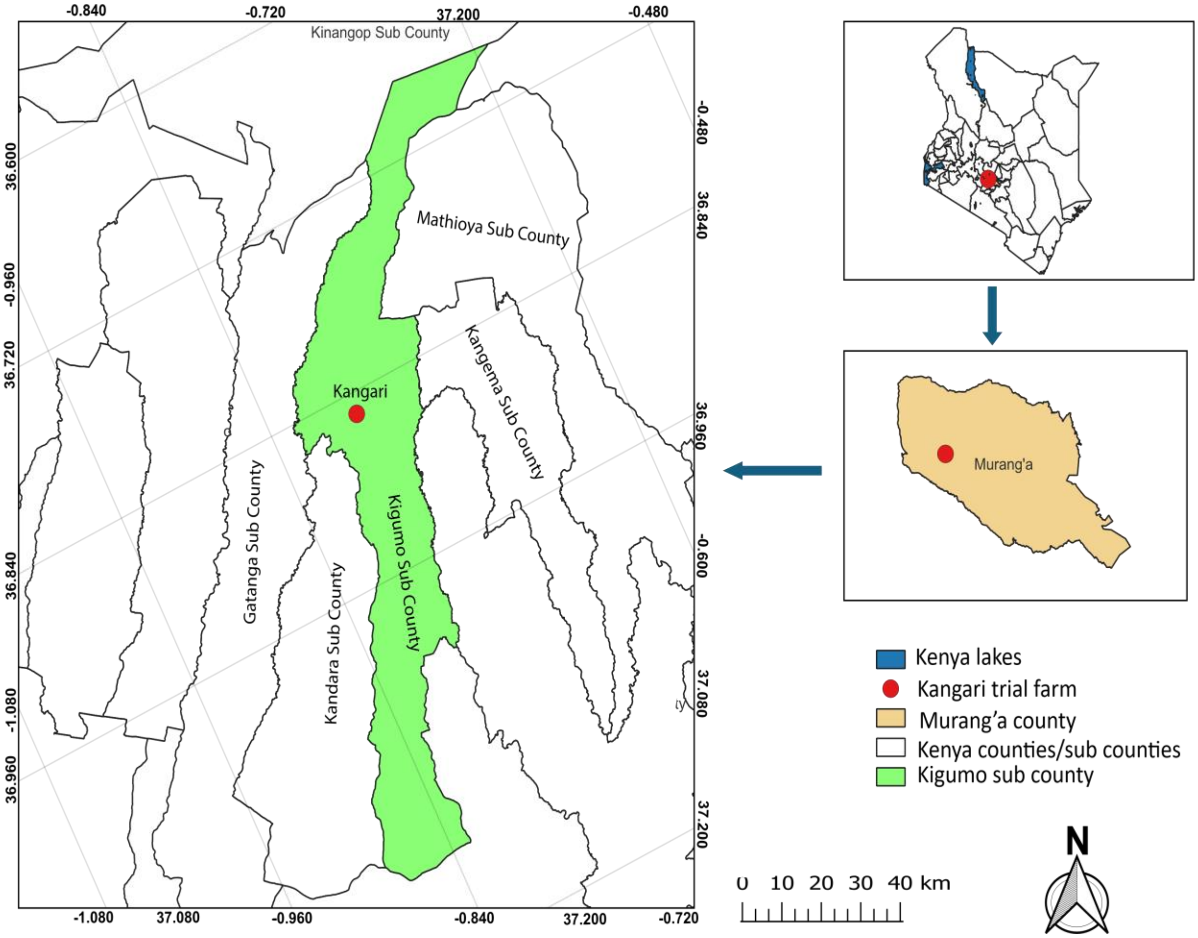

Figure 1: Map of Kenya showing the position of Kangari in Murang'a county, Kigumo sub-county.

3.7.2 Study design

This study was set in a Randomized Complete Block Design (RCBD) experimental setup. The setup included three treatments; *B. oleracea* var. acephala and *R. officinalis* intercrop, *B. oleracea* var. acephala monocrop, and a conventionally treated *B. oleracea* var. acephala control. A local variety of *B. oleracea* var. acephala commonly planted by the farmers (simlaw select) was used. There were three treatments each replicated three times in plot sizes of 3.5 by 3.5 meters. Manure was incorporated into the soil before planting the *Brassica oleracea* var. acephala and *R. officinalis* plots, and *Brassica oleracea* var. acephala monocrop. Top dressing was done after four weeks using compost manure made from Tithonia (*Tithonia diversifolia*) leaves and animal manure. Diammonium phosphate (DAP) was applied in the conventional control two weeks after planting and thereafter, top dressing using Calcium Ammonium Nitrate (CAN). A biopesticide (nimbecidine), was applied in the conventional plots at an interval of 14 days. An alley of 1m was maintained between the plots and the blocks. A spacing of 50 cm was maintained between rows in all the treatments. The plant-to-plant spacing for *B. oleracea* var. acephala and *R. officinalis* was 50cm each. The treatment plots had four rows of *Brassica oleracea* var. acephala with six plants each and three rows of *R. officinalis*. The control plots had seven rows each with six *B. oleracea var. acephala* plants per row. *Rosmarinus officinalis* established in the field three months before transplanting *B. oleracea* var. acephala seedlings at the same spacing as in *B. oleracea* var. acephala. Both the treatments were rainfed and did not receive any manual watering. Both the plots and the alleys were kept weed-free throughout the two seasons.

3.7.3 Abundance of *Brevicoryne brassicae* and its natural enemies

The *B. oleracea* var. acephala plants were checked regularly at two-week intervals for determination of *B. brassicae* and its natural enemies' abundance. The *B. oleracea* var. acephala cropping period lasted for 12 weeks and it was divided into 5 segments of data collection after planting (4,6,8,10,12 weeks). After every two weeks, data on the number of *B. brassicae*, predators and parasitoids were collected starting at the fourth week after planting until the twelfth week. *B. brassicae* population and infestation levels were assessed by counting the number of aphids that occurred singly or in their colonies and estimating their sizes on the upper 10 leaves of each *Brassica oleracea* var. acephala plant in the respective treatments. The total and the average yield per plot of each treatment i.e., intercrop, monoculture, and the positive control was determined by counting and weighing the total edible leaves until the final harvest of the second season. Direct observation was utilized to observe and count the number of mummified aphids in the colonies to determine the parasitism level across all the treatments. The mummies were left on the plants to ensure a subsequent generation of parasitoids.. Other arthropod diversity in each treatment such as the predators was also recorded.

3.8 Data analysis

Data analysis was conducted using R statistical software (R Core Team, 2022) version 4.2.3. The time spent by *B. brassicae* and *A. colemani* in each olfactometer arm was converted into proportions to address the dependence of visiting duration, with log-ratio transformations applied for compositional data analysis (Piepel and Aitchison, 1988; Mutyambai *et al*., 2015). The normality of the data was assessed using the Shapiro-Wilk test. Due to the non-normal distribution of the data for aphid and parasitoid responses (Shapiro-Wilk test: $P < 0.05$), a non-parametric Kruskal-Walli's test was used, followed by the Student-Newman-Keuls (SNK) test for mean separation. P values ≤ 0.001 were considered statistically significant. The time spent by parasitoids in the olfactometer, when exposed to headspace volatiles, was normally distributed (Shapiro-Wilk test: $P < 0.05$); thus, a parametric test ANOVA was used.

Gas Chromatography-Mass Spectrometry data were analyzed using MSD Chemstation software (v F.01.00.1903; Agilent Technologies). Compounds were identified by comparing their mass spectra with authentic standards and mass spectra databases (Adams2, Chemecol, NIST11) and the NIST Chemistry WebBook. Retention indices were determined using a mixture of n-alkanes (C8-C23). Further confirmation was done by co-injection with authentic standards under the same experimental conditions. Volatile organic compounds quantification involved dividing peak areas by known quantities of external standards. The emission rate (ng-1plant-1h-1) was calculated by multiplying the reciprocal of the proportion of total headspace utilized and dividing by the number of sampling hours. Contaminants detected in control samples were disregarded.

Data on the emission of compounds from all the test plants, due to non-normal distribution (Shapiro-Wilk test: $P < 0.05$), were analyzed using the Kruskal-Walli's test followed by Dunn's multiple pairwise comparisons to differentiate means. Volatile organic compounds contributions to plant dissimilarities were visualized using a heatmap.

A generalized linear model was used to compare the abundance of aphids, the abundance of the predators and *A. colemani* across the three treatments at different sampling days post-planting. Data was encoded in Microsoft Office Excel 2010 for calculating means, standard errors, and for graph visualization.

CHAPTER FOUR

4.0 RESULTS

4.1 Behavioral responses of *Brevicoryne brassicae* and its parasitoid to *Brassica oleracea* var. acephala and *Rosmarinus officinalis* plants and their headspace volatiles

It was observed that *B. brassicae* spent significantly more time in the olfactometer arm containing *B. oleracea* var. acephala relative to the arms containing *R. officinalis* or clean air (Kruskal–Wallis χ^2 = 20.38, df = 2, $P < 0.001$, **Fig. 2A**). Additionally, the aphids preferred the arm with *Brassica oleracea* var. acephala odor sources over clean air (Kruskal–Wallis χ^2 = 9.36, df = 1, $P < 0.001$, **Fig. 2A**). The preference for *B. oleracea* var. acephala volatiles was also significant relative to *R. officinalis* volatiles and clean air (Kruskal–Wallis χ^2 = 13.42, df = 2, $P < 0.001$, **Fig. 2B**). However, no significant difference was observed between the time spent in the *Brassica oleracea* var. acephala volatile extracts arm and the clean air arm (Kruskal–Wallis χ2 = 4.56, df = 2, P=0.12), though it was significantly different from the *R. officinalis* arm (Kruskal–Wallis χ^2 = 4.56, df = 2, $P < 0.001$, **Fig. 2B**). The aphids showed a significant preference for the *B. oleracea* var. acephala arm alone relative to the combined plant arm and clean air ($P < 0.001$, **Fig. 2C**), and there was a significant difference between the *B. oleracea* var. acephala arm and the clean air arm ($P = 0.05$, **Fig. 2C**).

Aphidius colemani parasitoids spent significantly less time in the *R. officinalis*-containing olfactometer arm when the whole plants were used , relative to the *B. oleracea* var. acephala and clean air arms (Kruskal–Wallis χ^2= 17.929, df = 2, $P < 0.001$, **Fig. 3A**).In the experiment with headspace volatiles of *R. officinalis* and *B. oleracea* var acephala, the time spent by *A. colemani* in all olfactometer arms was not statistically different (Kruskal–Wallis χ^2 =, df = 2, $P = 0.124$, **Fig. 3B**). When both plants were combined, *A. colemani* spent significantly more time in the *B. oleracea* var. acephala arm alone compared to the combined plant's arm and the clean air arm (Kruskal–Wallis χ^2 =22.66, df =2, $P < 0.001$, **Fig. 3C**). There was no significant difference between the time spent in the olfactometer arm with both *R. officinalis* and *B. oleracea* var acephala plants, relative to the clean air arm (Kruskal–Wallis χ^2 = 15.87, df= 1, $P = 0.271$, **Fig. 3C**).

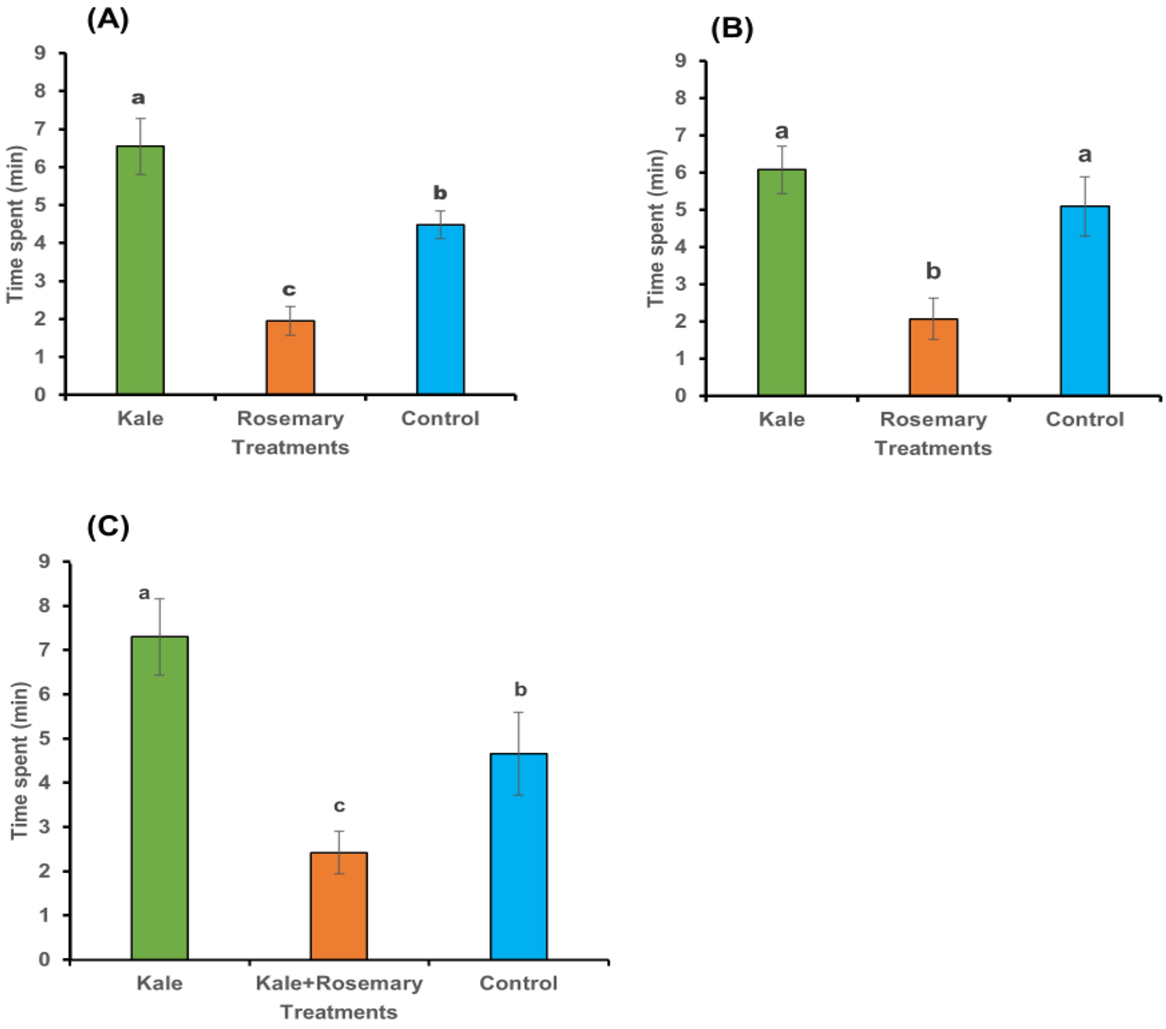

Figure 2 (A,B,C): Behavioral response of *Brevicoryne brassicae* to naturally emitted constitutive volatiles from *Brassica oleracea* var. acephala and *Rosmarinus officinalis* plants **(A)** their headspace volatiles **(B)** and a combination of the two plants tied together (C) in a four-arm olfactometer. Each *Brevicoryne brassicae* was observed for 20 min (N=12). Means (± SE) with different letters above the bars are significantly different at *P*<0.

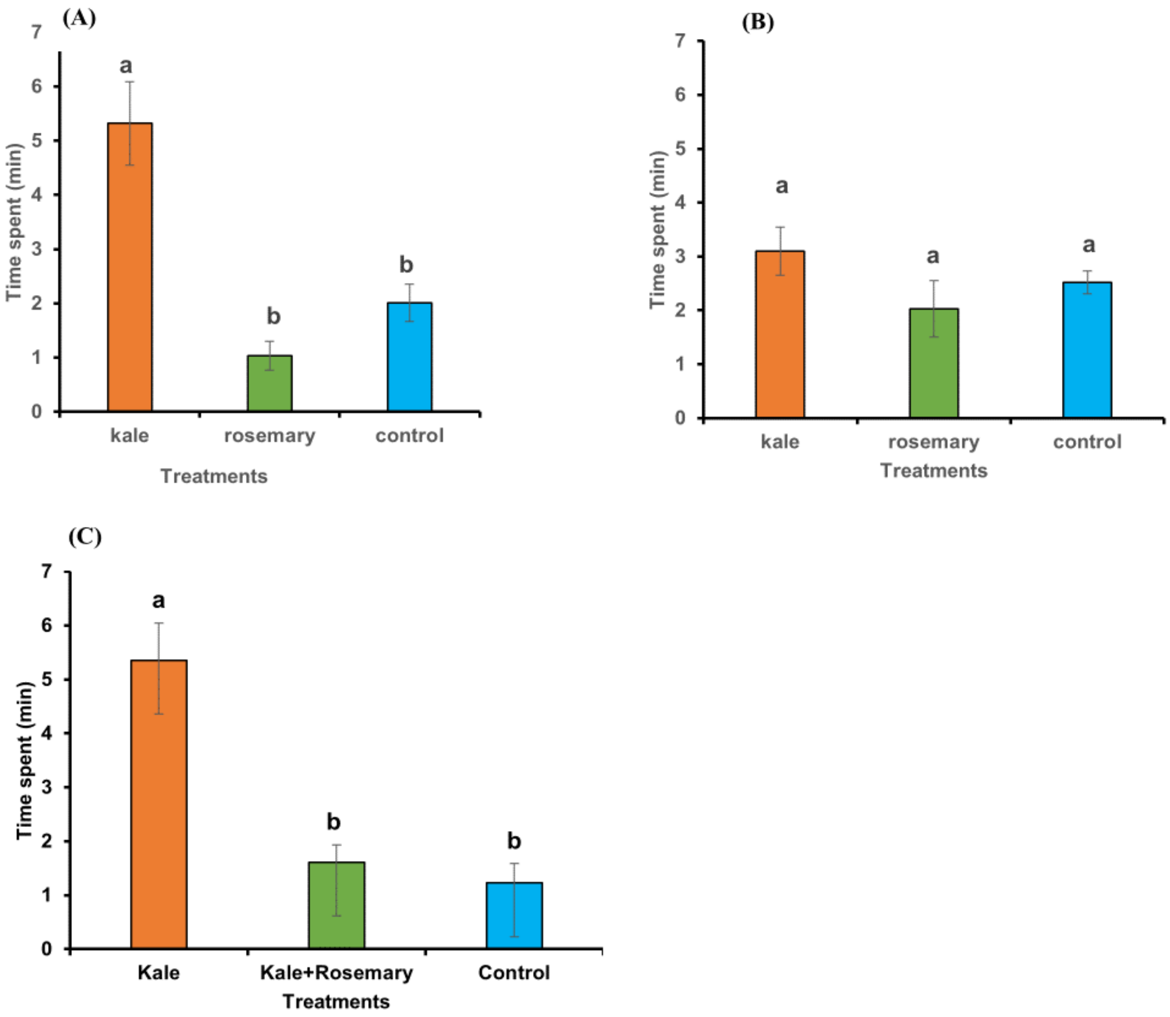

Figure 3 (A,B,C): Behavioral responses of *Aphidius colemani* to naturally emitted constitutive volatiles from *Brassica oleracea* var. acephala and *Rosmarinus officinalis* plants **(A)**, their headspace volatiles **(B),** and a combination of the two plants **(C)** in a four-arm olfactometer. Each parasitoid was observed for 12 min (N=12). Means (± SE) with different letters above the bars are significantly different at *P<0.05*

4.2 Volatile profiles from *Brassica oleracea* var. acephala and *Rosmarinus officinalis* plants

Gas Chromatography-Mass Spectrometry analysis identified 20 major compounds *R. officinalis* and 9 in *B. oleracea* var acephala plants' headspace samples, categorized into three chemical classes: monoterpenes (17), ketones (1), and sesquiterpenes (4) (**Table 1, Figures 4 and 5**). Both *R. officinalis* and *B. oleracea* var acephala plants shared common volatiles such as α-Pinene, β-Pinene, myrcene, 1,8-Cineole, γ-Terpinene, camphor, and β-Caryophyllene. *Rosmarinus officinalis* notably produced significantly higher amounts of these compounds compared to *B. oleracea* var. acephala, specifically producing 57, 61, 6, 36, 10, 106, and 274 times more α-Pinene, β-Pinene, myrcene, 1,8-Cineole, γ-Terpinene, camphor, and β-Caryophyllene, respectively ($P< 0.001$, **Table 1**). Volatile organic compounds found in *R. officinalis* but not in *B. oleracea* var. acephala included camphene, α-Phellandrene, δ-2-carene, (Z)-Sabinene hydrate, linalool, borneol, α-Terpineol, verbenone, citronellol, geraniol, bornyl acetate, α-Humulene, and caryophyllene oxide. Conversely, compounds present in *B. oleracea* var. acephala but absent in *R. officinalis* were sabinene and limonene (**Table 1**). Heatmap clustering revealed that volatiles from *R. officinalis* were more concentrated than those from *B. oleracea* var. acephala. It also highlighted that 1,8-Cineole, β-Pinene, myrcene, and sabinene were the most abundant volatiles in *B. oleracea* var. acephala, while γ-Terpinene, camphor, limonene, and α-Pinene were the least abundant. Additionally, 1,8-Cineole was the most abundant volatile in both *B. oleracea* var. acephala and *R. officinalis*, while α-Pinene, β-Caryophyllene, camphor, bornyl acetate, and verbenone were the most abundant VOCs in *R. officinalis* (**Figure 4**).

Table 1: **Average concentration (ng/plant/h) of volatile organic compounds detected in the headspace samples collected from *Brassica oleracea* var. acephala and *Rosmarinus officinalis* plants (n=4)**

No	RT (min)	Compound Name[1]	RI_{alk}[2]	RI_{L}[3]	*Brassica oleracea* var. acephala	*Rosmarinus officinalis*	*P*-value[4]
1	9.74	*α*-pinene*	931	934	410.54±159.48^{b}	23,465.15± 4393.73^{a}	0.002
2	10.03	Camphene	945	944	nd	5,914.963± 607.05	-
3	10.55	Sabinene	969	974	1,274.64± 746.55	nd	-
4	10.61	*β*-Pinene*	972	978	87.00± 50.44^{b}	5,379.80± 496.49^{a}	<0.001
5	10.93	Myrcene*	987	981	953.28± 527.07^{b}	5,983.69± 831.85^{a}	0.002
6	11.17	*α*-Phellandrene	998	1005	nd	1,429.39± 253.42	-
7	11.39	*δ*-2- Carene	1011	1011	nd	1,555.28± 289.47	-
8	11.65	Limonene*	1026	1030	1,457.36± 854.58	nd	-
9	11.79	1,8-Cineole	1032	1036	1,232.56± 622.18^{b}	40,197.45±14913.86^{a}	0.009
10	12.29	*γ*-Terpinene*	1061	1060	381.39±359.18^{b}	3,907.73±632.33^{a}	< 0.001
11	12.44	*(Z)*-Sabinene hydrate	1069	1092	nd	3,955.11± 1072.60	-
12	12.92	Linalool*	1096	1101	nd	7,470.31± 2507.75	-
13	13.73	Camphor	1146	1146	118.25± 53.30^{b}	12,642.43±3081.30^{a}	0.007
14	14.11	Borneol	1167	1167	nd	9,645.39± 1169.14	-
15	14.66	*α*-Terpineol*	1204	1189	nd	3,666.03± 1261.86	-
16	14.85	Verbenone*	1218	1209	nd	11,939.37± 2333.98	-
17	15.00	Citronellol*	1228	1230	nd	1,143.08±347.97	-
18	15.45	Geraniol*	1259	1253	nd	4,401.97±1092.20	-
19	15.90	Bornyl acetate	1290	1295	nd	12,775.81±2801.34	-
20	17.79	*β*-Caryophyllene	1428	1430	69.90±26.39^{b}	19,141.41±3947.36^{a}	< 0.001
21	18.17	*α*-Humulene	1462	1465	nd	4,706.05±1147.31	-
22	19.77	Caryophyllene oxide*	1593	1588	nd	2,971.13±491.12	-

1. * Indicates compound confirmed with authentic standards.
2. Means (± SE) with different superscript letter(s) within the rows are significantly different at the *P* < 0.05 level.
3. "nd" indicates not detected

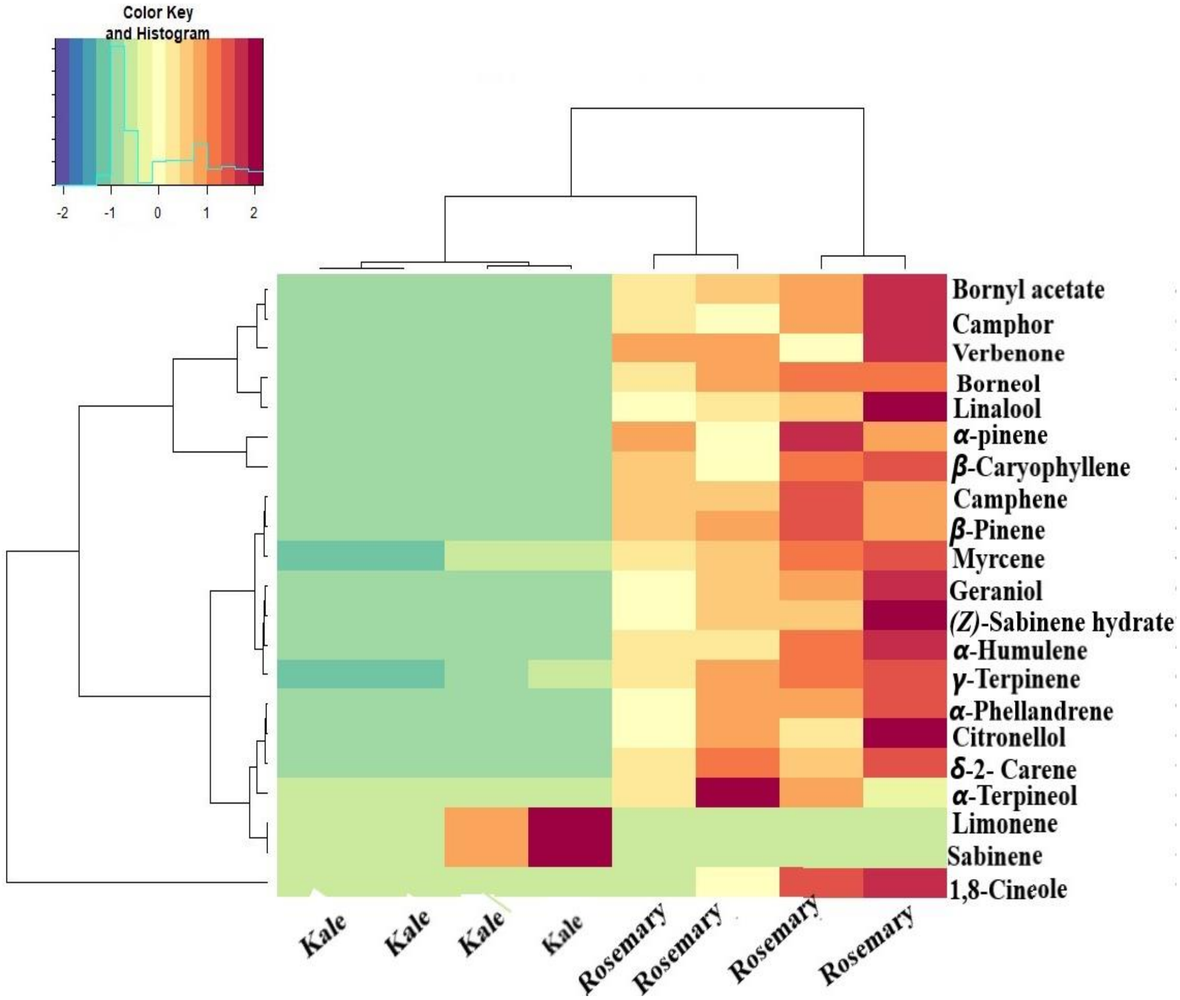

Figure 4: Heatmap clustering showing the abundance (in decreasing color intensity) of volatile organic compounds across replicates of *Brassica oleracea* var. acephala and *Rosmarinus officinalis* plants as per the colour key.

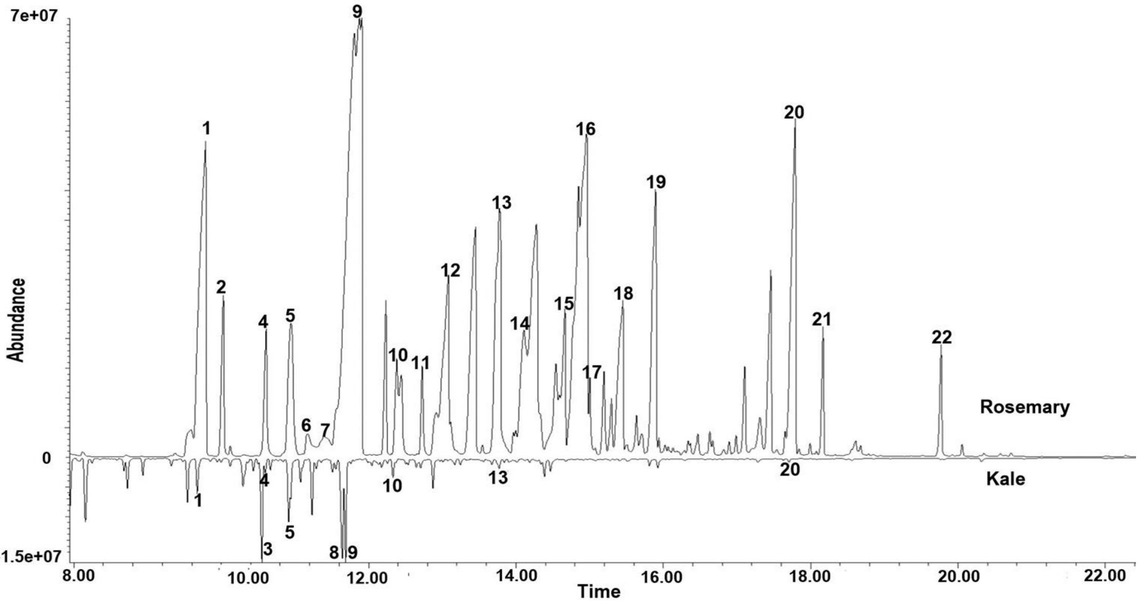

Figure 5: Representative gas chromatography-mass spectroscopy chromatogram of *Brassica oleracea* var. acephala and *Rosmarinus officinalis* plants. Identities of labeled peaks are presented in **Table 1**.

4.3 Gas chromatography-electroantennographic responses of *Brevicoryne* b*rassicae* to *Rosmarinus officinalis* and *Brassica oleracea* var. acephala headspace volatiles

The GC-EAD recordings showed that *B. brassicae* elicited an antennal response to three compounds from *B. oleracea* var. acephala namely sabinene, *γ*-terpinene and *β*-caryophyllene (**Fig. 6A**), and six active compounds from *R. officinalis* namely linalool (12), camphor (13), borneol (14), *α*-terpineol (15), verbenone (16) and geraniol (18) (**Fig. 6B** and **C**)

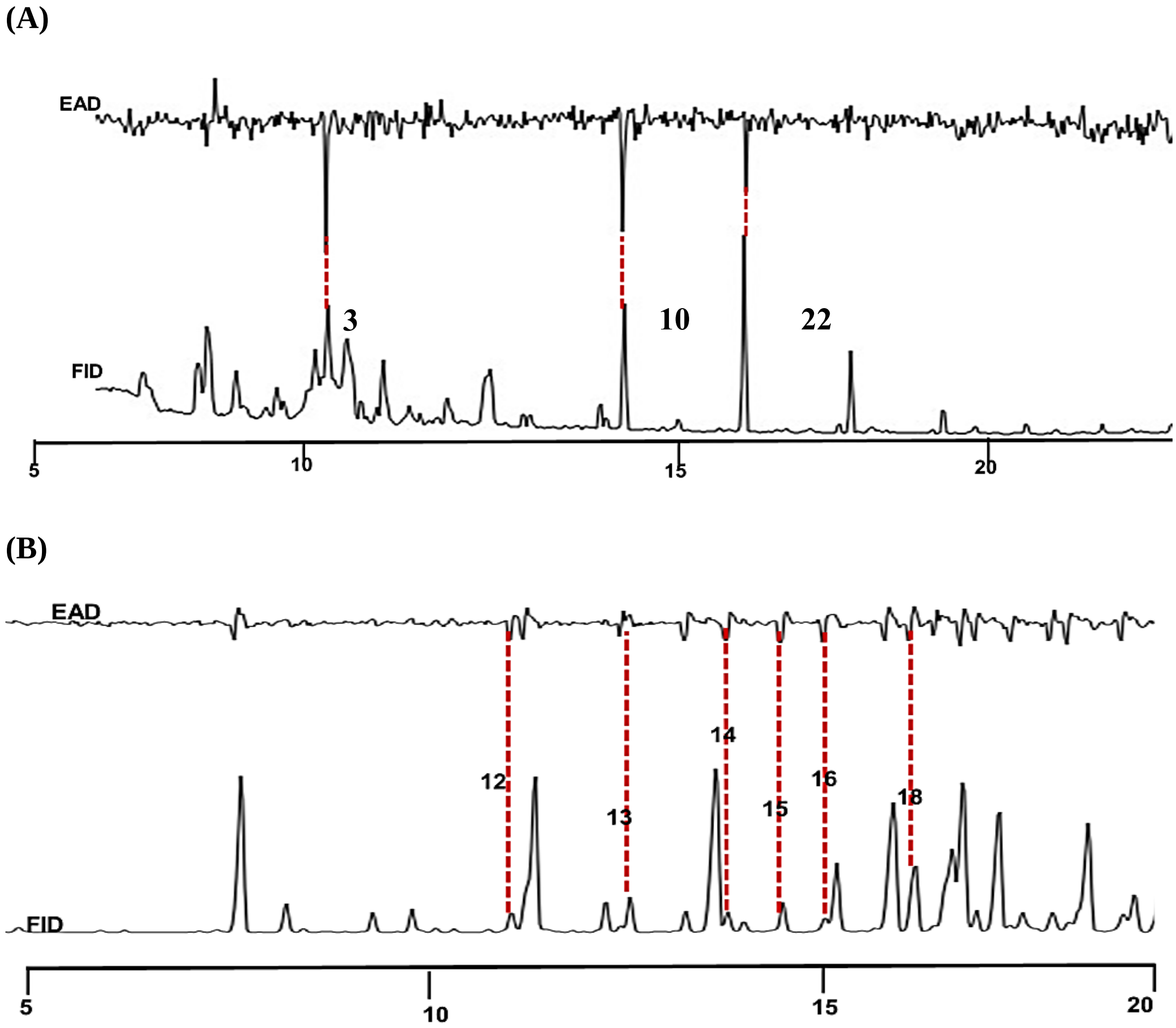

Figure 6 (A, B): Gas chromatography-electroantennography active compounds from *Brassica oleracea* var. acephala **(A)** and *Rosmarinus officinalis* **(B)** plant volatiles to *Brevicoryne brassicae* antenna. Identities of labeled peaks are represented in **Table 1.**

4.4 Effects of intercropping *Brassica oleracea* var. acephala with *Rosmarinus officinalis* on the abundance of *Brevicoryne brassicae* and its natural enemies

4.4.1 Abundance of *Brevicoryne brassicae*

Intercropping *R. officinalis* with *B. oleracea* var. acephala significantly reduced the number of *B. brassicae* across different weeks after sowing, compared to conventional and the monocrop controls (χ^2=50.57, df= 2, *P*<0.001) in the first cropping season. Suppression of *B. brassicae* was more pronounced from the 8th to 12th week (χ^2=33.59, df =2, *P*<0.001). The 6th week recorded the highest number of aphids in both the *R. officinalis* intercrop and the monocrop(χ^2=30.42, df=8, P<0.001). Additionally, in the first cropping season, the number of *B. brassicae* drastically reduced after the application of a biopesticide (nimbecidine) in the conventional plots, after which the numbers remained low across all the observation weeks (P<0.001). The number of *B. brassicae* in the conventionally treated plots was initially the same as the monocrop at the beginning of the first season (χ^2=1.64, df=1, P=0.20), after which it went down due to the effect caused by nimbecidine (χ^2=21.91, df=1, P<0.001) (**Fig 7**). However, the numbers did not differ in the 4th and the 8th week (χ^2=0.14, df=1, P=0.71). The number of aphids observed in the 6th week in both the *R. officinalis* intercrop and the conventional control did not significantly differ (χ^2=3.02, df=1, P=0.08). *Brevicoryne brassicae* were higher in the monocrop throughout the 10th and the 12th week respectively, as opposed to the other treatments (χ^2= 19.59, df=2, P<0.001, χ^2=13.15, df=2, P<0.001). In the second cropping season, *B. brassicae* in *R. officinalis* and *B. oleracea* var. acephala intercrop remained significantly lower across the whole cropping season compared to monocrop and conventional control (χ2=17.89, df=2, P<0.001). The number of aphids in the monocrop and the conventionally treated plots differed from the first season only in the sixth week, but not in the subsequent three sampling weeks .

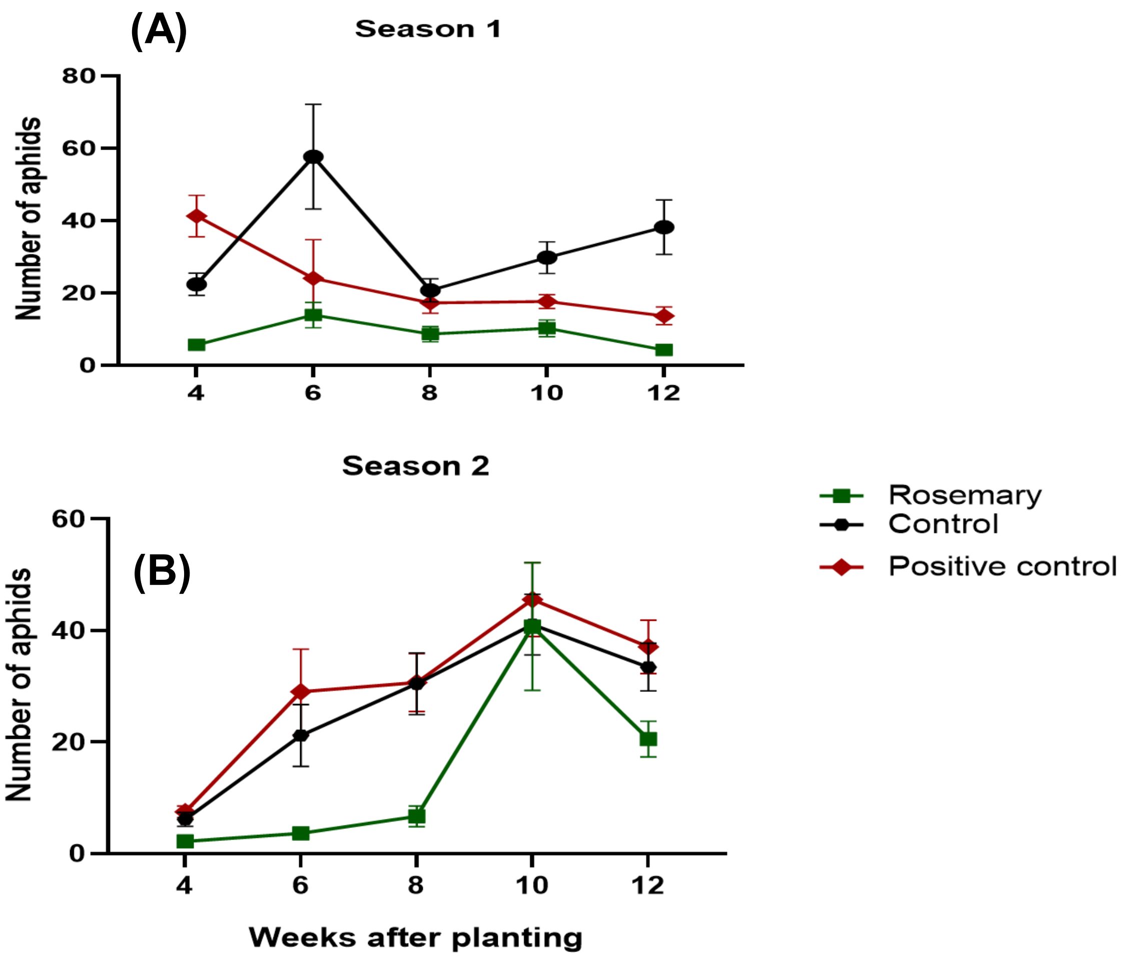

Figure 7 A,B: Mean number of *Brevicoryne brassicae* per plant under different cropping systems in two cropping seasons. Season 1; March to June 2023 (**A**) and season 2; October to January 2024 (**B**).

4.4.2 Abundance of natural enemies

The number of live *A. colemani* was slightly significant in the first cropping season (χ^2=5.80, df = 2, *P*=0.05) with the highest being recorded in *R. officinalis* intercrop, but the numbers did not differ in the second cropping season (χ^2=2.58, df = 2, *P*=0.27). The highest number of spiders (predators) in the first season were recorded in the monocrop control, which was significantly different from the other treatments (χ^2=7.94, df = 2, *P*<0.05*)*. Furthermore, there was no difference in the number of spiders in the three treatments in the second cropping season (χ^2=2.41, df = 2, *P*=0.30*)*. Percentage parasitism was highest in *R. officinalis* intercrop in the two cropping seasons whereby, in the first cropping system, it recorded 13.6% parasitism which was high compared to 2.0% of monocrop control and 3.6% of the conventional control (χ^2=11.62, df = 2, P<0.05). In the second season, *R. officinalis* intercrop recorded 1.3% parasitism which was higher than 1.1% of monocrop control and 0.6% of the conventional control although they were not significantly different (χ^2=0.85, df = 2, P=0.65).

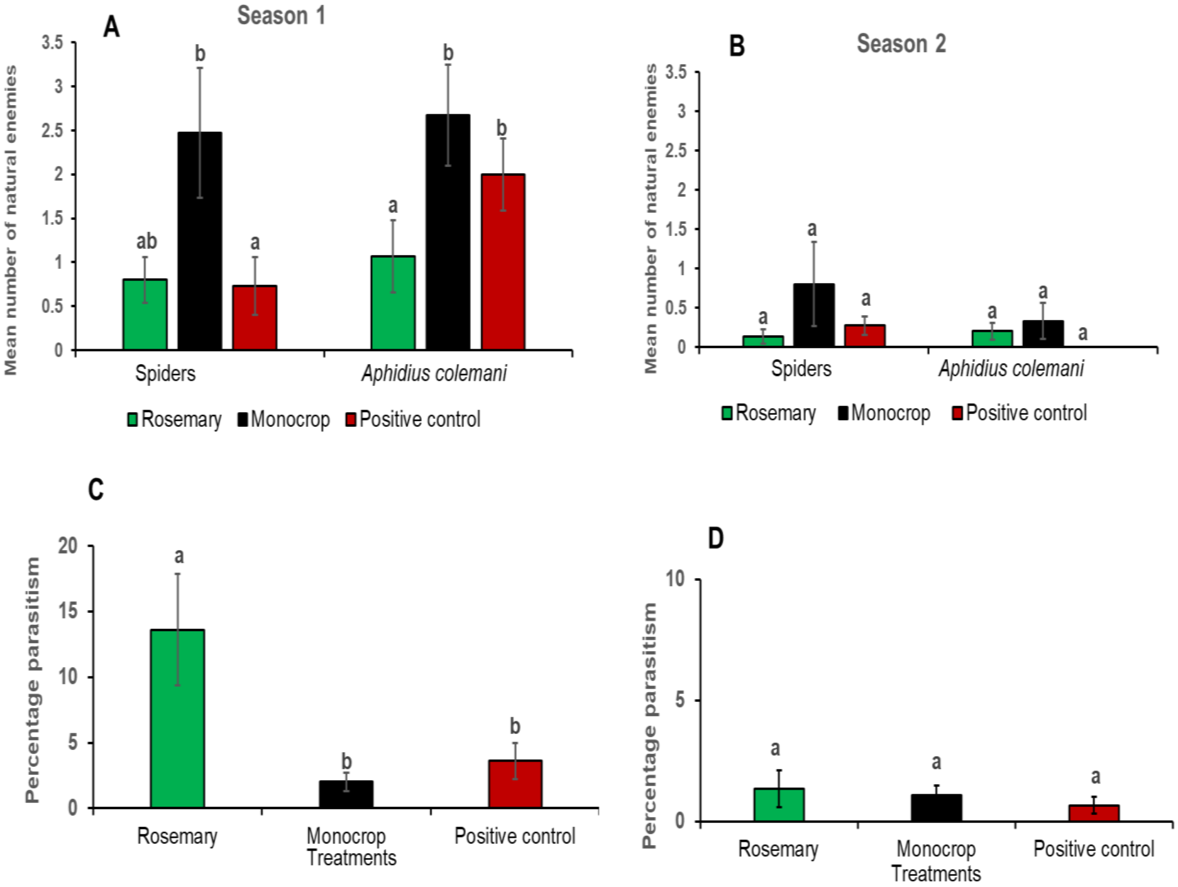

Figure 8 (A,B,C,D): The Mean number of natural enemies (Spiders and *Aphidius colemani* (**A** and **B**)) and percentage parasitism per treatment (**C** and **D**) in two cropping seasons: Season 1 (March to June 2023) and season 2 (October 2023 to January 2024).

CHAPTER FIVE

5.0 DISCUSSION

Insects rely on olfaction and visual cues to find suitable hosts and navigate their environment (Zhang and Chen, 2015) . Conversely, they tend to avoid plants emitting VOCs that are unfavorable for their survival as observed when desmodium was used to control stemborer moth (Khan *et al.*, 2000). Other plants produce a blend of VOCs as a defense against herbivory or in response to biotic stress (Mutyambai *et al*., 2019; Piesik *et al.*, 2016). These VOCs tend to be highly concentrated than the naturally emitted ones thereby disrupting the settling, feeding, and oviposition of the target pest (Ben-Issa *et al*., 2017). The findings of the current study showed that the constitutive VOCs from the *B. oleracea* var. acephala plant attracted *B. brassicae*, as opposed to control treatments (air and solvent) and displayed a reduced preference for *R. officinalis* as a whole as well as its volatiles. The findings of the current study collaborate with those of Cai *et al.* (2018), who investigated the response of *Myzus persicae* to *R. officinalis* volatiles and clean air and found out that the oduor of *R. officinalis* repelled the *B. brassicae* at different doses except when the weight of *R. officinalis* was reduced to 0.5 grams. The current findings are also supported by a study that investigated the orientation of *M. persicae* in the presence of different *R. officinalis* species in enclosed chambers and reported that the aphids preferred the control chamber over the ones containing *R. officinalis*, which emitted VOCs in relatively higher amounts (Dardouri *et al*., 2019). When both *R. officinalis* and *B. oleracea* var. acephala plants were tied together, *B. brassicae* showed more preference for the arm containing *B. oleracea* var. acephala alone. This is an indication that *R. officinalis* volatiles could mask those from *B. oleracea* var. acephala thereby making it difficult for *B. brassicae* to perceive. Similar to this observation, the *R. officinalis* plant has demonstrated its ability to mask the host plant attractive volatiles (Zhang and Chen, 2015), given its distinct aroma and previously demonstrated repellent properties.

Aphidius colemani is a parasitic wasp that is commonly used as a biological control agent against different species of aphids in horticultural settings (Douloumpaka and Van Emden, 2003). They parasitize different species of aphids thereby indirectly controlling the population of aphids in agro-ecosystem. Olfactometry bioassay with the *R. officinalis* and *B. oleracea* var. acephala plants indicated that *A. colemani* showed less preference for the olfactometer arm containing the *R. officinalis* plant than the arm-holding *B. oleracea* var. acephala plant. Furthermore, olfactometer bioassay with individual plants' headspace volatiles showed that *A. colemani* preferred volatiles from *B. oleracea* var. acephala to those from *R. officinalis*. This indicates that *A. colemani* was more attracted to volatiles emitted by *B. oleracea* var. acephala compared to *R. officinalis*. However, the time spent in the control arm containing air did not differ from that spent in the *R. officinalis* arm. These findings suggest that *R. officinalis* being a non-host plant of *B. brassicae* may have a repellent impact on *A. colemani*. Given that *A. colemani* is a generalist parasitoid, its host location involves the perception of multiple host-plant odors (Ameixa and Kindlmann, 2012). Interestingly, an olfactometer bioassay involving a combination of *B. oleracea* var. acephala and *R. officinalis* in one arm and *B. oleracea* var. acephala in the other arm, showed that *A. colemani* was more attracted to *B. oleracea* var. acephala than the combination of the two plants. Quantitatively higher amounts of volatiles emitted by *R. officinalis* could surpass low attractive volatiles emitted by *B. oleracea* var. acephala. Thus, the strength of repulsive and attractive headspace volatiles could explain this phenomenon. Electrophysiological responses of *A. colemani* to volatiles from each plant could provide a better understanding of the volatiles responsible for the behavioral response of the parasitic wasp.

Brevicoryne brassicae use their antenna's sensilla to detect plant volatiles to locate their suitable hosts in search of food resources (Mutyambai *et al.*, 2015). By using these antennae, the GC-EAD makes it possible to identify which volatiles and their blends cause the insect's behavior. The results of the current study show that *B. brassicae* antenna was able to detect sabinene, γ-terpinene and β-caryophyllene from *B. oleracea* var. acephala. One of the major constituents of *B. oleracea* var. acephala was Sabinene, which was absent in *R. officinalis*. Additionally, *B. brassicae* antenna did not detect γ-terpinene and β-caryophyllene when *R. officinalis* volatiles were used, despite these two compounds being present in both plants. However, *B.brassicae* antenna demonstrated antennal detection of linalool, camphor, borneol, α-terpineol, verbenone, and geraniol from *R. officinalis*.

Among the *R. officinalis* VOCs that elicited antennal detection, camphor, and α-terpineol have been reported to reduce the activities of *M. persicae* and other insects such as mosquitoes (Dardouri *et al*., 2019). The insect's antenna did not detect 1,8-cineole despite it being a major constituent of *R. officinalis* oil. However, some studies have reported its insecticidal activity against onion aphid, *Neotoxoptera formosana* (Takahashi) (Hemiptera: Aphididae) (Hori, 1998; Elhalawany *et al*., 2019). Camphor, citronellal, and geraniol have also been reported to have high insecticidal activity against *Aphis fabae* by disrupting their digestive and neurological enzymes hence leading to death (Chalise and Dawadi, 2019). Therefore, the presence of these compounds in the volatiles emitted by *R. officinalis* could have contributed to the observed behavior exhibited by *B. brassicae*.

Chemical analysis of headspace volatiles showed that *R. officinalis* produced more terpenes as compared to *B. oleracea* var. acephala plant. The most abundant VOCs in *R. officinalis* included 1,8-cineole, camphor, verbenone, bornyl acetate, linalool, and citronellol. The majority of these compounds have been associated with repellence properties against different insect species when used as plant extracts and essential oils (Miresmailli and Isman, 2006; Cloyd *et al*., 2009; Webster, 2009; Dayaram and Khan, 2016). Comparable results on *R. officinalis* essential oils were reported by Elhalawany *et al.* (2019), who observed that the major constituents of *R. officinalis* oil were mostly made of linalool, *α*-pinene, limonene, bornyl acetate, and β-caryophyllene. In another study, verbenone, 1-8 cineole, and linalool were found to be the major constituents of *R. officinalis* volatiles and their oil extracts (Hori, 1998), which aligns with the quantities of these compounds observed in the study in which 1,8-cineole was produced more than 200 folds the amount produced by *B. oleracea* var. acephala. *R. officinalis* emits a higher concentration of volatiles as compared to *B. oleracea* var. acephala, which can explain the basis of its characteristic aroma. However, the quantity of production of these compounds is key as a higher abundance of the specifically active compound translates to a more discrete response (Bruce *et al*., 2005). Additionally, some compounds may be produced at low rates but are the most influential to the behavior of the insect (Dardouri *et al*., 2019). The high abundance of these major compounds is evidence that *R. officinalis* being an aromatic herb produces such compounds in very high amounts, which the insect can perceive from afar and avoid while masking the host plant volatiles (Zhang and Chen, 2015). This is also the basis of intercropping *R. officinalis* with other crops of economic importance to control different insect pests. The number of VOCs produced by *B. oleracea* var. acephala across was relatively lower in concentration. This underscores the fact that *B. oleracea* var. acephala is not as aromatic as *R. officinalis* and therefore its volatiles are released in low quantities. Different chemical concentrations in *R. officinalis* demonstrate the need for assessment of optimal chemical concentration that offers maximum protection of *B. oleracea* var. acephala against *B. brassicae*.

Field experiments demonstrated that intercropping *B. oleracea* var. acephala with *R. officinalis* provided vegetation diversification for the host location. Consistently, throughout the cropping seasons, *R. officinalis* and *B. oleracea* var. acephala intercrops were observed to have lower *B. brassicae* populations as relative to the monocrop and the positive control treatments. This was possibly due to the strong oduor emitted by *R. officinalis* which affected *B. brassicae* thereby protecting *B. oleracea* var. acephala from attack. This is supported by the results reported by Zhang and Chen (2015) whereby *R. officinalis* showed the strongest repellence against the tea green leafhopper, *Empoasca vitis* from the lowest to the highest concentration compared to other essential oils used. The lack of a oduor to deter aphids from settling and colonization could likely explain the higher number of *B. brassicae* in monocrops as observed in the study. Additionally, considering that aphid species are more guided by olfaction and visual cues (Döring, 2014), the dense green canopy formed by the *B. oleracea* var. acephala in the monocrop control may have provided an ideal place, due to its visibility and attractiveness for settlement without any odor alteration or chemical treatment. Moreover, fertilization led to high soil nitrogen concentrations in the soil leading to more attacks by *B. brassicae*, as observed in intercropped fields by Van Emden and Harrington (2007). Intercropping mustard with different spices was found to be an effective way of suppressing aphids, except in the sole crop (Noman *et al*., 2013). The high numbers of *B. brassicae* in the sixth week may be explained by the tender developmental stage of *B. oleracea* var. acephala plants, which formed an ideal host for colonization since the plants were soft and easy to pierce and a subsequent volatile production as a defense mechanism. After six weeks of growth, the numbers remained the same as those in *R. officinalis* intercrop throughout the cropping season, which may suggest that *R. officinalis* is an effective biocontrol push plant for intercropping for the management of *B. brassica*e.

Despite the equal number of *B. brassicae* in the conventionally treated *R. officinalis* intercrop plots, the monocrop *B. oleracea* var. acephala maintained a higher number of *B. brassicae*. In the second season, *R. officinalis* showed the same trend observed in the first cropping season explained by the presence of a less attractive oduor. Unlike the first cropping season, the numbers observed in the first observation period were all different since minimal settling occurred when the crop was still young. After the sixth week of the second cropping season, the number of *B. brassicae* in the conventional plots did not reduce as expected. This could be explained by the fact that the *B. brassicae* antenna sensory mechanism became used to the biopesticide and may have developed resistant traits against the biopesticide (Ahmad and Aslam, 2005; Mutiga *et al*., 2010). Another reason could be due to the soft and succulent leaves of the plants in this treatment, which made it favorable for *B. brassicae* to colonise and formed a good canopy for some *B. brassicae* to hide such that the biopesticide did not get into contact with them, ensuring continuity of their reproduction (Mutiga *et al*., 2010).

The survival and parasitizing of *A. colemani* were notably high in *R. officinalis* intercrop compared to the other treatments throughout the two cropping seasons. However, the number of *A. colemani* and the mummified aphids reduced during the second season. This could be attributed to the fact that in the first season, the plants were still young and the volatiles emitted were not as much as those in the second season. The high emission of the VOCs from *R. officinalis* in the second season reduced the number of *B. brassicae* in this intercrop, which in turn reduced the number of *A. colemani* as a result of their reduced prey. Moreover, changes in weather and other environmental factors could have contributed to the reduction in the numbers of *A. colemani,* given the fact that the second season was planted during heavy rains which could have washed away some mummified aphids. Despite this reduction, the percentage of parasitism remained high in *R. officinalis* intercrop, an indication that the few *B. brassicae* available were parasitized, further reducing the numbers. This result contradicts those of Boivin *et al*. (2012) who explained that parasitism is influenced by the quality and size of the colony hence small colonies lead to fewer parasitoids, which was not the case in the *R. officinalis* intercrop.

Predators such as spiders, which feed on *B. brassicae* were more in the monocrop control throughout the first and the second cropping seasons. The increased number of predators in the monocrop could have been due to prey abundance as reported by Finch and Kienegger (1997). The presence of non-host oduors in *R. officinalis*-*B. oleracea* var. acephala intercrop may have influenced the presence of spiders, which also rely on olfaction to locate their host (Piesik *et al*., 2016). Although the conventionally treated plots had many *B. brassicae*, the numbers of the spiders remained low due to the interference caused by the biopesticide applied which could have killed the predators or caused migration in search of a habitable host (Bale *et al*., 2008; Peris and Kiptoo, 2017). The presence of many predators in monocrop control is an indicator that *R. officinalis* intercrop greatly influenced the settlement and feeding of these predators due to odor alteration. More studies should be conducted to assess potential intercrops with higher predator influence that can extend to even monocrop.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion of the study

Based on the findings of this study**,** the following conclusions were made.

1. *Rosmarinus officinalis* has a characteristic odor that can repel *B. brassicae* and which has a masking effect over *B. oleracea* var. acephala VOCs, making it difficult for the pest to perceive the host plant VOCs.
2. *Rosmarinus officinalis* emits many volatiles compared to *B. oleracea* var. acephala. Additionally, the volatiles emitted by *R. officinalis* are more highly concentrated than the ones in *B. oleracea* var. acephala. The two plants only share a few common volatiles.
3. The repellency of *R. officinalis* is attributed to the production of distinct VOCs, which elicit antennal detection when the insect is exposed to them.
4. Intercropping *R. officinalis* with *B. oleracea* var. acephala leads to a reduction in the number of *B. brassicae* and natural enemies. The efficacy of *R. officinalis* in field settings however is prone to the influence of environmental factors and interaction with other organisms such as the parasitoids.

6.2 Recommendations

1. Behavioral responses with the available synthetic standards are required to validate the results obtained using the volatiles from *R. officinalis*.
2. Electrophysiological responses of *A. colemani* are required to determine the concentrations of *R. officinalis* volatiles that can be detected by its antenna.
3. As much as *R. officinalis* may offer benefits in controlling aphids, its activity on the non-target organisms such as the parasitoids and the predators should be carefully evaluated to determine the optimal stage that is favorable for their survival and minimize its effects on these organisms and the ecosystem at large.
4. Long term studies are required to establish which cultivars of *R. officinalis* work best in aphids' control, as well as the pattern of planting that can offer maximum

protection i.e. how many rows of *R. officinalis* can offer maximum protection to a given number of rows of *B. oleracea* var. acephala.

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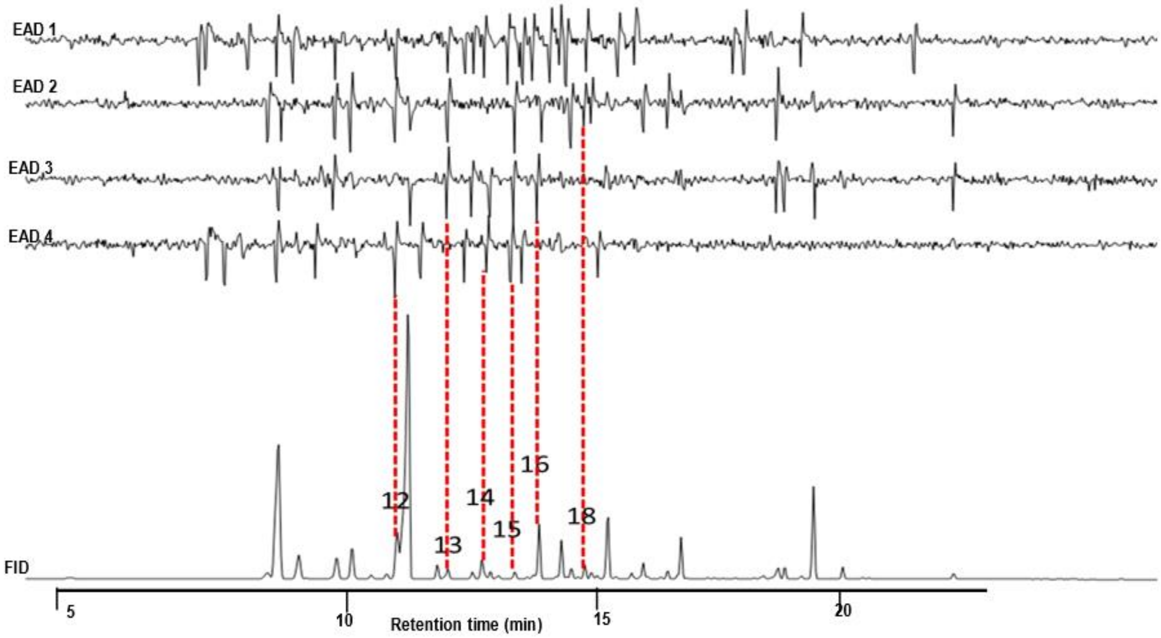
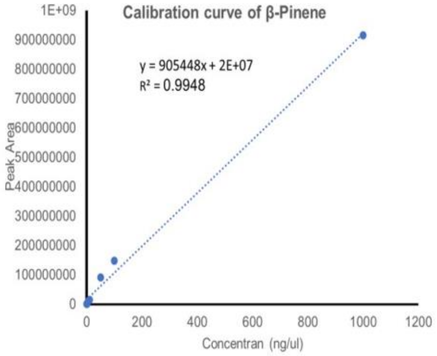
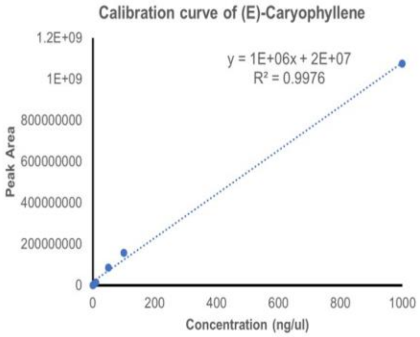
APPENDICES

Appendix i: Representative EAD responses of cabbage aphid antenna to *R. officinalis* volatiles. A compound was considered electrophysiologically active if the antenna detected it in 3 or more replicates. 20 replicates were carried out in order to come with a conclusive EAD response**.**

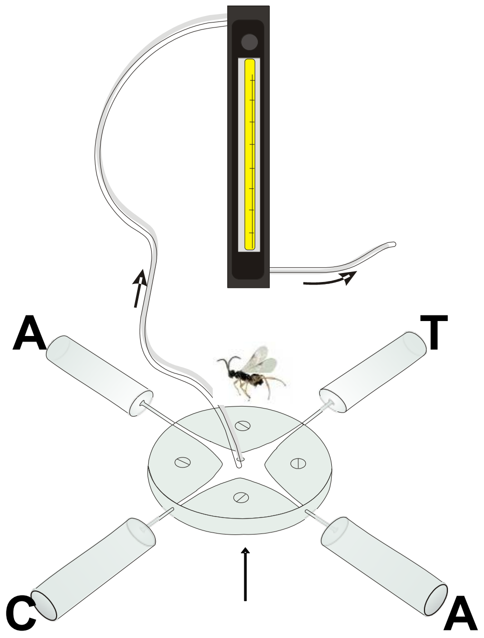


Appendix ii: Calibration curves for the standards of β-Pinene and *β*-Caryophyllene for quantification, as a representative of the chemical classes. The curve covers a concentration range of 1-1000 µg/mL.

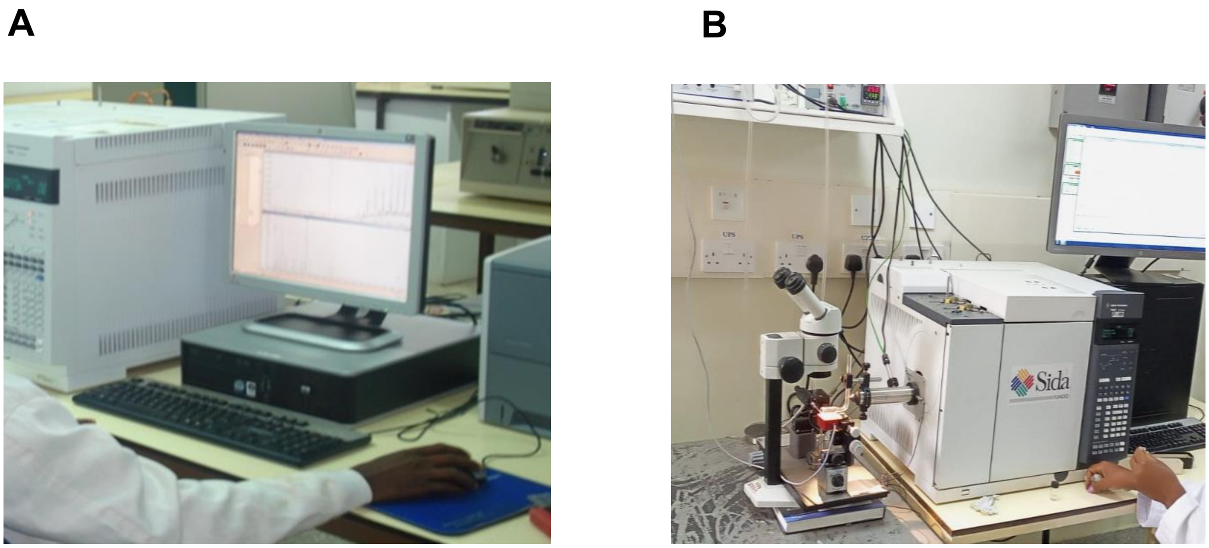




Appendix iii: Perspex four arm olfactometer used for behavioural responses of cabbage aphid and its parasitoid

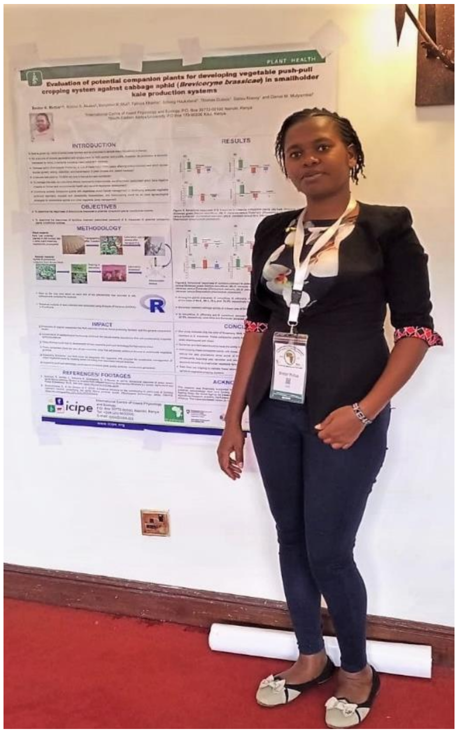


Appendix iv: Gas Chromatography- Mass spectrometer used for identification and quantification of volatile organic compounds (A) and Gas Chromatography-Electroantennographic detector used for antennal responses(GC-EAD).

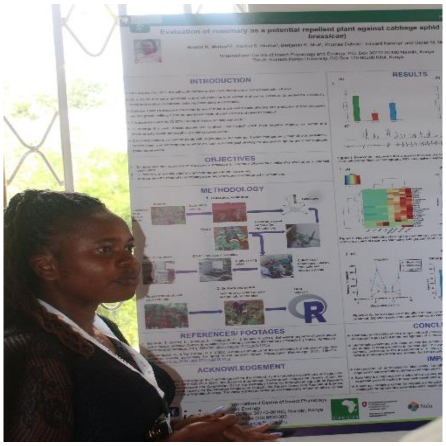


Appendix v: OUTPUTS

1. Poster presentation at the 1st Eastern Africa Agroecology conference held at Safari Park Hotel, Kenya from 21st to 24th March, 2023.



2. Poster presentation at the 23rd Annual Workshop of the Horticultural Association of Kenya (HAK) held at the International Centre of Insect Physiology and Ecology from 13th to 17th November 2023. Best poster Award received at this conference.



3. Publication

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RESEARCH

Electrophysiological and Behavioral Responses of Cabbage Aphid (*Brevicoryne brassicae*) to Rosemary (*Rosmarinus officinalis*) Volatiles, a Potential push Plant for Vegetable push-pull Cropping System

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Abstract

The cabbage aphid (*Brevicoryne brassicae*) is a major pest of kale (*Brassica oleraceae* var. *acephala*), an important vegetable that is grown worldwide due to its high nutritional and economic value. *Brevicoryne brassicae* poses a great challenge to *B. oleraceae* var. *acephala* production, causing significant direct and indirect yield losses. Farmers overly rely on synthetic insecticides to manage the pest with limited success owing to its high reproductive behavior and development of resistance. This necessitates a search for sustainable alternatives to mitigate these challenges. This study assessed behavioral responses of *B. brassicae* to odors from rosemary (*Rosmarinus officinalis*) and *B. oleraceae* var. *acephala* headspace volatiles in a Perspex four-arm olfactometer. We identified and quantified volatiles emitted by each of the two plants and those eliciting antennal response using coupled gas chromatography-mass spectrometry (GC-MS) and GCelectroantennograhic detection(GC-EAD), respectively. Our findings revealed that *B. brassicae* spent more time in the arms of the olfactometer that contained *B. oleraceae* var. *acephala* volatiles compared to the arm that held *R. officinalis* volatiles. Additionally, *B. brassicae* spent more time in the olfactometer arms with *B. oleracea var. acephala* compared to the arms holding *B. oleracea var. acephala* and *R. officinalis* enclosed together and clean air. GC-MS analysis revealed diverse and higher quantities of volatile compounds in *R. officinalis* compared to *B. oleraceae* var. *acephala*. GC-EAD analysis showed that antennae of *B. brassicae* detected Linalool, α-Terpineol, Verbenone, Geraniol, Camphor, and Borneol from the volatiles of *R. officinalis*, and Sabinene, γ-Terpinene, and β-Caryophyllene from *B. oleraceae* var. *acephala* volatiles. Our findings demonstrate the potential of *R. officinalis* as a repellent plant against *B. brassicae* and could be utilized as a 'push' plant in an intercropping strategy against this pest.

Keywords Agroecology · Cabbage Aphid · Integrated pest Management · Kale · Rosemary

Introduction

Kale (*Brassica oleracea* L. var. *acephala*) is a leafy vegetable of global importance, primarily cultivated by smallscale farmers for both subsistence and income generation, particularly in tropical and subtropical regions (Mutiga et al. 2011; Peris and Kiptoo 2017; Šamec et al. 2019). According to the Center for Disease Control (CDC), *B. oleracea* var. *acephala* was ranked 15th of the 47 powerhouse fruits and vegetables, producing more than 17 essential nutrients (CDC 2014). *Brassica oleracea* var. *acephala* has garnered significant attention recently owing to its notable health advantages. It contains phytochemicals that have been linked to reduced risk of cancer and other chronic diseases, due to antioxidant properties and high dietary fiber content (Šamec et al. 2019). Additionally, *B. oleracea* var. *acephala* is known for its resilience to adverse effects of climate change, rendering it adaptable to extreme climatic conditions (Lagerkvist et al. 2012). In Kenya, *B. oleracea* var. *acephala* has become increasingly popular due to its ability to maximize land use and address food security

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