Ecological and bio-based strategies against *Brassica* insect pests in eastern Democratic Republic of Congo

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Photo de couverture : Une plante de chou-fleur sévèrement attaquée par des pucerons cendrés *Brevicoryne brassicae*. © Patient Niyibizi Gakuru

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À la mémoire de ma chère mère Madeleine MANABI

partie au début du parcours doctoral

Abstract

Brassica vegetables are important crops in the eastern Democratic Republic of Congo (DRC), as they provide food and income for producers and other actors in the supply chain. The main cultivars in the region include cabbage, cauliflower, Chinese cabbage, and broccoli. Insect pests are a major challenge for vegetable production, as they can affect plant growth and thereby reduce crop yields. Chemical control remains the most widely used method in pest management. However, the use of synthetic pesticides raises concerns about human health, the environment, and beneficial organisms. In this context, the objective of this thesis is to develop innovative strategies for managing insect pests in *Brassica* crops by evaluating alternative methods, including agroecological practices and biological approaches based on the use of pesticidal plants and entomopathogenic fungi.

In **Chapter 1,** the scientific literature was reviewed to provide an overview of *Brassica* cultivation and the main constraints in pest management. It describes the attack mechanisms of the main pests and the adverse effects associated with the intensive use of synthetic insecticides. This chapter also analyzes various alternative strategies to reduce dependence on synthetic pesticides and promote sustainable vegetable production.

The prior identification of pests is a fundamental step in their effective management and understanding their economic impact. For this reason, the functional diversity in cabbage crops in eastern DRC was investigated **(Chapter 2)**, which involved identifying both pests and their natural enemies. Insect samplings were conducted on smallholder farms using yellow traps, sweep nets, and direct plant observations. The collection areas were selected based on their importance as suppliers of market garden produce to Goma city. The areas studied include the periurban area of Lac Vert in Goma and the territories of Nyiragongo, Masisi, Rutshuru, and Kalehe. Lepidopteran caterpillars and aphids were found to be the predominant pests causing economic losses. The cabbage aphid, *Brevicoryne brassicae*, was distinguished by its rapid reproduction, both sexual and asexual, and its presence at all stages of plant growth. Natural enemies identified include predators such as Coccinellidae, Syrphidae, and Chrysopidae, as well as parasitoids mainly from the Braconidae and Ichneumonidae families.

After identifying the pests, it was necessary to analyze the control methods used by farmers in eastern DRC to understand local challenges. Accordingly, an assessment of farmers' perceptions of cabbage pests and their management strategies was conducted (**Chapter 3)**. Interviews and field observations were conducted in Goma and in the territories of Nyiragongo, Masisi, and Kalehe. Synthetic pesticides were found to be the most used control method. Many of the active substances identified pose risks to human health and the environment, ranging from class II (moderately toxic) to class Ib (highly toxic) according to the World Health Organization (WHO) classification. The lack of personal protective equipment and farmers' failure to comply with prescribed doses increase the risk of exposure to hazardous substances and consumption of contaminated vegetables.

Given the concerning situation, it is necessary to promote alternative, less harmful control strategies. In **Chapter 4**, the potential of three aromatic plants, *Cymbopogon citratus*, *Lantana camara,* and *Lippia alba,* in managing the cabbage aphid, was assessed. First, the repellency of these plants when associated with cabbage was examined. It was found that the behavior of *B. brassicae* is influenced by the presence of aromatic plants, particularly *C. citratus,* with aphids preferring to settle on cabbage far from these plants. Next, the direct toxicity and repellent effect of essential oils from these three aromatic plant species were also evaluated. High toxicity of *C. citratus* essential oil and marked repellent effects of *L. camara* essential oil were found. Phytotoxicity analyses on cabbage seedlings indicated the following toxicity gradient: *C. citratus* > *L. alba* > *L. camara*. Integrating companion aromatic plants within *Brassica* crops as a preventive measure, and applying their essential oils as a curative measure, could contribute to the sustainable management of cabbage aphids.

In addition to the potential of pesticidal plants, it remained essential to evaluate the effectiveness of natural enemies of pests, particularly entomopathogenic fungi. **Chapter 5** analyzes the potential of different strains of *Beauveria bassiana* against *B. brassicae*. Three strains were studied: the commercial strain GHA, the strain GxABT-1 isolated from soil samples in Belgium, and the strain KA14 isolated from insect cadavers collected in eastern DRC. Promising biocontrol potential was revealed for all three strains, with the indigenous strain KA14 standing out for its high toxicity against *B. brassicae*. The median lethal times (LT_{50}) for KA14 were 3 days after direct application of fungal solution and 4 days after feeding insects with fungus-inoculated plants. This strain also had strong endophytic potential, colonizing the host plant's tissues and promoting upward growth. The GHA strain demonstrated efficacy equivalent to the chemical insecticide Cypermethrin in field trials in eastern DRC, highlighting the viability of microbial control under local conditions.

Finally, a general discussion of the results is presented (**Chapter 6),** leading to the development of a management plan for sustainable control of the cabbage aphid in eastern DRC. This management is based on an integrated approach combining preventive cultural measures, including varietal selection, appropriate choice of sowing dates, crop rotations and intercropping, particularly with repellent plants, as well as the use of physical barriers. These practices are supplemented by curative biological measures, particularly plant- and microbe-based biopesticides. This thesis demonstrates the effectiveness and synergy of agroecological measures and biocontrol interventions, generating original and actionable knowledge to sustainably improve productivity, food quality, and the resilience of vegetable production systems in DRC.

Résumé

Les cultures maraîchères du genre *Brassica* constituent des productions de grande importance dans l'Est de la République Démocratique du Congo (RDC), en raison de leur contribution à la sécurité alimentaire et aux revenus des producteurs et autres acteurs de la chaîne d'approvisionnement. Les principaux cultivars produits dans la région sont le chou pommé, le chou-fleur, le chou chinois et le brocoli. Les insectes ravageurs constituent un défi important pour la production maraîchère, car ils affectent la croissance des plantes et entraînent une baisse des rendements. La lutte chimique reste la méthode de lutte la plus utilisée dans la gestion des ravageurs de ces cultures. Cependant, l'usage de pesticides de synthèse soulève des préoccupations relatives à la santé humaine, à l'environnement et aux organismes bénéfiques. Dans ce contexte, cette thèse a pour objectif de développer des stratégies innovantes pour lutter contre les ravageurs des cultures du genre *Brassica* en évaluant des méthodes alternatives, notamment des pratiques agroécologiques et des approches biologiques reposant sur l'utilisation de plantes pesticides et de champignons entomopathogènes.

Dans le **chapitre 1,** la littérature scientifique offre une vue d'ensemble de la culture des crucifères et des principales contraintes liées à la gestion de leurs ravageurs. Il décrit les mécanismes d'attaque des principaux ravageurs et les effets négatifs associés à l'utilisation intensive d'insecticides de synthèse. Ce chapitre présente également différentes stratégies alternatives visant à réduire la dépendance aux pesticides de synthèse et à favoriser une production maraîchère durable.

L'identification préalable des ravageurs constitue une étape fondamentale pour une gestion efficace et la limitation des dégâts économiques. Dans ce cadre, la diversité fonctionnelle dans les cultures de chou à l'Est de la RDC a été examinée, en identifiant à la fois les ravageurs et leurs ennemis naturels (**chapitre 2)**. Les échantillonnages d'insectes ont été réalisés dans des exploitations paysannes à l'aide de pièges jaunes, de filets-fauchoirs et d'observations directes sur les plantes. Les zones de collecte ont été sélectionnées en fonction de leur importance en tant que fournisseurs de produits maraîchers à la ville de Goma. Ces zones étudiées comprennent la zone périurbaine de Lac Vert à Goma ainsi que les territoires de Nyiragongo, Masisi, Rutshuru et Kalehe. Les chenilles et pucerons ont été identifiés comme étant les ravageurs predominants et responsables de pertes économiques. Le puceron cendré du chou, *Brevicoryne brassicae*, s'est démarqué par sa reproduction rapide, tant sexuée qu'asexuée, et par sa présence à tous les stades de croissance des plantes. Les ennemis naturels identifiés incluent des prédateurs tels que les Coccinellidae, Syrphidae et Chrysopidae, ainsi que des parasitoïdes principalement issus des familles des Braconidae et Ichneumonidae.

Après l'identification des ravageurs, il s'avérait nécessaire d'analyser les méthodes de lutte adoptées par les producteurs de l'Est de la RDC afin de comprendre les défis locaux. Ainsi, une évaluation de la perception paysanne des ravageurs des choux et des pratiques de gestion adoptées a été menée **(chapitre 3)**. Des entretiens et observations directes sur le terrain ont été réalisés à Goma et dans les territoires de Nyiragongo, Masisi et Kalehe. Il s'est avéré que l'usage des pesticides de synthèse constitue la principale méthode de lutte contre les ravageurs. De nombreuses substances actives identifiées présentent des risques pour la santé humaine et l'environnement, allant des classes II (modérément toxiques) aux classes Ib (hautement toxiques) selon la classification de l'Organisation Mondiale de la Santé (OMS). L'absence d'équipements de protection individuelle et le non-respect des doses prescrites augmentent les risques d'exposition aux substances dangereuses et de consommation de légumes contaminés.

Face à cette situation préoccupante, la promotion de stratégies alternatives de contrôle moins nocives s'avère nécessaire. Le potentiel de trois plantes aromatiques, *Cymbopogon citratus*, *Lantana camara* et *Lippia alba,* a été évalué dans la gestion du puceron cendré du chou **(chapitre 4)**. D'abord, le pouvoir répulsif de ces plantes en association avec chou a été examiné. Il a été constaté que le comportement du puceron cendré est influencé par la présence de plantes aromatiques, en particulier *C. citratus,* les individus préférant s'installer sur des choux éloignés de ces plantes. Ensuite, la toxicité directe et le pouvoir répulsif des huiles essentielles issues de ces trois espèces de plante aromatique ont également été évalués. Une forte toxicité pour l'huile essentielle de *C. citratus* et des effets répulsifs marqués pour celle de *L. camara* ont été observés. Les analyses de phytotoxicité sur les plantules de chou ont indiqué le gradient de toxicité suivant : *C. citratus* > *L. alba* > *L. camara*. L'association de plantes aromatiques avec les légumes crucifères, en tant que mesure préventive, ainsi que l'utilisation de leurs huiles essentielles, en tant que mesure curative, pourraient contribuer à une gestion durable du puceron du chou.

Outre le potentiel des plantes pesticides, l'évaluation de l'efficacité d'ennemis naturels des ravageurs, notamment les champignons entomopathogènes, demeurait essentielle. Le potentiel de différentes souches de *Beauveria bassiana* a été determiné dans le contrôle de *B. brassicae* (**chapitre 5**). Trois souches ont été étudiées : la souche commerciale GHA, la souche GxABT-1 isolée à partir d'échantillons de sol prélevés en Belgique et la souche KA14 isolée de cadavres d'insectes collectés à l'Est de la RDC. Un potentiel de biocontrôle prometteur a été observé pour les trois souches; la souche indigène KA14 se distinguant par sa toxicité et son efficacité contre *B. brassicae*. Les temps létaux médians (TL_{50}) pour KA14 sont de 3 jours après pulvérisation directe de la solution fongique et de 4 jours après alimentation des insectes avec des plantes inoculées au champignon. Cette souche présente également un fort potentiel endophyte, colonisant les tissus de la plante hôte et favorisant la croissance en hauteur. La souche GHA a démontré une efficacité équivalente à celle de l’insecticide chimique à base de cyperméthrine sur le terrain à l’Est de la RDC, mettant en évidence la viabilité de la lutte microbienne dans les conditions locales.

Finallement, une discussion générale des résultats est présentée, aboutissant à l’élaboration d’un itinéraire technique pour la gestion durable du puceron cendré du chou à l’Est de la RDC (**chapitre 6)**. Pour y parvenir, cela implique une approche intégrée combinant des mesures culturales preventives, notamment la sélection variétale, le choix judicieux de la date de semis, les rotations et les associations culturales, en particulier avec des plantes à effet répulsif, ainsi que l’utilisation de barrières physiques. Ces pratiques sont complétées par des mesures curatives biologiques, notamment l’usage de biopesticides d’origine végétale et microbienne. En mettant en évidence l’efficacité et la synergie de mesures agroécologiques et d’interventions de biocontrôle, cette thèse génère des connaissances originales et opérationnelles susceptibles de renforcer durablement la productivité, la qualité des aliments et la résilience des systèmes maraîchers en RDC.

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Table of contents

List of figures

List of tables

List of acronyms

ANOVA : Analysis of variance
DAI : Days after inoculation
DAT : Days after transplanting
DBM : Diamondback moth
DRC : Democratic Republic of Congo
EO : Essential Oil
EPF : Entomopathogenic fungi
EU : European Union
FAO : Food and Agriculture Organization
GC-MS : Gas chromatography – Mass spectrometry
GLM : Generalized Linear Model
GLMM : Generalized Linear Mixed-Model
HIPVs : Herbivore-Induced Plant Volatiles
IPM : Integrated Pest Management
LC_{50} : Lethal concentration for fifty percent of test population
LC_{90} : Lethal concentration for ninety percent of test population
LD_{50} : Lethal dose for fifty percent of test population
LRS : Long Rainy Season
LT_{50} : Lethal time for fifty percent of test population
NGO : Non-Governmental Organization
PCoA : Principal Coordinate Analysis
PPE : Personal Protective Equipment
RDA : Redundancy analysis
RI : Repellent Index
SRS : Short Rainy Season
VOCs : Volatile Organic Compounds
WHO : World Health Organization

Chapter 1

General introduction

A colony of cabbage aphids on a cabbage leaf. © P.N. Gakuru

Chapter 1. General introduction

1.1. Research background

1.1.1. Vegetable farming in Democratic Republic of Congo

The world's rapid population growth (8.23 billion in 2025 according to the United Nations Population Fund) (UNFPA, 2025) and the fastest increase of urbanized areas are driving a sharp rise in demand for agricultural products, particularly vegetables. Developing countries are particularly affected by the need to improve food security and nutrition, as many regions struggle to meet the dietary needs of their growing populations (Ochieng et al., 2020). The Congolese population is estimated at 112.8 million people (UNFPA, 2025) and is expected to continue growing. This growth will further strain agricultural production systems, necessitating innovative solutions to support this demographic growth and ensure food availability. One of the most significant challenges in modern agriculture is to ensure sufficient food production while minimizing environmental impact (Brévault and Clouvel, 2019). To address these issues, vegetable farming is increasingly recognized as a viable strategy to boost local food production and improve livelihoods. Farmers can enhance vegetable productivity and resilience against climate-related challenges by implementing sustainable practices (Reza et al., 2025).

Vegetable farming is an important component of world agriculture, enhancing household food security and income generation for farmers across Sub-Saharan Africa (Edmondson, 2024). It not only supports local economies but also contributes to the gross domestic product and addresses food security challenges faced by communities in the Global South (Machekano et al., 2017). Moreover, the diverse functions of market gardening contribute significantly to poverty reduction and enhance the socio-economic conditions of households in the region. This sector is particularly essential in the Democratic Republic of Congo (DRC), where most people live in a subsistence economy and rely heavily on agriculture for their livelihoods (Ochieng et al., 2020). Market gardening not only employs people in rural areas but also benefits urban households, as it is well-suited to small-scale farms in urban areas. Overall, the impact of market gardening extends beyond mere food production, fostering resilience and improving the quality of life for many households in urban and peri-urban areas of DRC (Machekano et al., 2017). In big cities like Kinshasa, Lubumbashi, and Goma, urban agriculture plays a vital role in community livelihoods by offering diverse vegetable options and creating economic opportunities for local farmers (Balasha and Mwine Fyama, 2020; Bazungula et al., 2025). This multifaceted approach addresses both food access and income generation for smallholder farmers in these urban settings. Growing vegetables in and around large urban areas increases the supply of fresh, high-quality produce in short supply chains. Since start-up costs are lower and the value of the products is high, urban and peri-urban agriculture provides a livelihood for vegetable growers, particularly low-income city dwellers (FAO, 2010). The development of sustainable practices in vegetable farming is crucial for enhancing these benefits and promoting long-term resilience among farming households in DRC. In light of these factors, promoting agroecological and innovative agriculture practices could further enhance the sustainability and productivity of market gardening systems in DRC (Karume et al., 2022).

Vegetable farming involves cultivating plants whose leaves, fruits, and roots are used in crudités and in protein- and vitamin-rich sauces that complement calorie-dense foods, such as cassava, rice, and maize. Vegetables are used not only as condiments or for snacking but also as an essential part of the food base (Ndjadi et al., 2020). They can be consumed fresh or cooked in different preparations, contributing to a diverse and balanced diet essential for the well-being of the population. Vegetables provide essential nutrients that support overall health and nutrition, addressing dietary needs that are often unmet by staple foods alone. They contain proteins, vitamins, folic acid, minerals such as iron and calcium, and other crucial elements in the human diet. Furthermore, the integration of vegetables into daily diets supports individual health and enhances community resilience against food insecurity and malnutrition. Vegetable consumption in DRC involves traditional vegetables based on local cultures, such as African eggplant (*Solanum aethiopicum* L.), black nightshade (*Solanum nigrum* L.), and amaranth (*Amaranthus* spp.), and exotic vegetables linked to the colonial past and, more recently, to world trade, such as carrot and cucumber (Bokombola et al., 2018; Kpadenou et al., 2020). Certain introduced crops were brought to Africa for non-vegetable uses. However, over the years, their use as vegetables increased through adaptation and culinary innovation (Shackleton et al., 2009). Such is the case of cassava (*Manihot esculenta* Crantz) and sweet potato (*Ipomoea batatas* L.), both introduced from Latin America for their tubers and roots, respectively, but whose leaves are now an essential source of vegetables and are commonly incorporated into gardens (Tabaglio et al., 2023). Depending on their climatic characteristics, certain vegetables originate in tropical regions, including tomatoes, peppers, onions, and okra. Others originate in temperate regions, such as lettuce, leeks, and *Brassica* crops, which currently account for a significant portion of the vegetable supply.

1.1.2. Overview of *Brassica* vegetables

Brassica crops are cultivated plants belonging to the genus *Brassica* within the family Brassicaceae (formerly Cruciferae) (Mpumi et al., 2020). Brassicaceae is a cosmopolitan family comprising more than 340 genera and 4,636 known species distributed worldwide (Wu et al., 2022). Members of this family are characterized by herbaceous morphology and cross-shaped flowers. Their geographic origin is not clearly defined, but many hypotheses place the Mediterranean/Middle East region as the first domestication area (Cai et al., 2022). The genus *Brassica* includes six economically important species, which are divided into two genetic groups: three are diploids, namely *Brassica rapa* L. (AA genome, n=10), *Brassica nigra* (L.). Koch (BB genome, n=8), and *Brassica oleracea* L. (CC genome, n=9), while three others are allotetraploids: *Brassica juncea* (L.) Czern & Coss (AABB genome, n=18), *Brassica napus* L. (AACC genome, n=19), and *Brassica carinata* (A. Braun) (BBCC genome, n=17). These species share complex genomic relationships explained by the classical “U-triangle” model, which describes the amphidiploid origins of cultivated *Brassica* species (Wu et al., 2022; Zhang et al., 2025). The genus *Brassica* comprises three main important groups based on the consumed parts: vegetables, oilseeds, and condiments. It also includes ornaments, medicinal plants, and others that provide fodder and green manure. The economically important vegetables are cabbage (*Brassica oleracea* var. *capitata*), cauliflower (*Brassica oleracea* var. *botrytis*), broccoli (*Brassica oleracea* var. *italica*), Chinese cabbage (*Brassica rapa* var. *pekinensis*), and Brussels sprouts (*Brassica oleracea* var. *gemmifera*) (Bruckner et al., 2024). Oilseeds are mainly rapeseed (*B. napus*), while condiments are mostly derived from the seeds of mustard *B. juncea* and black mustard *B. nigra* (Zhang et al., 2025). The diversity of morphotypes within species of the *Brassica* genus is the result of artificial selection during domestication worldwide (Wang et al., 2023).

Brassica vegetables are an economically important group, with an estimated production of over 100 million tons in 2023, cultivated on nearly 4 million hectares (FAO, 2025). Cabbage and other under-cultivated brassicas represent three-quarters of the total production, while cauliflower and broccoli represent the remaining quarter. China is the leading producer of brassicas, representing 45% of global production. Cabbage remains widely produced in Africa, with Kenya ranking first at 1,042,800 tons, while DRC production was estimated at 20,509.56 tons in 2023 (FAO, 2025). These Congolese yields remain below market requirements, highlighting the need for improved agricultural practices and support for farmers to enhance production in the DRC. Cultivating *Brassica* vegetables is a promising strategy for strengthening rural food security and improving nutritional outcomes. Owing to their rapid growth and high productivity, they contribute to year-round food availability, dietary diversity, and income generation (Lekamoi et al., 2025). These crops are globally valued for their nutritional richness, adaptability, and short growth cycles, making them key components of sustainable food systems.

Regarding nutrient content, *Brassica* vegetables are rich in vitamins A, C, and K, as well as minerals such as iron, calcium, and potassium. They also contain glucosinolates, antioxidant compounds, and phenolic compounds that promote immune health and reduce risks of chronic diseases such as various cancers, cardiovascular disease, and diabetes (Baladia et al., 2024; Zhang et al., 2025). For example, broccoli is one of the so-called “superfoods” because of its exceptional nutritional value (Gupta and Mishra, 2021).

The most cultivated *Brassica* vegetable crop in DRC is cabbage, followed by cauliflower, Chinese cabbage, and broccoli. Cabbage, also known as head cabbage, is widely accepted in local diets. It is productive, fairly storable, and easily integrated into small-scale farming systems (Lekamoi et al., 2025). It is commonly grown in both rural and peri-urban areas and used in diverse local dishes, providing essential micronutrients that complement local carbohydrate-based staples such as maize, potato, and banana. Brassicas, domesticated initially in the temperate areas, have since adapted well to the high-altitude and cooler agroecological areas of eastern and southern DRC (Balasha and Mwine Fyama, 2020; Gakuru et al., 2025b). Brassicas can contribute to more sustainable pest and soil health management through rotations. Indeed, cabbage residues can improve soil fertility through green manure effects and biofumigant properties resulting from glucosinolate degradation. Despite these benefits, realizing the full potential of cabbage in DRC requires overcoming challenges such as limited access to quality seed, high pest pressure, and weak postharvest infrastructure and markets. Strengthening local seed systems, promoting integrated pest management, and supporting farmers' training in vegetable value chains are therefore crucial for scaling up the contribution of *Brassica* crops to food security, nutrition, and sustainable livelihoods in DRC.

The growth and development of cabbage are significantly influenced by several ecological factors, including soil quality, temperature, nutrient availability, and water management (Saha et al., 2022). For optimal growth, cabbage requires well-drained, fertile soil with a pH between 6.0 and 7.0, which supports nutrient uptake and root development. The crop also needs loamy or silty loam soils with a high organic matter content. Light and temperature also play crucial roles; cabbage thrives best at air temperatures of 15–25°C with an optimal range of 18 to 20°C. Cabbage can tolerate some frost, although extreme cold can hinder head formation (Daniel et al., 2023; Kadzere et al., 2024). Nutrient management is vital, as cabbage has high nutrient needs, particularly for nitrogen, phosphorus, and potassium. Regular monitoring of these nutrients is essential for maximizing growth and yield. In terms of water, cabbage is considered a high-water-requirement vegetable. It requires 380-500 mm of rainfall throughout the growing period (Gelaye and Tadele, 2022). Watering practices are thus important; consistent moisture is necessary to prevent head splitting and to maintain optimal soil moisture levels. Cabbage is also sensitive to flooding, which can cause root rot and lead to plant death. It requires draining to remove excess water during heavy rainfall (Saha et al., 2022; Daniel et al., 2023).

1.1.3. Common *Brassica* insect pests

Cabbage production is severely constrained by a range of insect pests that affect all growth stages of the crop (Lekamoi et al., 2025). Depending on their feeding capacity, phytophagous insects can belong to a range of categories from monophagous, feeding on a single host plant species or on narrow host plants within a genus, to polyphagous, feeding on several plant species that belong to two or more different families. Oligophagous insects are intermediate between the two categories, feeding on host plants belonging to the same family or closely related families. *Brevicoryne brassicae* (L., 1758) and *Plutella xylostella* (L., 1758) are typical examples of oligophagous insects that feed on host plants within the Brassicaceae family (Mpumi et al., 2020). Regarding polyphagous insects, several species, including *Myzus persicae* (Sulzer, 1776) (Hu et al., 2023) and *Tricoplusia ni* (Hübner, 1803) (Rajashekarappa et al., 2025), can feed on cruciferous crops and many other host plant species, such as tomato and cotton.

The cabbage aphid

The cabbage aphid, *Brevicoryne brassicae* (L., 1758) (Hemiptera: Aphididae), is a native to Europe, but has become a cosmopolitan pest, now widely distributed across temperate and tropical regions, including sub-Saharan Africa (Mutua et al., 2024), where it is commonly reported in highland vegetable systems of East and Central Africa such as Uganda (Lubanga et al., 2012), Tanzania (Mpumi et al., 2020), Kenya (Mutua et al., 2024), and DRC (Gakuru et al., 2025b).

B. brassicae belongs to the family Aphididae within the order Hemiptera (Moorthy et al., 2022). *B. brassicae* was first described as *Aphis brassicae* in 1758 by Linnaeus, based on specimens collected in Sweden (Nematollahi, 2022). The name *Brevicoryne* is derived from two Latin words, "brevi" and "coryne", which may be translated as "small pipes". These pipes refer to organs at the posterior end of the insect, called cornicles or siphunculi (tailpipe-like appendages), which can be seen with a hand lens (Gill et al., 2013). Cornicles secrete various compounds that play crucial roles in the survival of aphids, particularly in defense against predators. These specialized structures release volatile compounds that function as alarm pheromones, alerting and repelling nearby aphids. They also produce nonvolatile compounds, which are defensive secretions that can reduce predators' foraging efficiency. The cornicles thus serve as a vital adaptation for aphids, enhancing their survival in various ecological contexts (Michaud, 2022). The cornicles of *B. brassicae* are black and about 0.25 mm long, longer than the cauda, and look swollen in the middle. The cauda is black, short, and cone-shaped. (Edde, 2022). The cornicles of cabbage aphid are relatively shorter than those of other aphids, except for the turnip aphid *Lipaphis erysimi* (Kaltenbach, 1843). However, the cabbage aphid is distinctive for its body, which is coated with a waxy covering that gives it a grayish-white appearance (Pal and Singh, 2013). This wax coating provides microclimate isolation and serves as a protective barrier against various environmental threats and predators (Smith, 1999). Without the waxy covering, the body of the cabbage aphid is greenish gray with eight black spots located on either side of the dorsal part of the abdomen. These spots increase in size towards the cauda (Edde, 2022). The short cornicles and the waxy coating help to differentiate the cabbage aphid from other aphids that attack *Brassica* crops. Regarding body length, *B. brassicae* is relatively longer (approximately 2.0 – 2.5 mm long) compared to *L. erysimi* (1.6 – 2.2 mm long) (Gill et al., 2013). The body form of *B. brassicae* is evident in apterous individuals, which are oval-shaped and 1.26 to 1.27 mm wide (Edde, 2022). Morphological variation could be influenced by the environment, and particularly the host plants. The study by Ruiz-Montoya et al. (2005) on *B. brassicae* revealed significant morphological variation associated with the use of different host plants. Aphids collected from *Brassica campestris* (syn. *Brassica rapa*, turnip) were observed to be bigger and with longer appendages than those found on *B. oleracea* (green cabbage). Winged aphids have black heads, thoraxes, and eyes, while the entire body is yellow green. The wings have thick, coarse veins and a dark stigma. The antennae are about 1.54 mm long, and the cornicles are dark brown (Edde, 2022). Aphids feed using their mouthparts, specially fitted with a rostrum. The rostrum consists of a long labium and specialized stylets, allowing the aphid to reach deep into plant tissues and to extract phloem sap. The rostrum has three segments, enhancing flexibility during feeding, and includes a tube-like canal that facilitates fluid intake (Krenn, 2019).

Adult aphids can be found either winged or wingless and can reproduce in two ways. In warm conditions, wingless females commonly produce female nymphs parthenogenetically, *i.e.*, without mating (viviparity), while in cooler conditions, winged males and apterous oviparous females mate for sexual reproduction (Ruiz-Montoya et al., 2005; Pal and Singh, 2013). This dual reproductive capacity allows aphids to spread rapidly, with up to 15 – 20 generations per growing season (Javed et al., 2022). An adult aphid can produce 2 to 5 live nymphs per day, and these nymphs reach maturity in 5 to 7 days. Moreover, a mature cabbage aphid may reproduce for up to 30 days (Munthali and Tshegofatso, 2014). The average life cycle ranges from 16 to 50 days, and it is strongly influenced by temperature. Warmer temperatures and dry conditions result in shorter cycles (Gill et al., 2013). Aphids are ectothermic organisms whose physiological processes depend heavily on climatic variables, especially temperature (Brodeur et al., 2013). Research by Soh et al. (2018) evaluated the effects of temperature on *B. brassicae*. They found that temperature variation significantly affects the growth, survival, and reproduction of aphids. For instance, the development dropped sharply from 18.45 days at 10 °C to 5.05 days at 30 °C, with no development occurring at 35 °C. Mortality was highest at 10 °C and above 30 °C, and lowest at 15–25 °C. Aphid fecundity was maximal around 20 °C but null at 30 °C, and population growth reached its optimum near 25 °C before collapsing at 30 °C. Hence, cabbage aphids cause more damage in the tropical and subtropical conditions of Africa where annual temperature favor their development (Munthali and Tshegofatso, 2014).

The cabbage aphid is a highly specialized, oligophagous aphid species that feeds exclusively on host plants in the Brassicaceae family (Soh et al., 2018), whose secondary compounds, the glucosinolates, may influence host recognition by these aphids (Mezgebe and Azerefegne, 2021). Host plants include many economically important vegetables, such as cabbage, cauliflower, broccoli, Chinese cabbage, Brussels sprouts, and kale (Zanardi et al., 2024). The aphid forms dense colonies on the undersides of leaves, leaf axils, stems, floral buds, and within the cabbage head (Munthali and Tshegofatso, 2014). *B. brassicae* can affect all growth stages but prefers young plants and developing tender tissues. Losses caused by cabbage aphids are so high that they can reach up to 80% (Mezgebe and Azerefegne, 2021). Direct damage involves feeding on phloem sap through their needle-like mouthparts (A. K. Patel et al., 2024), resulting in yellowing, leaf curling, reduced photosynthetic efficiency, stunted growth, deformation of cabbage heads, and, in severe cases, plant death (Hao et al., 2019). Large infestations also produce abundant honeydew, which promotes the development of fumaginous saprophytic fungi, affecting photosynthetic activity and thus further reducing market value (Zanardi et al., 2024). Indirect damage refers to the transmission of plant viruses, including the cauliflower mosaic virus (CaMV) (Bruckner et al., 2024) and the turnip mosaic virus (TuMV) (Hao et al., 2022), which severely worsen yield losses. For example, a study in Kenya demonstrated that TuMV significantly reduced cabbage yield, causing 50% losses in head production (Spence et al., 2007). An earlier study in eastern DRC evaluated the impact of major insect pests on cabbage production. They found *B. brassicae* among the most feared pests, and this aphid was the leading cause of plant death (Walangulu and Mushagalusa, 2000). Another major challenge is the development of resistance by this aphid, which makes its control more difficult (Hamedani Radja et al., 2020). The management of cabbage aphids is thus a significant challenge for stable cabbage production and market availability.

The diamondback moth

Plutella xylostella (L., 1758) (Lepidoptera: Plutellidae), commonly known as the diamondback moth (DBM), is a globally distributed lepidopteran pest and is considered the most destructive insect pest of *Brassica* crops (Furlong et al., 2012). The female lays eggs on cabbage leaves, and a few days later, they hatch. The larvae that emerge are highly voracious and feed on plant pods, leaves, and stems (Paul et al., 2024). In cases of severe damage, the crops are unusable, and losses could reach 100% of production (Machekano et al., 2017). Morphologically, the larvae are pale green and tapered at both ends. They are highly mobile and, when disturbed, wriggle and may fall from the plant. Adults are small moths (6 to 8 mm long; wingspan 12 to 15 mm) with a slender gray-brown body and narrow forewings marked with a distinctive pattern of three or four pale yellow diamond-shaped forms when the moth rests, thus the common name "diamondback moth" (Mason, 2022).

The species likely originated in the Mediterranean (South Europe) or South Africa region, before spreading worldwide through trade and continuous crop cultivation (Wei et al., 2013). Nowadays, *P. xylostella* is a cosmopolitan pest, particularly prevalent in tropical and subtropical regions where climatic conditions allow up to 20 generations per year (Askri et al., 2025). In these regions, continuous year-round *Brassica* cultivation, elevated temperatures, and overlapping generations favor persistent DBM outbreaks (Wei et al., 2013). Studies in southern, eastern, and central Africa, including DRC, indicate that DBM is the primary constraint to cabbage production, often forcing farmers into frequent pesticide applications (Badenes-Perez and Shelton, 2006; Machekano et al., 2017; Mpumi et al., 2020; Gakuru et al., 2025b). DBM is also known for its high resistance to insecticides, with reports of resistance to nearly all major chemical classes (Roy et al., 2023). The pest's high reproductive rate, migratory potential, and adaptive capacity make it a central focus of pest management programs for *Brassica* vegetables.

Other insect pests affecting Brassica vegetables

Cabbage is affected by several other economically important insect pests that significantly reduce yield and market quality. The cabbage webworm, *Hellula undalis* (Fabricius, 1781) (Lepidoptera: Crambidae), is one of the most destructive pests in tropical Africa. Its larvae bore into the growing tip, leaf stalks, and tender heads, causing wilting, tunneling damage, and failure of head formation (Moorthy et al., 2022). Other lepidopteran pests that cause economic damage to cabbage and hinder its cultivation in tropical regions include the cabbage looper *Tricoplusia ni* (Hübner, 1803) (Lepidoptera: Noctuidae), the cabbage white butterfly *Pieris brassicae* (L., 1758) (Lepidoptera: Pieridae), the tobacco caterpillar *Spodoptera litura* (Fabricius, 1775) (Lepidoptera: Noctuidae), and cutworms *Agrotis* spp (Lepidoptera: Noctuidae) (Mpumi et al., 2020; Mayanglambam et al., 2021; Moorthy et al., 2022; Daniel et al., 2023). Regarding sucking insects, other aphids feed on *Brassica* crops, including the green peach aphid *M. persicae* and the turnip aphid *Lipaphis erysimi* (Kaltenbach, 1843) (Edde, 2022; Yang et al., 2025). Flea beetles, *Phyllotreta* spp. and *Psylliodes* spp. (Coleoptera: Chrysomelidae), are another important group that affects cabbage crops, boring small, round, or irregular holes in the leaf epidermis (Edde, 2022). Other cabbage pests, such as the cabbage root maggot, *Delia radicum*, L., 1758 (Diptera: Anthomyiidae), affect the root system, causing wilting and poor nutrient uptake (Badenes-Perez and Shelton, 2006).

1.1.4. Challenges of pesticide use in vegetable farming

Overview of pesticide use in agriculture

The broad-scale use of chemical pesticides emerged since the Second World War, when they were widely introduced to control emerging pests and increase agricultural production (Belmain et al., 2013). A chemical pesticide is defined as any chemical substance or combination of substances intended to repel, destroy, or control harmful organisms that can include insects and other animals, micro-organisms (fungi, bacteria, and viruses), and weeds (Bennett et al., 2010). Chemical pesticides are widely used in conventional agriculture for their advantages, including reducing labor requirements, minimizing harvest loss, increasing farmers' income, and lowering food costs for consumers (Bennett et al., 2010). However, pesticide use poses negative externalities, including environmental pollution, health risks for farmers and consumers (Ahouangninou et al., 2011; Desye et al., 2024), development of pest resistance, and destruction of beneficial insects (Brévault et al., 2002; Williamson et al., 2008).

Recent studies indicate a worrying situation in the DRC and Central Africa more generally, as farmers do not wear personal protective equipment or adhere to recommended doses due to limited awareness and low levels of education (Balasha and Nsele, 2019; Gakuru et al., 2025c). According to Bayendi Loudit et al. (2017), insecticides containing lambda-cyhalothrin, commonly used in Gabon, were applied at doses ranging from 10 to 20 ml per 10 liters of water, corresponding to three to six times the recommended dose. Moreover, valuable information on pesticide containers is often written in foreign languages, and pictograms complying with international standards are poorly understood, leading to the abusive and hazardous use of chemical products (Ahouangninou et al., 2011). Research also highlights the risk of non-compliance with pre-harvest intervals, particularly for short-cycle crops such as vegetables (Muliele et al., 2018; Kalala et al., 2025). A study in Kinshasa, DRC, revealed that over 60% of market gardeners harvest vegetables less than 14 days after the last application of toxic pesticides with residual properties, such as thiodan (Ngakiama et al., 2019).

Human health risks and environmental impact

Although the use of synthetic pesticides in Africa accounts for only 2 to 4% of the global pesticide market (estimated at US$31 billion), this continent continues to have the highest risk of human mortality linked to synthetic pesticides (Williamson et al., 2008). In developing countries, farmers are unaware of the toxicity and persistence of the pesticides used in agriculture (Lekamoi et al., 2025). An assessment of pesticide residue in vegetables in Kinshasa and Lubumbashi revealed high levels of active substances, including dichlorodiphenyltrichloroethane (DDT), endosulfan, and malathion (Ndelo et al., 2023). Other studies have shown the accumulation of pesticides in common vegetables consumed in DRC, including amaranth (Ngweme et al., 2021), tomatoes (Kavatsurwa et al., 2014), and cabbage (Kipelo et al., 2025). However, pesticides are responsible for poisoning and many chronic health problems, including cancers, immune system and reproductive disorders, neurological dysfunction, metabolic diseases such as diabetes, dermatological issues, and disruption of the endocrine system (W. Zhou et al., 2025). Pesticides also act as inhibitors of acetylcholinesterase, a vital enzyme in the nervous system, and others affect neurobehavioral performance, particularly in children (Kushwaha et al., 2016). The situation is particularly critical for producers who frequently handle hazardous pesticides (Kapeleka et al., 2019).

The toxicity of a pesticide is influenced not only by its use but also by its persistence in the environment (Kushwaha et al., 2016). Its environmental half-life determines the extent to which the compound may accumulate in soils, water, or living organisms

(Ngweme et al., 2021). For example, pesticides that pollute the environment can indirectly affect human health through the consumption of food and drinking water (Desye et al., 2024). A typical example of a highly hazardous substance worldwide is DDT. It is not only toxic to humans but also highly persistent in the environment and food webs (Tshibanda et al., 2024). Environmental pollution from pesticide use is increasingly reported in DRC, particularly in urban and peri-urban areas where vegetables are grown, raising concerns. For example, a study of the N'djili River in Kinshasa detected organochlorine pesticides, including DDT, in surface water, indicating persistent environmental contamination from agricultural activities (Tshibanda et al., 2024). Persistent chemicals are so harmful because they disrupt biological balance and erode biodiversity, particularly soil and aquatic biodiversity (Ngweme et al., 2021). Collectively, studies provide consistent evidence of pesticide-related environmental pollution in Congolese agricultural production. Multi-level analyses are needed to assess their impact to ensure human health and environmental protection.

Impact on beneficial organisms and development of pest resistance

Broad-spectrum insecticides widely used in crop protection significantly harm non-target beneficial insects, which are crucial for ecosystem balance and agricultural productivity (Theenoor et al., 2024). Pesticide application can cause direct mortality by altering behavior and disrupting physiological functions in beneficial insects, including pollinators and natural enemies of pests (Samanta et al., 2023). A recent study of Knapp et al. (2025) reported that a broad-spectrum insecticide, gamma-cyhalothrin, commonly used against aphids, caused 100% mortality in the predator ladybird *Harmonia conformis* (Boisduval, 1835) (Coleoptera: Coccinellidae) within 24 hours. Predators and parasitoids are more susceptible to pesticide effects than phytophagous insects, which may have detoxification mechanisms (Samanta et al., 2023). Pesticides can directly affect beneficial insects, such as parasitoid wasps, by compromising their life-history traits, disrupting host-seeking behavior, and modulating the immune responses of their hosts, thereby reducing the effectiveness of natural pest regulation (Theenoor et al., 2024). Indirectly, systemic insecticides, such as neonicotinoids, organochlorines, organophosphates, and carbamates, are absorbed by plants and can persist in various plant tissues, including pollen and nectar, leading to inadvertent exposure of non-target insects. This exposure can impair the survival, reproductive capacity, and immune responses of beneficial insects, finally disrupting their function in pest control and pollination (Buszewski et al., 2019). Recent evidence shows that neonicotinoids, including imidacloprid and thiamethoxam, impair foraging behavior, learning, and colony growth in honeybees and wild bees (Woodcock et al., 2017). Overall, research indicates that pesticide exposure impairs biological control and pollination processes and contributes to ecological imbalance in agroecosystems.

Pesticides are also considered to be an important source of pest resistance. The main mechanisms underlying pest resistance are target-site resistance and metabolic resistance. In the case of target-site resistance, the insecticide's binding site is modified, mutated, or lost, rendering the target site incompatible with activation. In parallel, metabolic resistance occurs when enzymes that break down or detoxify insecticides are overproduced. In this last case, insecticides are broken down before reaching their target site, leading to pest resistance (Le Goff and Giraudo, 2019; Khan et al., 2020). For example, the first insecticide resistance mechanism reported in *M. persicae* was the increased production of carboxylesterases, which confer broad-spectrum resistance to organophosphates, carbamates, and, in some cases, pyrethroids (Bass et al., 2014). Many cases of resistance were observed in *P. xylostella* to old insecticides such as deltamethrin, chlorpyrifos (Agboyi et al., 2016), and abamectin (Pu et al., 2010), as well as to novel insecticides such as fluxametamide (Roy et al., 2023). Other cases of pest resistance have been reported in common *Brassica* pests, including *B. brassicae* to dimethoate (Hamedani Radja et al., 2020). Insect resistance to pesticides represents a significant challenge for modern agriculture, as it increases the frequency and volume of pesticide applications, resulting in higher costs, potential yield losses, and greater impacts on health and the environment (Le Goff and Giraudo, 2019).

Regulatory challenges in pesticide use in DRC

To address global challenges posed by pesticides, the World Health Organization (WHO) recommended classification of pesticides by hazard provides a global reference for acute toxicity categorization based on LD_{50} values. An LD_{50} (lethal dose 50) is an estimated quantity of toxicant per kg of body weight required to kill 50% of a large population of tested animals. This classification – ranging from Class Ia (extremely hazardous) to Class U (unlikely to present acute hazard) – is frequently used by national regulatory authorities and other stakeholders for pesticide registration, labeling, and risk communication (Table 1) (WHO, 2020). The International Code of Conduct on Pesticide Management, jointly developed by FAO and WHO, serves as a voluntary normative standard that guides governments, industry actors, users, and other stakeholders in the responsible management of pesticides (FAO and WHO, 2014).

In DRC, the existing regulations also promote the judicious use of pesticides. National regulation is primarily based on the agricultural law No. 11/022 of December 24, 2011 (Democratic Republic of Congo, 2011a), which governs agricultural inputs, and the law No. 11/009 of July 9, 2011 on the environment (Democratic Republic of Congo, 2011b), which addresses hazardous substances management. In addition, an earlier decree No. 05/162 of November 18, 2005 on phytosanitary regulations (Democratic Republic of Congo, 2005) defined the conditions for the marketing and use of pesticides. Moreover, DRC is a party to major international conventions that frame pesticide governance, including the Rotterdam Convention, on the procedure for international trade of hazardous pesticides (Rotterdam Convention, 2005). Although these regulations exist, the enforcement capacity remains weak, resulting in widespread circulation of counterfeit or highly hazardous pesticides (Minengu et al., 2021). Pesticide use is characterized by unregulated application, including reliance on banned or unregistered products such as endosulfan and dichlorvos (Balasha et al., 2023), excessive doses and unsafe handling practices, driven by low awareness and the dominance of illegal importations and informal markets (Balasha et al., 2023; Gakuru et al., 2025b). More mechanisms are needed to ensure farmers' security and consumer food safety.

Table 1. The World Health Organization recommended classification of pesticides by hazard

Class		**LD_{50} for the rat (mg/kg body weight)**	
		Oral	**Dermal**
Ia	Extremely hazardous	<5	<50
Ib	Highly hazardous	5-50	50-200
II	Moderately hazardous	50-2000	200-2000
III	Slightly hazardous	Over 2000	Over 2000
U	Unlikely to present acute hazard	>5000	>5000

1.1.5. Integrated management of vegetable pests

Cultural control

Human activities disturb ecosystems at all trophic levels and modify interactions among biological and ecological communities, including cultivated plants, natural habitats, and above-ground and soil biodiversity (Schowalter, 2012). Resilient farming systems and cultivation practices must be adapted for sustainable pest management. Cultural control covers all agricultural practices designed to reduce insect pest populations on crops by making the environment unfavorable to their survival or spread (Sorensen et al., 2016). This approach includes simple farming measures, such as selecting resistant or tolerant varieties and adjusting sowing dates, as well as much more complex practices, including the spatial and temporal configuration of agroecosystems (Walgenbach, 2018). For example, a study by Cokola et al. (2024) in eastern DRC showed that an early planting date for maize had a strong influence on reducing larval density and the severity of *Spodoptera frugiperda* (J.E. Smith, 1797) compared to a late planting period. The planting date can also significantly affect cabbage aphids, *Lipaphis pseudobrassicae* (Davis, 1914) and *B. brassicae*, as reported by Sidoine and Xavier (2022).

Furthermore, crop diversification through crop rotation and crop diversity at the plot and landscape levels is highly recommended to promote natural pest control (Otieno et al., 2020). Intercropping is a traditional agricultural practice observed in many Asian and African regions. It involves the simultaneous production of two or more species on the same plot for a significant period (without necessarily being sown or harvested simultaneously). This practice facilitates biological and cultural interactions that contribute to crop health (He et al., 2019). Various studies on cabbage crops have shown the positive effects of intercropping on reducing pest abundance, pest oviposition (Jankowska et al., 2009), and increasing the presence of natural enemies (Cai et al., 2010). The use of companion plants particularly plays a crucial role in agroecological production systems (Djian-Caporalino et al., 2020). Companion plants are defined as plants associated with the main cash crops to ensure ecosystem services, such as regulating pest populations by discouraging their establishment or by hosting their natural enemies (Zuma et al., 2023). For example, aromatic plants have demonstrated their effectiveness in pest regulation through the volatile organic compounds they emit. Research by Yang et al. (2025) showed that combining garlic with cabbage significantly reduced aphid survival, fecundity, and population growth. All these techniques that modify the agricultural landscape to reduce pest establishment on host plants or to create unfavorable conditions for their development are referred to as the "bottom-up" strategy. This strategy focuses on the indirect regulation of higher trophic levels (pests) by lower trophic levels (plants), primarily through plant chemical and visual cues (Almdal and Costamagna, 2023).

The use of trap crops is increasing interest in habitat manipulation against insect pests (Badenes-Perez and Shelton, 2006). Trap crops are naturally or artificially attractive plants grown to lure pests away from the target crop. Trap plants can be used to keep insects clustered in one part of the field, where they can be destroyed using physical or chemical methods (Walgenbach, 2018). For example, using white mustard as a trap crop has been effective in reducing DBM infestations in cabbage (Daniarzadeh et al., 2014).

Cultural control practices, including crop rotation, intercropping, trap cropping, and cover cropping, are essential in sustainable pest management. By altering crop environments and influencing interactions among plants, pests, and their natural enemies, these practices reduce pest establishment and survival (He et al., 2019). Cultural control contributes to ecosystem resilience, lower pesticide dependence, and enhanced biodiversity, making it a key component of environmentally friendly agriculture.

Physical control

Physical and mechanical methods aim to reduce pest populations through manual operations, machinery, and other devices that physically affect pests or their survival environment (Sorensen et al., 2016). Physical control includes passive techniques such as physical barriers (nets, mulches) and trapping techniques, as well as active methods such as hand-picking, thermal techniques, and destruction of infested plant residues (Vincent et al., 2003). In DRC context, vegetable growers commonly rely on manual methods to collect and destroy lepidopteran larvae. Active physical control can be effective when insects or their larvae are large, slow-moving, and few in number (Cokola et al., 2023b; Gakuru et al., 2025b).

Physical barriers refer to living or non-living materials used to delimit space and limit pest flows (Vincent et al., 2003). For example, insect-proof nets are recommended to protect sensitive crops, including fruit orchards and vegetable crops, as a generic protection against insect pests while providing sufficient natural ventilation (Nordey et al., 2017). Research by Kiptoo et al. (2015) revealed low larval infestations of *P. xylostella* on cabbage crops covered with pest exclusion nets and higher yields compared to no-net use plots. In addition, protective covers prevent infection by phytoviruses commonly carried by whiteflies and aphids such as *B. brassicae* and *M. persicae* (Sorensen et al., 2016). To increase the effectiveness of insect nets and other physical barriers, it is advisable to treat the nets with an insecticide or an insect repellent. This is particularly effective against sucking insects that seek tiny passageways through nets (Martin et al., 2013). Covering crops with nets for specific periods of the day may also increase protection. A study by Licciardi et al. (2008) examined the impact of tunnel screens on cabbage pests. They discovered that the temporary screens (from 05:00 pm to 09:00 am) provided more effective control against *P. xylostella* and *H. hundalis* and were more economically viable than permanent screens and conventional insecticide treatment. Other physical control methods for vegetable crops include traps impregnated with kairomone baits and sticky traps; for example, for leaf miners *Liriomyza* spp. (Diptera: Agromyzidae) are attracted to yellow sticky traps, which can be used for their monitoring and mass trapping (Schiffers and Wainwright, 2011).

Physical control methods are environmentally friendly and well-suited to small-scale vegetable growers in DRC, particularly when integrated with other IPM components adapted to local conditions. However, they can be labor-intensive and require good field monitoring to be effective. Without adequate resources, local farmers in DRC mainly rely on mosquito nets to protect their vegetable gardens.

Semiochemical control

Semiochemical control is based on chemical interactions between living organisms of the same or different species. Scientific advances have enabled understanding of the role of semiochemicals in pest-host plant recognition relationships (Smart et al., 2014). Phytophagous insects use visual and chemical stimuli to locate their host plants. For the visual stimuli, phenomena such as phototaxis and geotaxis are useful for short-distance recognition of the host plant. Phototaxis is a reaction towards or away from a light source (positive and negative phototaxis, respectively) (Kim et al., 2019), while geotaxis is an orientation reaction triggered by gravity, *i.e.*, towards or away from the earth (positive and negative geotaxis, respectively) (Bae et al., 2016). The detection of a host plant by phytophagous insects over long distances requires perception of semiochemicals (Cook et al., 2007). Semiochemicals are classified into pheromones, which act within the same species, and allelochemicals, which facilitate interspecific communication (Smart et al., 2014). Semiochemicals can be used to manage pests in two ways: to monitor pest presence and to reduce pest infestations (e.g., mass trapping, mating disruption) (Belmain et al., 2013). For example, the use of pheromone traps has become an interesting strategy because they can predict pest abundance (e.g., based on the number of individuals captured by the trap) and guide decision-making to implement an appropriate control method (Smart et al., 2014). It can be highly effective for predicting aphid infestations in vegetable crops.

More recent research in chemical ecology has focused on understanding plant production systems that use naturally produced allelochemicals. The group of allelochemicals produced by plants that affect the behavior of other organisms, including insects, is known as volatile organic compounds (VOCs) (Khan et al., 2008). For example, plants responding to insect attacks produce a group of VOCs, often referred to as Herbivore-Induced Plant Volatiles (HIPVs), that can act as repellents for other phytophagous and as attractants for beneficial organisms such as predators and parasitoids (Xiu et al., 2019). Other VOCs can naturally be produced by non-attacked plants, affecting insect behavior. For example, VOCs emitted by aromatic plant species, such as onion and marigold, have been reported to impact the host-finding behavior of cabbage aphids, reducing infestation (Mutiga et al., 2010; Jankowska et al., 2009).

"Push-pull" and "attract-and-kill" strategies are typical applications demonstrating the role of semiochemicals in crop protection (Rodriguez-Saona and Stelinski, 2009). The principle of the "Push-pull" approach is the combination of negative signals that repel pests away from the main crop ("*push*" effect) and attractive stimuli that divert pests away from the protected resource towards a trap ("*pull*" effect) (Cook et al., 2007). The push-pull strategy concerns not only semiochemical release but can also be implemented by linking repellent and attractive plants or by combining intercropping with semiochemical release (Xu et al., 2018). Push-pull applications in cabbage crops offer positive outcomes, as demonstrated by da Silva et al. (2022). In the "attract-and-kill" strategy, target organisms are attracted to an attractant (a visual and/or semiochemical cue), and an associated insecticide then destroys them (Vernon et al., 2016). The attract-and-kill strategy differs from the mass trapping method in that it relies on an attractant combined with a toxic factor rather than a physical retention (e.g., a sticky surface or liquid holder) (Rodriguez-Saona and Stelinski, 2009). In a plant mediated variant of attract-and-kill, Zhu et al. (2021) demonstrated that faba bean plants treated with aqueous extract of Chinese kale acted as dead-end trap plants, attracting *P. xylostella* for oviposition while inhibiting larval development. Semiochemical-based approaches offer several advantages, including species specificity, reduced risks to beneficial organisms, and compatibility with smallholder farming systems. However, their adoption in Congolese vegetable production remains limited by the lack of technical knowledge and access to standardized inputs.

Plant-based biopesticides

For millennia, pesticidal plants were widely promoted in agriculture until the 1940s, when synthetic pesticides were developed (Anjarwalla et al., 2020). In Africa, using these plants is an ancestral practice whose knowledge was passed down from generation to generation. Pesticidal plants are plants whose chemical properties of organs, secretions, or emissions can be exploited to control harmful organisms (Yarou et al., 2017b). They are plant species that naturally produce bioactive compounds that can deter, repel, or kill agricultural pests. Pesticidal plants can be used in various forms, including aqueous extracts, powders, and essential oils (Park and Tak, 2016).

The use of botanical insecticides is highly promoted in integrated pest management because they are often effective at low rates, offer less exposure, and break down faster than synthetic pesticides (Stevenson et al., 2017). In DRC, the use of pesticidal plants is increasingly recognized among smallholder vegetable farmers as a low-cost and eco-friendly alternative control method (Kyana et al., 2024). Local species such as neem (*Azadirachta indica* A. Juss), castor (*Ricinus* communis L.), papaya (*Carica papaya* L.), chili pepper (*Capsicum frutescens* L.), garlic (*Allium sativum* L.), lantana (*Lantana camara* L.), and *Eucalyptus* spp. are commonly used to control key pests of vegetable crops (Koleramungu et al., 2018; Cokola et al., 2023b). The neem *A. indica* is one of the pesticidal plants with various benefits for crop protection. Its compounds can act as repellents and anti-feeding effects, as well as affect egg-laying and larval growth in certain arthropods (Adusei and Azupio, 2022). Promising findings have been reported in DRC with different botanical extracts tested against *Brassica* pests, including *Tithonia diversifolia* (Hemsl.) A.Gray, *Tephrosia vogelii* (Hook.f.), and *Capsicum pubescens* (Ruiz & Pav.) against the cutworm *Agrotis ipsilon* (Hufn., 1766) (Byamungu et al., 2024), and *Capsicum frutescens* L. against cauliflower pests (Gakuru et al., 2019). While botanical insecticides offer promising pest suppression, challenges remain in standardizing extraction methods, ensuring consistent efficacy, and training farmers in safe preparation and application techniques.

Regarding the use of essential oils, recent research in DRC shows promising results with essential oils from indigenous plant species in the management of field pests. For example, a research by Mbula et al. (2023) on the essential oil of *Drypetes gossweileri* S. Moore (Putranjivaceae) showed significant biological activity against *Spodoptera littoralis* (Boisduval, 1833) and *M. persicae*, suggesting real potential for applications in Congolese agroecosystems. However, challenges remain about variability in oil composition (depending on plant species, harvest time, and extraction method) (Nea et al., 2020), limited field-based studies under Congolese farming conditions, and the need for standardized application protocols to achieve consistent efficacy. Great caution should be exercised when using essential oils, as some may be phytotoxic or harmful to humans (Werrie et al., 2020). For example, rotenone is a compound derived from *Tephrosia* spp. and several other plant species, and is effective against leaf miners, aphids, and bugs. However, it remains toxic to humans, fish, and other animals at high concentrations (Anjarwalla et al., 2020).

Natural pest regulation with natural enemies

Natural regulation is a key ecological process in IPM, relying on the activity of natural enemies to suppress pest populations below economic thresholds (Bellone et al., 2023). Beneficial organisms, such as predators and parasitoids, form an essential part of the natural balance in agroecosystems (Mkenda et al., 2019). Predation refers to the direct consumption of prey by entomophagous arthropods. In contrast, in parasitism, the natural enemy (parasitoid) develops on or inside the host's body, using it for oviposition and the emergence of its offspring, generally leading to the host's death (Stenberg et al., 2021). In vegetable systems, different arthropod groups regulate sucking pests and chewing lepidopterans. Generalist predators, including ladybirds (Coleoptera: Coccinellidae), hoverflies (Diptera: Syrphidae), and lacewings (Neuroptera: Chrysopidae), are voracious and have been reported to be effective in aphid management (Xiu et al., 2019). Other generalist biocontrol agents, such as predatory mites, are increasingly being implemented for their effectiveness against various insect pests (Francis et al., 2020). Hymenopteran parasitoids are extensively used as they are more specific than predators, resulting in a more restricted host range while minimizing non-target adverse effects. For example, *Diaeretiella rapae* (McIntosh, 1855) (Hymenoptera: Braconidae) has been shown to be effective against the cabbage aphid *B. brassicae* in cauliflower crops (Verma et al., 2019). Another case study of Cobblah et al. (2012) illustrated the parasitoid *Cotesia plutella* (Kurdjumov, 1912) (Hymenoptera: Braconidae) as an efficient control agent against *P. xylostella* in cabbage crops.

Natural pest regulation can be achieved either by importing and establishing permanently exotic species of natural enemies (*classical biological control*), by directly manipulating populations of natural enemies for temporarily establishment, e.g. release from mass-reared arthropods (*augmentative biological control*), or finally by managing habitat for enhancing populations of natural enemies, e.g. establishing flower strips (*conservation biological control*) (Stenberg et al., 2021). Hence, the top-down strategy supports the recruitment of natural enemies through good agricultural practices (Bellone et al., 2023). The introduction of exotic biocontrol agents may pose ecological risks, as released predators may attack non-target species and reduce biodiversity. Therefore, non-target effects must be carefully assessed prior to introduction. Preference should be given to specialist natural enemies over generalists, with limited dispersal capacity to reduce ecological threats (Francis et al., 2020). Closed vegetable production systems, particularly greenhouses, are ideal for augmentative biological control because they provide a favorable environment for natural enemies, limiting their dispersal and reducing the arrival of new pests (Walgenbach, 2018). Currently, no studies have documented the mass production of predators or parasitoids for biological control in DRC. However, recent observations in cabbage fields have reported the natural presence of natural enemies, including Syrphidae and Braconidae species, indicating that natural regulation processes are already functioning in smallholder systems (Gakuru et al., 2024).

Microbial control

Biopesticides are living organisms or their derived products used to control crop pests. Some research uses the expression biopesticides more specifically to refer to commercially available microbial agents, including bacteria, fungi, and viruses (Belmain et al., 2013). Microbial control agents play an important role in the natural regulation of insect populations in agroecosystems, thereby reducing the threat they pose to crop yields. Their use has several advantages, including easy dissemination, efficacy at low doses, and persistence after application (Quesada Moraga, 2020). Entomopathogenic fungi (EPF) are particularly preferred since they can infect insects through cuticle contact (Shang et al., 2024). They penetrate the insect's body, where they grow. They release toxic secondary metabolites, disrupt the circulatory system, and deplete nutrients, leading to host mortality. After the host's death, the fungus emerges through the cuticle, sporulates on the cadaver's surface, and releases new conidia that can infect other insects (Mantzoukas et al., 2022b). *Beauveria bassiana* (Bals.-Criv.) Vuill. and *Metarhizium anisopliae* (Metschn.) Sorokin are among the most studied EPF against a wide range of arthropods, including sap-sucking and chewing insects (Nouh et al., 2022). For example, *B. bassiana* is effective against economic *Brassica* pests, including *B. brassicae* (Gakuru et al., 2025a), *P. xylostella, P. rapae* (Nouh et al., 2022), and *M. persicae* (Dessauvages et al., 2024).

EPF of increasing interest in biological control are endophytes, which can colonize plants' inner tissues without causing external symptoms on the host plants (Jaber and Ownley, 2018). Endophytic fungi operate through multiple mechanisms, including the production of toxic metabolites, the induction of plant resistance, and the promotion of plant growth (Gupta et al., 2022). For example, *B. bassiana* has been shown to colonize plant tissues as an endophyte while providing beneficial effects on pest and abiotic stress resistance (Quesada Moraga, 2020). The increase in resistance due to endophytic EPF is attributed to food deterrence or antibiosis, which results from the production of induced metabolites. Additionally, endophytes may alter plant odor, thereby attracting natural enemies and contributing to pest control (Francis et al., 2020). The effectiveness of EPF can be enhanced when combined with other biocontrol agents, such as plant-based biopesticides. For example, the study by Gebreyohans et al. (2022) showed that the combination of EPF *B. bassiana* with plant extract of *A. indica* produced the highest reduction in *B. brassicae* populations, compared to the EPF and the plant extract alone. The efficacy of EPF can be significantly influenced by a complex of abiotic factors, including humidity (a high level is required), ambient temperature (23–28°C), rainfall (which can lead to significant removal of conidia from leaf surfaces), and sunlight (high radiation levels can cause conidial death). However, the large-scale use of microbial biopesticides raises questions about their high production costs, effectiveness across diverse ecosystems, potential toxicity, pathogenicity, and environmental persistence (Jaronski, 2010).

Control methods covered in this thesis

In this thesis, three main areas of integrated pest management were assessed. First, the study focused on the diagnosis of the entomofauna (both pests and natural enemies) associated with cabbage and farmers' pest management practices. Then, aromatic plants were evaluated both as part of cultural control by serving as repellent companion plants, and as plant-based biopesticides through the use of their essential oils. The final component explored was microbial control by assessing the biocontrol potential of endophytic entomopathogenic fungi. These combined approaches are part of an overall integrated pest management framework, as they include good farming practices, pest monitoring, and curative intervention using biological methods.

1.2. Thesis objectives and structure

The objective of this thesis is to develop alternative methods to control major pests affecting cultivated vegetables, in this case *Brassica* crops, in eastern DRC.

The specific objectives (SO) and corresponding hypotheses are as follows:

- **SO1**: Identify the main insect pests and associated natural enemies in *Brassica* crops in eastern DRC.

  Hypothesis 1: Lepidoptera larvae (*Plutella xylostella*, *Hellula undalis*, …) and aphids (*Brevicoryne brassicae* and *Myzus persicae*) mostly affect *Brassica* crops.

  Hypothesis 2: There is a diversity of predators (Coccinelidae, Syrphidae, and Chrysopidae) and parasitoids (Braconidae and Ichneumonidae) affecting *Brassica* phytophagous pests.

- **SO2:** Identify the control methods used by *Brassica* growers against insect pests.

  Hypothesis: The use of chemical control and certain ancestral strategies based on pesticidal plants.

- **SO3**: Assess the potential of aromatic plant species, both as companion plants and as essential oils, to manage cabbage aphids.

  Hypothesis 1: *Cymbopogon citratus*, *Lantana camara*, and *Lippia alba* can be associated with *Brassica* crops to reduce cabbage aphid infestations.

  Hypothesis 2: The essential oils from *C. citratus*, *L. camara*, and *L. alba* can induce mortality and repellent effects against cabbage aphids.

- **SO4**: Assess the efficacy of entomopathogenic fungi as biocontrol agents against cabbage aphids.

  Hypothesis: The indigenous strain *Beauveria bassiana* KA14 from DRC can be effective as the commercial strain *B. bassiana* GHA against cabbage aphids.

The research is structured into six chapters:

Chapter 1 focuses on a review of the literature. It provides an overview of vegetable crops in the DRC, with an emphasis on *Brassica* crops. This section highlights the damage caused by the main pests, the challenges of chemical control, and available alternatives for vegetable farming.

Chapter 2 assesses the entomofauna associated with cabbage to identify the main pests and natural enemies. Insects were collected using yellow pan traps and a sweep net and then brought to the laboratory for identification.

Chapter 3 covers farmers' knowledge of the main pests affecting cabbage and the control methods used to manage them. A survey of farmers was conducted, focusing on synthetic insecticides used and associated risks, as well as available alternative resources.

Chapter 4 focuses on the valorization of tropical aromatic plants (*Cymbopogon citratus*, *Lantana camara*, and *Lippia alba*) against cabbage aphid in two ways: firstly, associating these plants with cabbage to assess their repellent properties as companion plants; and secondly, using essential oils from these aromatic plants to assess their toxicity and repellent effects on cabbage aphids.

In **Chapter 5,** we examine the natural regulation of cabbage aphids using different strains of the entomopathogenic fungus *Beauveria bassiana*, particularly an indigenous strain isolated from insect cadavers collected in eastern DRC.

Finally, **Chapter 6** provides a general discussion of the results achieved, prospects for sustainable management of *Brassica* crop pests, and a conclusion.

Chapter 2

Cabbage insect pests and associated natural enemies in eastern DRC

Collecting insect samples with a sweep net on cabbage crops. © P.N. Gakuru

Chapter 2. Cabbage insect pests and associated natural enemies in eastern DRC

This section was adapted from original published article: **Gakuru, P.N.,** Noël, G., Muhashy Habiyaremye, F., Francis, F. (2024). Monitoring entomofauna associated with cabbage, *Brassica oleracea* L. var capitata in eastern DR Congo. *Journal of Insect Biodiversity and Systematics* 10(3), 453-466. https://doi.org/10.61186/jibs.10.3.453

Abstract

Cabbage is an important component of agriculture in the eastern Democratic Republic of Congo (DRC) for its various food uses. It is important to identify pests that affect its production, as this is still insufficient to meet the increasing consumer needs. Furthermore, insect data for tropical agroecosystems, particularly in the DRC, are limited, leading to a poor understanding of insect diversity. The objective of this study is to assess the diversity and abundance of entomofauna associated with cabbage across five cabbage-growing sites: Minova, Sake, Kibumba, Rutshuru, and Lac Vert. Insects were collected using a sweep net and yellow pantraps during 2021 and 2022. A total of 1109 insects from 9 orders and 48 families were collected. The most abundant pest family was Aphididae. Other important families were Cicadellidae (8%), Coreidae (7%), Chrysomelidae (5%), and Pentatomidae (4%). The prominent families of beneficials (natural enemies and pollinators) identified were Syrphidae (7%), Carabidae (4%), Coccinellidae (3%), and Apidae (3%). Insect communities across different locations are both similar and diversified. Given the functions these functional groups play as ecological indicators, it is important to preserve entomological biodiversity. This is achieved through the rational selection of pest management methods to optimize ecosystem services provided by beneficials.

Keywords: cabbage, insects, pests, beneficials, Democratic Republic of Congo

2.1. Introduction

Cabbage, *Brassica oleracea* L. (Brassicaceae), is an important vegetable widely grown in the eastern Democratic Republic of Congo (DRC). Consumed in both raw and cooked form, cabbage contains several vitamins and other essential nutrients. It is also an important source of income for many producers (Macharia et al., 2005). Cabbage is preferred for its relatively short growth cycle and year-round production. However, the quantities produced remain insufficient to meet the growing number of consumers. In 2020, annual cabbage production in DRC was 20,554 tonnes (FAO, 2020).

The Cabbage crops are seriously affected by attacks of many pests, including aphids (Hemiptera: Aphididae) and caterpillars such as *Plutella xylostella* (L., 1758) (Lepidoptera: Plutellidae), *Hellula undalis* (Fabricius, 1781) (Lepidoptera: Crambidae), and others from the genus *Spodoptera* (Guenée, 1852) (Lepidoptera: Noctuidae) (Ngom et al., 2020). Aphids are also harmful pests for cabbage as they suck sap useful for plant growth, transmit both toxins and viruses (Fidelis et al., 2018; Sarwar, 2020). Some aphids, such as *Brevicoryne brassicae* (L., 1758), attack a specific host range (Brassicaceae) while others, such as *Myzus persicae* (Sulzer, 1776), attack hundreds of plant species from different families (Nalam et al., 2019). Other hemipterans belonging to the Aleyrodidae and Pentatomidae families, also polyphagous, can cause significant damage to cabbage (Capinera, 2020). The diamondback moth, *P. xylostella*, without adequate protection, can cause losses up to 90% or more (Macharia et al., 2005; Mondedji et al., 2014). It is present in almost all regions of the world and attacks mainly Brassicaceae plants to which it is attracted by glucosinolates, sulphur compounds characteristic of this family (Arvanitakis, 2013).

To control pests, farmers from DRC usually use chemicals. Unfortunately, these products are toxic to humans, animals, and the environment (Kumar et al., 2019). In addition, chemicals cause resistance in pests and lead to the death of non-target organisms, such as pollinators and natural enemies (Tsaboula et al., 2016). The reported effects on beneficials can be direct (physiological and behavioral disorders) or indirect (destruction of oviposition and mating sites) with serious consequences for their survival and the ecosystem services they provide (Desneux et al., 2007).

The insect class is the largest group of multicellular organisms in the world, with a diversity of species that provide many ecosystem services. The most well-known services are pollination, natural pest regulation, and decomposition of organic matter (Losey and Vaughan, 2006). Quantitative and economic assessments of these services enable a better understanding of the contribution of functional groups. Gallai et al. (2009) estimated, for example, that pollinator services worldwide are worth 153 billion euros, representing 9.5% of the value of global food production. In this context, hoverflies (Diptera: Syrphidae) provide both pollination and biological control. Adults pollinate plants by visiting flowers, where they feed on nectar and pollen, while their larvae feed mainly on small insect pests such as aphids and thrips (Dunn et al., 2020). Hymenopteran parasitoids also contribute to the natural regulation of pests. For example, aphid parasitoids belonging to the Braconidae and Aphelinidae families have been shown to be effective as they mainly attack target pests. Many insect species decompose plant and animal waste, thereby recycling nutrients. Beetles, termites, ants, and springtails improve soil structure and fertility (Chagnon et al., 2001; Deprince, 2003).

Many farmers in the DRC are unaware of the benefits of maintaining entomological diversity and the roles that beneficials play in agroecosystems. Scientifically, very little research exists on the inventory of entomological diversity in vegetable crops. For cabbage, no studies have been found that assess insect abundance, diversity, or functional groups in DRC. In such a context, it is challenging to adopt sustainable pest management models without first knowing the pests and natural enemies present in the environment. The purpose of this study is to assess the diversity and abundance of insect pests and beneficials associated with cabbage crops in the eastern DRC. In addition, the presence of different insect groups is assessed based on the geographical differences among the sampling sites.

2.2. Materials and methods

Study sites

The research was carried out in eastern DRC in five locations: Sake, Kibumba, Rutshuru, Lac Vert (North-Kivu province), and Minova (South-Kivu province) (Figure 1). These areas have a humid tropical climate and/or a relatively temperate climate, particularly in Kibumba. The coordinates collected indicate altitudes ranging from 1100m to 2200m. Table 2 provides latitude, longitude, and climate information of the study areas. The existing soils are Ferralsols and especially Andosols of volcanic origin. These materials are porous and rich in amorphous minerals (Ngongo et al., 2009). Local growing seasons are characterized by alternating dry and rainy periods. Samplings were conducted during the 2021 short rainy season (March to August) and the 2022 long rainy season (September to February).

Table 2. Geographical coordinates and Climate information of the study areas

Sites	Geographical coordinates	Climate information
Minova	S 01° 42' 42,7" E 029° 00' 47,6" Alt 1489m	Highland agricultural area with a tropical climate bordering Kivu Lake
Rutshuru	S 01° 08' 47,8" E 029° 25' 32,9" Alt 1113m	Mid-altitude zone with a humid tropical climate
Kibumba	S 01° 30' 42,1" E 029° 19' 49,9" Alt 2028m	High altitude region with a relatively temperate climate, bordering the Virunga National Park
Sake	S 01° 33' 56,6" E 029° 03' 11,0" Alt 1495m	Humid tropical zone located to the west of Goma city
Lac vert	S 01° 33' 53,2" E 029° 09' 16,4" Alt 1543m	Peri-urban site in Goma city with a humid tropical climate and volcanic soil.

Collection and identification of insects

For each location, 10 elementary plots of 10 m² each, selected from cabbage farms of at least 500 m², were monitored (Yarou et al., 2018). Plots were selected at a minimum distance of 3 meters from field edges to limit the border effect (Polo Lozano et al., 2013). Each plot served as an experimental unit and was trapped using two methods to collect as many insects as possible from different groups. Three samplings were carried out per site per cropping season following the cabbage growth. They were mainly carried out using yellow pan traps and sweep nets as proposed by Campbell & Hanula (2007) and Ghani & Maalik (2020), respectively. For the first method, yellow pan traps (diameter 27cm, height 10cm) were placed in the elementary plots with one trap per plot. Traps were placed in the morning and removed in the evening. They were adjusted to absolute crop height to favor insect capture (O'Connor et al., 2019). They contained an aqueous solution that did not exceed the dipstick, with a few drops of dishwashing liquid added to reduce the surface tension of the water and increase the retention of trapped insects. The solution was renewed for each monitoring day. The trapped insects were collected with a sieve, a brush, and soft tweezers to preserve their condition (Chmelíková and Wolfrum, 2019; Mignon et al., 2003). For the second trapping method, each elementary plot (10m²) was swept with a net (Figure 2) for an average of fifteen minutes. This method is mainly used to catch flying insects. The collection consisted of moving through the field, mowing any insects flying over the cabbage plants. To trap as many insects as possible, the surface vegetation was also mowed with rapid lateral movements (Yarou et al., 2018). All insects collected (sweep net & yellow trap) were placed in plastic bottles containing 70% ethanol and then brought to the laboratory for identification. Insects were identified to the family level using the keys of Delvare and Aberlenc (1989) and Mignon et al. (2016). The main functional groups considered in the identification were pests, predators, parasitoids, pollinators, mixed (i.e., those grouping both genera and/or species of beneficials and phytophagous), and neutrals (i.e., insects with various functions without direct agronomic impact or not yet defined).

Due to the high abundance of apterous aphids (Hemiptera: Aphididae) on cabbage leaves, direct observations on plants in the elementary plots were used to express infestation levels (Lopes et al., 2012). The evaluation scale was designed based on the models of Banks (1954), Fening et al. (2014), and Lopes et al. (2012). It is presented as follows: 0: total absence of aphids; 1: presence of one aphid to a few individuals (<20 aphids); 2: a few isolated colonies (20 to 50 aphids); 3: several isolated colonies (50 to 200 aphids); 4: strong presence in diffuse colonies (200 to 500 aphids); 5: very dense aphids (> 500) infesting several parts simultaneously.

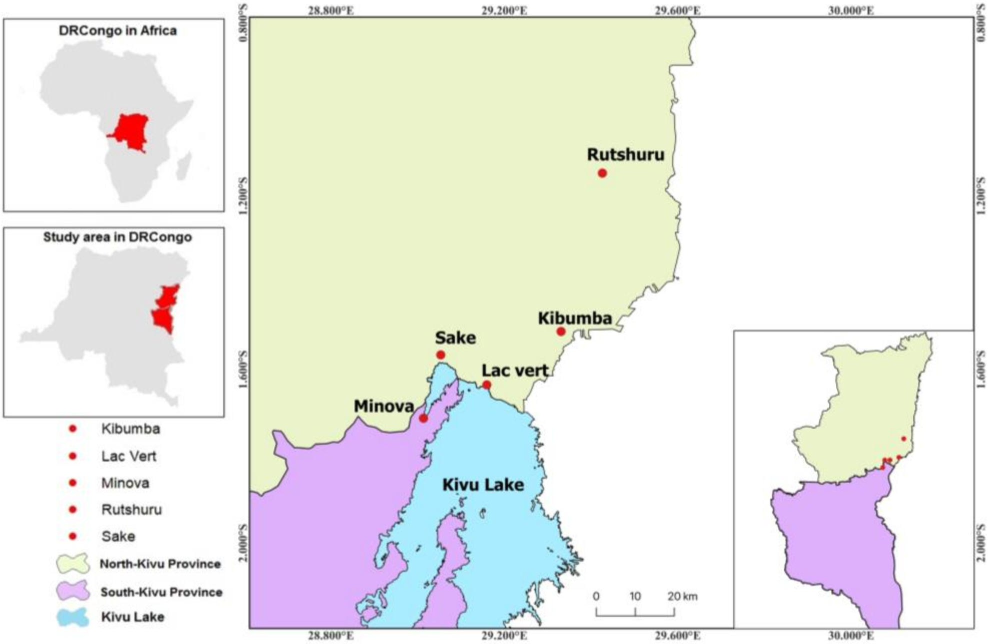

Figure 1. Location of cabbage entomofauna collection sites in eastern DRC

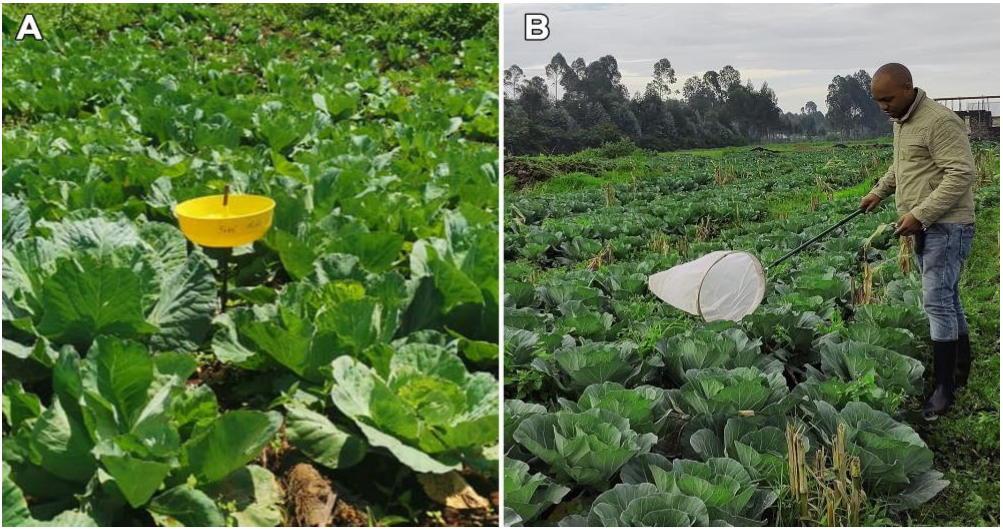

Figure 2. Methods used during cabbage entomofauna collection: A. yellow trap; B. sweep net

Data analysis

The relative abundance (**F**) was calculated using the formula **F (%) = *ni/N*100***, where ***ni*** is the number of individuals in a family, and **N** is the total number of individuals excluding Aphididae because of their high abundance. To assess the diversity of family taxa, the Shannon Index was calculated as follows:

$$\boldsymbol{H'} = -\sum_{i=1}^{S} pi.\,log_2(pi)$$

Where pi = ni/N; ni is the abundance of family i and N is the total abundance. The Pielou equitability (E) associated with the Shannon index was calculated as follows: **E** =$\boldsymbol{H'}/\boldsymbol{H'max}$ with $\mathrm{H'max} = \log_2(\mathrm{S})$, where $\boldsymbol{H'max}$ is the maximum Shannon diversity, and ***S*** represents the total number of families. The **E** value varies from 0 (dominance of one family) to 1 (equitable distribution of individuals across families) (Buckland et al., 2005).

To study the effect of sampling location on insect family diversity, we performed multivariate statistics, using two techniques: Principal Coordinate Analysis (PCoA) and Redundancy Analysis (RDA). To determine whether insect communities differed across sites, a PCoA was performed using the packages *Vegan* (Oksanen et al., 2015), *ape* (Legendre and Gallagher, 2001), and *ggplot2* (Guilherme, 2011). PCoA is a nonlinear method for exploring and visualizing similarities and dissimilarities in data, and it has the advantage of applying to all types of variables. PCoA analyses generate ordination diagrams that allow comparison of how closely related the different sites are in terms of insect family composition (Chahouki, 2011; Paliy and Shankar, 2016). RDA was carried out to determine the effect of environmental variables (elevation, latitude, longitude) on insect family richness and abundance using the packages *ggvegan* and *ggord*. RDA is a method for extracting and summarizing the variation in a set of response variables that can be explained by a set of explanatory variables (Bellamy et al., 2018; Dyola et al., 2022). An Analysis of variance (ANOVA) was computed to test the overall significance of environmental variables (p-value < 0.05). All tests were based on the Bray-Curtis distance and performed in R 4.1.3.

2.3. Results

Abundance and diversity of insect orders

A total of 1109 insects were collected in 2021 and 2022 at the five sampling locations: Minova (282 insects), Sake (239 insects), Kibumba (187 insects), Rutshuru (226 insects), and Lac Vert (175 insects). These insects are distributed in 9 orders, namely Hemiptera (30%), Coleoptera (21%), Diptera (19%), Orthoptera (10%), Hymenoptera (10%), Lepidoptera (8%), Thysanoptera (1%), Neuroptera (1%), and Odonata (<1%). Even without considering the Aphididae, Hemiptera is the most numerous order, accounting for nearly a third of the total number of insects, and are most important in Minova, Sake, and Rutshuru. Coleoptera, second in total abundance (21%), are most abundant in Kibumba, while Diptera, with almost one-fifth of the total number of individuals, have the highest abundance in Lac Vert (Figure 3A).

Abundance and diversity of insect families

Referring to Table 3, 48 insect families were identified, excluding the Aphididae which is the most abundant family (Figure 3B). With respectively 12 and 11 families, Hemiptera and Coleoptera orders rank first and second with the highest number of identified families. Except for the Aphididae, the most abundant families in Hemiptera order are the Cicadellidae, Coreidae and Pentatomidae, while among Coleopterans, Chrysomelidae and Carabidae are the most abundant. Hymenoptera order have seven families with predominance of Apidae and Formicidae. Dipterans (six families) are largely represented by Syrphidae and Sepsidae. Lepidopterans include five families among which Plutellidae and Noctuidae are the most numerous. The Tetrigidae and Tettigoniidae have the highest abundance of the 4 families found in Orthoptera order. Lastly, the single families representing the Thysanoptera, Neuroptera and Odonata orders are respectively Thripidae, Chrysopidae and Coenagrionidae.

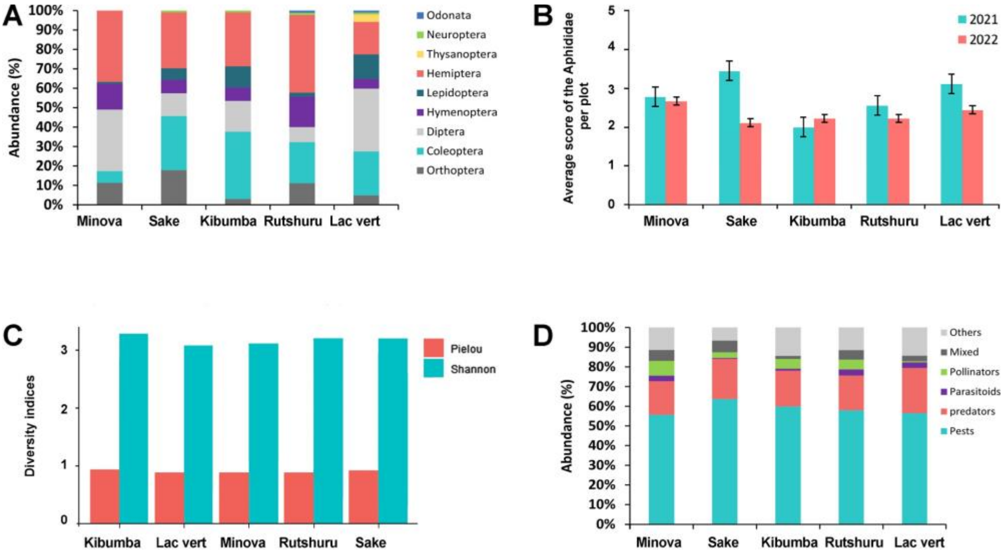

Figure 3. The relative abundance and diversity indices of entomofauna associated with cabbage in the studied areas. **A:** Relative abundance of insect orders per site; **B:** Aphids (Hemiptera: Aphididae) abundance per site per year; **C:** Shannon Index diversity and Pielou Equitability within sampled sites; **D:** Relative abundance of functional insect groups per site

Distribution of entomofauna according to site characteristics

The Shannon indices across sites show little difference, with Kibumba having the highest (3.25), followed by Sake (3.18), Rutshuru (3.14), Minova (3.09), and Lac Vert (3.03). All values are close to 3, meaning that all sites have high species diversity and are inhabited by nearly the same number of insect families. The Pielou equitability values are basically the same and tend to 1. Therefore, insect communities across different localities are similarly distributed with respect to abundance and rarity within families (Figure 3C).

The PCoA results show similarities among the insect communities from the different sampling locations (Figure 4). The lowest dispersion of insect families is observed in Kibumba. The redundancy analysis (RDA) shows a correlation between environmental factors and the distribution of insect families (Figure 5). The full RDA model is statistically significant ($p = 0.001$). Latitude, i.e., the distance of a sampling position from the equator, is the variable that significantly influences ($p = 0.001$) the distribution of insect families in the study sites.

Distribution of entomofauna in functional groups

Overall, phytophagous insects are the most widespread across the cabbage crops in the survey areas. They cover more than half of the samples at each site. The 23 families (excluding Aphididae) that group the phytophagous insects together represent 58% of the samples, including Cicadellidae (8%), Coreidae (7%), and Chrysomelidae (5%) (Table 3). Although less numerous than the pests, predators rank second in both the number of families found (9) and relative abundance (19%) (Figure 3D). Syrphidae (7%), Carabidae (4%), and Coccinelidae (3%) are the most abundant. Pollinators (Apidea, Andrenidae, and Megachilidae) account for 5% while parasitoids (Braconidae and Ichneumonidae) account for 2%. Mixed families and neutrals represent 5% and 11% respectively.

Table 3. Abundance and diversity of insect families per site

	Minova		**Sake**		**Kibumba**		**Rutshuru**		**Lac vert**		**Total**	
	N	F(%)	N	F(%)	N	F(%)	N	F(%)	N	F(%)	N	F(%)
Orthoptera	**32**	**11**	**42**	**18**	**6**	**3**	**23**	**10**	**8**	**5**	**111**	**10**
Acrididae *	4	1	6	3	0	0	13	6	1	1	24	2
Gryllidae *	6	2	15	6	0	0	0	0	0	0	21	2
Tetrigidae *	13	5	16	7	3	2	1	0	4	2	37	3
Tettigoniidae *+	9	3	5	2	3	2	9	4	3	2	29	3
Coleoptera	**18**	**6**	**68**	**28**	**66**	**35**	**44**	**19**	**40**	**23**	**236**	**21**
Chrysomelidae *	5	2	8	3	18	10	7	3	21	12	59	5
Coccinellidae +	1	0	18	8	1	1	11	5	5	3	36	3
Cryptophagida±	3	1	5	2	0	0	3	1	1	1	12	1
Dryophthoridae*	0	0	1	0	6	3	1	0	0	0	8	1
Scarabaeidae *	1	0	17	7	13	7	2	1	1	1	34	3
Geotrupidae ±	0	0	0	0	3	2	0	0	2	1	5	0
Lampyridae *+	5	2	2	1	0	0	0	0	1	1	8	1
Malachiidae *+	2	1	7	3	0	0	2	1	1	1	12	1
Staphylinidae +	0	0	0	0	8	4	1	0	2	1	11	1
Oedemeridae ±	0	0	0	0	5	3	4	2	1	1	10	1
Carabidae +	1	0	10	4	12	6	13	6	5	3	41	4
Diptera	**88**	**31**	**28**	**12**	**29**	**16**	**16**	**7**	**57**	**33**	**218**	**19**
Diopsidae *	15	5	4	2	4	2	2	1	0	0	25	2
Tipulidae *	14	5	2	1	3	2	4	2	14	8	37	3
Rhinophoridae ±	2	1	0	0	4	2	0	0	4	2	10	1
Sepsidae ±	18	6	7	3	5	3	3	1	8	5	41	4
Syrphidae +	34	12	14	6	5	3	5	2	25	14	83	7
Rhagionidae ±	5	2	1	0	8	4	2	1	6	3	22	2
Hymenoptera	**38**	**13**	**16**	**7**	**13**	**7**	**31**	**14**	**8**	**5**	**106**	**10**
Andrenidae Ω	11	4	1	0	1	1	7	3	0	0	20	2
Apidae Ω	10	4	5	2	8	4	4	2	1	1	28	3
Braconidae σ	0	0	1	0	0	0	1	0	2	1	4	0
Formicidae ±	4	1	3	1	2	1	12	5	2	1	23	2
Megachilidae Ω	0	0	1	0	0	0	0	0	0	0	1	0
Sphecidae +	3	1	0	0	0	0	1	0	0	0	4	0
Vespidae +	2	1	5	2	0	0	0	0	0	0	7	1
Ichneumonidae σ	8	3	0	0	2	1	6	3	3	2	19	2

Lepidoptera	**3**	**1**	**14**	**6**	**20**	**11**	**27**	**12**	**23**	**13**	**87**	**8**
Crambidae *	0	0	5	2	5	3	0	0	0	0	10	1
Noctuidae *	0	0	8	3	4	2	2	1	4	2	18	2
Papilionidae *	0	0	0	0	0	0	7	3	3	2	10	1
Pieridae *	0	0	0	0	3	2	2	1	9	5	14	1
Plutellidae *	3	1	1	0	8	4	16	7	7	4	35	3
Hemiptera	**101**	**36**	**69**	**29**	**52**	**28**	**81**	**36**	**29**	**17**	**332**	**30**
Acanthosomatidae *	0	0	3	1	8	4	0	0	0	0	11	1
Cicadellidae *	12	4	17	7	14	7	38	17	11	6	92	8
Reduviidae +	6	2	0	0	7	4	5	2	0	0	18	2
Cydnidae *	3	1	0	0	3	2	0	0	0	0	6	0
Cercopidae *	0	0	6	3	1	1	1	0	0	0	8	1
Coreidae *	39	14	19	8	6	3	9	4	1	1	74	7
Delphacidae *	17	6	11	5	0	0	2	1	0	0	30	3
Dictyopharidae *	5	2	0	0	0	0	5	2	7	4	17	1
Membracidae ±	0	0	0	0	0	0	2	1	1	1	3	0
Miridae *	8	3	1	0	7	4	15	7	0	0	31	3
Pentatomidae *	11	4	12	5	6	3	4	2	9	5	42	4
Thysanoptera	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**7**	**4**	**8**	**1**
Thripidae *	1	0	0	0	0	0	0	0	7	4	8	1
Neuroptera	**1**	**0**	**2**	**1**	**1**	**1**	**2**	**1**	**1**	**1**	**7**	**1**
Chrysopidae +	1	0	2	1	1	1	2	1	1	1	7	1
Odonata	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**1**	**2**	**1**	**4**	**0**
Coenagrionidae +	0	0	0	0	0	0	2	1	2	1	4	0
Total	**282**	**100**	**239**	**100**	**187**	**100**	**226**	**100**	**175**	**100**	**1109**	**100**

N: number, F: frequency, *Pests, +Predators, σ Parasitoid, Ω Pollinators, *+ mixed, ± Neutral insects.

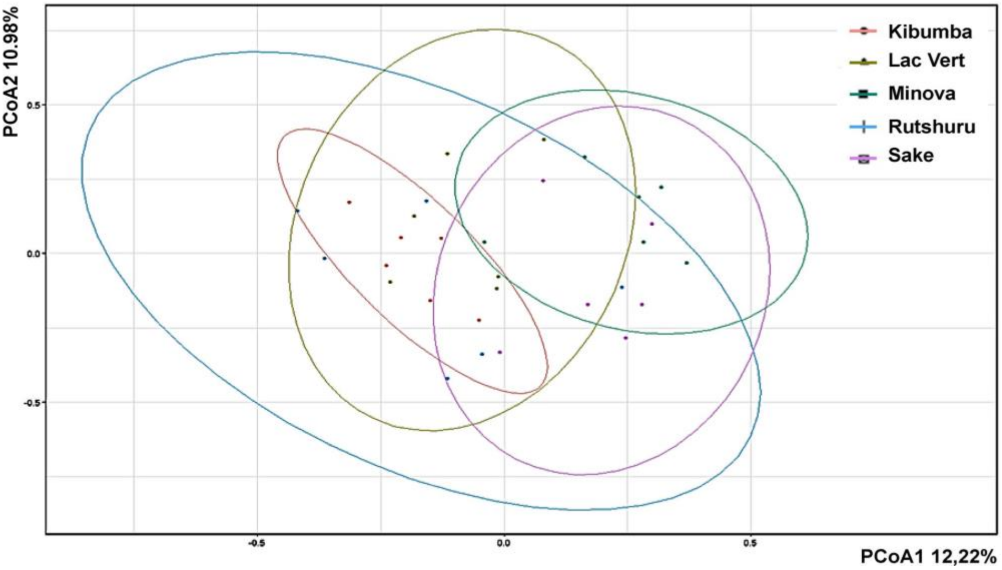

Figure 4. Principal Coordinate Analysis (PCoA) showing the community structure of insect families in five different habitats. Points in the PCoA plot represent insect samples, each colored and shaped according to the sampling site.

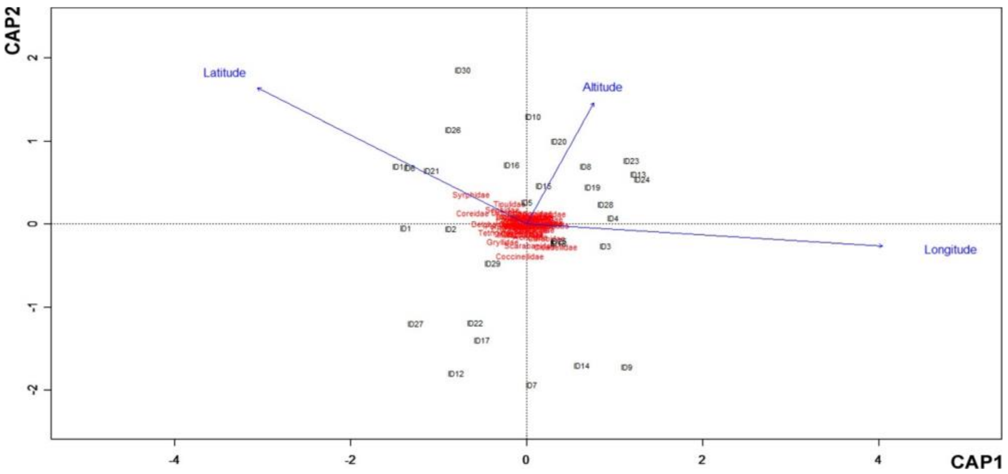

Figure 5. Redundancy Analysis (RDA) - biplot showing the correlation between cabbage insect families and the environmental variables. Red names represent insect families. Blue arrows correspond to the intensity of the environmental variables.

2.4. Discussion

Findings indicate a dominance of Hemipteran insects. Hemipterans include many biting and sucking insects and are among the most damaging pests in vegetable crops. Similar studies (Vayssières et al., 2001; Tendeng et al., 2017) conducted in tropical regions have shown a high prevalence of hemipterans in vegetable crops. Regarding the identified insect families, our results support those of Bayendi et al. (2018) in Gabon, based on an inventory of entomofauna on different vegetable crops (amaranth, tomato, cabbage, and sorrel). As with other crops, they found that Aphididae were the most abundant pests on cabbage. Aphididae are destructive pests because they suck sap from plants and transmit viral diseases. Chrysomelidae were the first most abundant family of Coleoptera. Known for their small holes drilled through the leaf blade, the damage caused by Chrysomelidae increases with their abundance (Capinera, 2020).

The results for the two diversity parameters (Shanon Index and Pielou Equitability) are similar to those observed in other agricultural ecosystems. In the PCoA, similarities among insect families across the sampled sites are likely due to similar agricultural practices that can influence entomological diversity, such as monoculture and the use of the same chemical inputs.

Among the identified functional groups, phytophagous insects are clearly the most abundant, as they are primary consumers of plant resources. Phytophagous insects are therefore the first biotic factor causing yield losses in agriculture (Prabaningrum and

Moekasan, 2020). Our results are close to (Cai et al., 2010) on cabbage cultivation in subtropical China. They found that neutral insects were more abundant, but phytophagous insects ranked second and were more numerous (27.24%) than predators and parasitoids combined (10.63%).

Among the collected insects, Hemiptera dominated because most (leafhoppers, bugs, etc.) are polyphagous and reproduce rapidly when resources are available. Regarding Aphididae, *B. brassicae* was found at all sites, whereas *M. persicae* was rarely found. *B. brassicae* mainly feeds on Brassicaceae, particularly cabbage, as found by (Byamungu et al., 2019) in South Kivu (DRC), while *M. persicae* has hundreds of hosts in different plant families (Nalam et al., 2019). High presence of *B. brassicae* would be justified because, apart from the Kibumba site, where another *Brassica* cultivar (Cauliflower) is grown, in all other sites, cabbage is the only host on which *B. brassicae* can feed. Leafhoppers (Hemiptera: Cicadellidae), which are also abundant, are considered important crop pests because they serve as disease vectors (Sarwar, 2020). Although limited in number, Lepidoptera include many damaging insects found on crops, especially, *P. xylostella* (Plutellidae), *H. undalis* (Crambidae), *Spodoptera* sp. (Noctuidae). Lepidopterans are most found in their larval stage on crops, where they cause considerable economic losses. These larvae are voracious and highly destructive, particularly in the last larval stage (Wagner, 2013). Many studies have highlighted the damage these pests cause to cabbage in tropical environments (Macharia et al., 2005; Labou et al., 2017; Mpumi et al., 2020).

Aphids and other sucking insects attract predators, especially gravid hoverflies that seek oviposition sites and food sources for their larvae (Colley and Luna, 2000). This aphid presence may explain the high presence of Syrphidae during our investigations. Regarding ladybirds, one of the major groups of natural enemies recorded, both adults and larvae are predators of aphids, whiteflies, and other phytophagous insects. This pest consumption is advantageous for biological control because it is not common for fully metamorphosing insects to maintain the same feeding regime across developmental stages (Kundoo and Khan, 2017). Findings by Fidelis et al. (2018) confirm the predatory status of the found taxa in tropical regions. They noticed that the main predators, including Coccinellidae and Syrphidae, settled where aphid colonies were located, and that predators were the leading cause of aphid mortality.

Pollinators were less numerous, possibly because cabbage plants lack inflorescences, and during our research, there were no flowering plants in the sampling farms. The same observation applies to parasitoids. Recent studies showed that open-flowering plants favor the establishment of parasitoids, wasps, and bees by providing them with nectar (Harrison et al., 2019; Herrera et al., 2022).

2.5. Conclusion

This research aimed to assess the entomofauna associated with cabbage under the growing conditions in eastern DRC. Aphids and lepidopteran larvae were among the main pests found, because they cause severe losses on cabbage. Besides plant pests, pollinators, predators, and parasitoids were recorded. These insects provide important ecosystem services (pollination, biological control), and it is important to preserve them through careful selection of control methods. Good cultural practices positively influence the recruitment of beneficial insects. In agroecosystems, this involves, for example, introducing aromatic or flowering plants to provide beneficials with shelter, oviposition sites, alternative prey, and food resources. This research provided a general overview of the cabbage entomofauna in the investigated areas and served as a necessary basis for future investigations of insect dynamics in the DRC. For future studies, we suggest monitoring all plant growth stages to optimize temporal sampling and better understand insect dynamics.

Based on this entomological assessment, a critical evaluation of the pest control methods currently used by farmers is required to assess their sustainability and their compatibility with the conservation of beneficial insects.

Chapter 3

Insights on farmers' knowledge and management strategies of cabbage pests

On the left: A farmer preparing an insecticide solution without personal protective equipment; on the right: application of insecticide with a backpack sprayer.
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Chapter 3. Insights on farmers’ knowledge and management strategies of cabbage pests

This chapter was adapted from original published article: **Gakuru, P. N.**, Muhashy Habiyaremye, F., Noël, G., Caparros Megido, R., Francis, F. (2025). Assessment of cabbage (*Brassica oleracea* Linnaeus) insect pests and management strategies in eastern Democratic Republic of Congo. *Agriculture* 15(21), 2203. https://doi.org/10.3390/agriculture15212203

Abstract

Cabbage (*Brassica oleracea* L.) is an important vegetable crop for food security and income generation for farmers in the Democratic Republic of Congo (DRC). However, production is severely undermined by a complex of insect pests. This study investigates farmers’ knowledge, perception, and pest management practices in key cabbage-growing areas surrounding Goma city in Eastern DRC. A total of 430 farmers were interviewed using a structured survey administered via the KoboToolbox platform. The diamondback moth (*Plutella xylostella* L., 1758) and the cabbage aphid (*Brevicoryne brassicae* L., 1758) were identified as the main pests, with peak incidences reported during the dry mid-season. Pest damages are most frequently observed at the post-transplanting and heading stages of cabbage. Although chemical control was the dominant strategy (69.4%), concerns arise due to the widespread use of moderately to highly hazardous insecticides, including pyrethroid, organophosphorus, and avermectin-based formulations. The insufficient use of personal protective equipment (PPE) and limited training on safe pesticide handling remain further challenges. While indigenous practices, such as crop rotation, handpicking of insects, and the use of botanical extracts, are employed to a lesser extent, awareness and implementation of biological control are almost nonexistent. The findings underscore the need to promote integrated pest management (IPM) approaches based on agroecological principles, including the safe use of (bio-) pesticides, training programs, and stakeholder engagement to enhance sustainable cabbage production.

Keywords: cabbage pests, IPM, indigenous practices, botanicals, pesticides, Democratic Republic of Congo

3.1. Introduction

Cabbage (*Brassica oleracea* L.) plays a vital role in household food security and income generation for smallholder farmers across sub-Saharan Africa. In the Democratic Republic of Congo (DRC), where agriculture is largely subsistence-based, cabbage plays a crucial role in improving nutrition and livelihoods (Ndjadi et al., 2020). It is rich in essential nutrients and bioactive compounds, making it vital for enhancing dietary quality and health outcomes in the region (Francisco et al., 2017).

The infestation of cabbage by insect pests is a major concern for smallholder farmers in the region. Phytophagous insects not only reduce crop yields but also affect the quality of the products, thereby threatening food security. Major pests affecting cabbage crops include aphids (A. K. Patel et al., 2024), moth caterpillars (Mpumi et al., 2020), and flea beetles (Y. Zhou et al., 2025), which cause significant damage at different growth stages of the crops. Aphids, including the cabbage aphid *Brevicoryne brassicae* (L., 1758) and the green peach aphid *Myzus persicae* (Sulzer, 1776), mainly feed on the undersides of cabbage leaves, extracting sap and nutrients, which can lead to stunted growth and reduced yield (Malik et al., 2025; A. K. Patel et al., 2024). The cabbage aphid can reduce growth by up to 35%, and infestations can result in 85% yield losses (Embaby and Lotfy, 2015). Also, several lepidopteran species pose significant threats (Badenes-Perez and Shelton, 2006), particularly in the larval stage, affecting cabbage crops globally and leading to substantial yield losses (Mpumi et al., 2020). For example, the larvae of the diamondback moth (DBM), *Plutella xylostella* (L., 1758), feed on cabbage leaves, leading to distinct diamond-shaped holes and reduced plant vigor (Paul et al., 2024). The estimated annual economic loss due to *P. xylostella* infestations on cabbage farmers globally is up to $US 4–5 billion, highlighting the significant threat this pest poses to global food security and agricultural productivity (Paul et al., 2024; Zalucki et al., 2012). Climate change is expected to alter the distribution and severity of pest infestations, potentially increasing the risk of pest outbreaks in the Great Lakes countries of Africa (Mouafo-Tchinda et al., 2025). Global warming can potentially create opportunities for pests to thrive in regions where they were less prevalent because of former unsuitable climatic conditions (IPPC, 2021). Infestation peaks during specific seasons are directly correlated with climatic factors, mainly temperature and humidity. Higher temperatures promote pest populations, particularly the flea beetle and DBM, which peak during warmer months (Lal et al., 2020).

To manage cabbage pests, Congolese farmers mainly resort to broad-spectrum insecticides. The main synthetic chemicals applied against a range of pests in DRC include organophosphates (e.g., profenofos, chlorpyrifos), pyrethroids (e.g., cypermethrin), and avermectin-based formulations (e.g., abamectin) (Labou et al., 2017; Rubabura et al., 2022; Balasha et al., 2023). The chemical control, while aimed at effectively managing pests, significantly contributes to biodiversity loss, environmental pollution, pest resistance, and higher production expenses for farmers (Matubi et al., 2020; Warra and Prasad, 2020). This reliance raises concerns about the health risks to consumers and farmers associated with pesticide misuse (Gilden et al., 2010; W. Zhou et al., 2025). Farmers who frequently and closely work with pesticides face an increased risk of exposure, by contact and inhalation (Kapeleka et al., 2019). Recent studies reveal an alarming situation in DRC, particularly as many farmers do not use personal protective equipment (PPE) and have not received specific training on the use of pesticides (Muliele et al., 2018; Ngakiama et al., 2019; Cokola et al., 2023b).

Additionally, smallholder farmers rely on traditional knowledge, although these methods are often insufficient alone. Farmers employ crop rotation, weeding, and handpicking to manage pest populations (Mangaza et al., 2021; Mpumi et al., 2020). Plant diversification through crop rotation, intercropping, and mixed cropping enhances biodiversity and reduces pest populations (Badenes-Pérez et al., 2017; Huss et al., 2022). An interesting variant of crop association for pest control is companion planting, where plants are grown next to the main cash crop to ensure ecosystem services such as pest regulation, by discouraging pest establishment or hosting natural enemies (Parker et al., 2013; Zuma et al., 2023). Another ancestral approach to control pests is the use of plant-based pesticides (Stevenson et al., 2017). Many recent studies in DRC have assessed pesticidal properties of several species, including *Azadirachta indica* (A.Juss.), *Ricinus communis* (L.) (Badinenganyi et al., 2023), *Nicotiana tabacum* (L.), *Allium cepa* (L.), *Piper nigrum* (L.) (Nsomue et al., 2020), *Tephrosia vogelii* (Hook.f.), *Tetradenia riparia* (Hochst.) Codd, *Tithonia diversifolia* (Hemsl.) A.Gray (Koleramungu et al., 2018), and *Capsicum frutescens* (L.) (Gakuru et al., 2019). Traditional methods, such as using ash, smoking, and storing crops in pods, are standard for managing storage pests, highlighting the importance of indigenous knowledge in reducing crop losses (Anjarwalla et al., 2020).

The transmission of indigenous knowledge is often community-based, with a risk of loss due to the increasing adoption of modern practices. Therefore, it is essential to understand farmers' knowledge and perceptions of cabbage pests and current management practices to develop a suitable approach to pest management. Such information is largely lacking for eastern DRC, particularly in Goma and surrounding areas, highlighting the need for locally grounded evidence.

This study aims to investigate insect pests affecting cabbage in eastern DRC and pest control strategies adopted by farmers. Specifically, we assess (i) smallholder farmers’ knowledge and perceptions of insect pests, (ii) the types of control methods used, and practices applied to manage pest damage. Understanding farmers’ perspectives and practices is critical for designing sustainable and context-specific IPM interventions.

3.2. Materials and methods

Study area

Surveys were conducted in the Eastern part of the DRC, focusing on vegetable-growing areas surrounding Goma (1°41′01″ S, 29°14′07″ E, 1502.09 m altitude), the capital of the North-Kivu province. Besides the peri-urban areas of Goma city, three administrative territories across two provinces were covered. In North-Kivu province, the Nyiragongo and Masisi territories were surveyed, while in South-Kivu province, the Kalehe territory was surveyed (Figure 6). In DRC, a “territory” is a second-level administrative division under a province. The selected areas are the leading suppliers of market garden produce, including *Brassica* crops, to Goma. A tropical Afro-mountain climate dominates this high-altitude region. It is characterized by mild temperate conditions with a correlation between temperature and elevation, and the area is endowed with rich volcanic soil (Baributsa et al., 2021), making it ideal for growing cabbage. Most of the population in the regions surveyed relies on small-scale agriculture and livestock farming, fishing, and informal trade. The main grown cruciferous vegetables are cabbage and cauliflower.

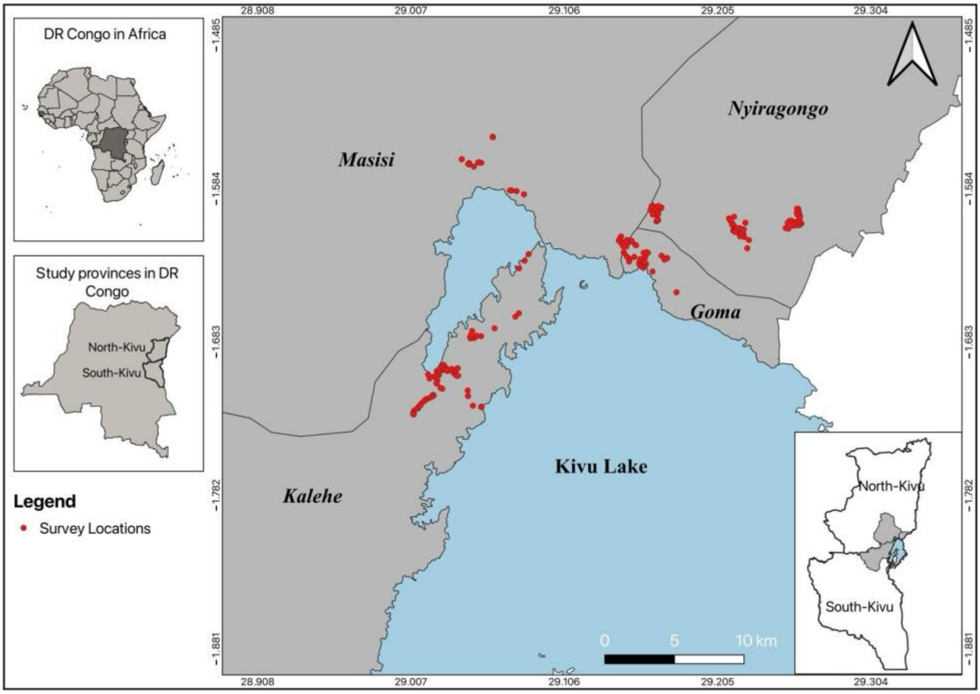

Figure 6. Map showing the survey locations (red dots) in the North-Kivu and South-Kivu provinces, Democratic Republic of Congo. *Generated in QGIS 3.34 based on GPS coordinates collected with KoboCollet Application.*

Data collection

The survey was conducted using a structured questionnaire. Before its deployment, the questionnaire underwent a pre-validation process involving a pilot test with a sample of farming households from the target survey areas. This pre-test served to assess the clarity, relevance, and consistency of the instrument, allowing for modifications to enhance its reliability and validity (Halilou et al., 2022). After revision, the final version of the questionnaire was administered to a total of 430 farmers. The number of respondents per territory was determined based on estimates of cabbage producers and accessibility to the area. Participants were randomly selected from distinct villages within each study territory. Only farmers who had cabbage and/or another cruciferous cultivar among their cultivated crops were selected.

To facilitate fieldwork with producers, the questionnaire was encoded on the KoboToolbox platform “https://www.kobotoolbox.org/ (accessed on 26 April 2023)”. The survey was conducted using smartphones programmed with the KoboCollect application, which allowed for the import of the questionnaire form. This setup enables rapid data collection, automatic recording of GPS coordinates, online data storage and transmission, and real-time monitoring of the survey progress (Gangopadhyay et al., 2024). The collected GPS coordinates were used to generate Figure 6 in QGIS 3.34. Investigations were carried out in 2023 and 2024 during growing seasons (short rainy season and long rainy season, respectively). The survey consisted of open- and closed-ended questions, administered face-to-face in French and Swahili to ensure the farmers' understanding. The survey covered the socioeconomic profiles of farmers (e.g., gender, education, and family size), farm characteristics (e.g., farm size, cabbage cropping systems), knowledge and perceptions of the main pests affecting cabbage, including insect identification, seasonal pest incidence, information and training about insect pests received from public services and non-governmental organizations (NGOs). The survey also focused on the management strategies undertaken by smallholder farmers (e.g., control methods, cultural practices, chemical pesticides used, and the safety precautions during application). Most farmers were interviewed on their fields to gather more information on key elements, including pests and pesticides used. In addition, clear printed pictures of the cabbage pests and their damage were provided to ensure their recognition by farmers. Empty bottles of chemical pesticides were checked to confirm the active ingredients and their concentration.

Data analysis

Data processing and statistical analyses were conducted using RStudio 4.4.1. Chi-square tests of independence were used to assess relationships between categorical variables, including socioeconomic characteristics, farmers' perceptions of cabbage pests, and pest control strategies, across different regions. The same analytical approach was consistently applied across all relevant qualitative variables. Given the non-normal distribution and heterogeneity of variances among groups, a non-parametric approach was used to assess differences across territories for the quantitative variables, including the household size, household active members, and the insecticide use patterns. A Kruskal–Wallis test assessed overall differences among territories for each response variable. When significant, Dunn's post hoc test was used for pairwise comparisons with Bonferroni correction applied to adjust *p*-values. Analyses were conducted using "dunn.test" package (Dinno, 2024) and the "multcompView" package (Graves et al., 2024). All tests were set at the significance level of 5%.

3.3. Results

Socioeconomic and farm characteristics

Significant socioeconomic variations were observed across geographical regions. The gender distribution reveals that males were predominant (76.3%) in all survey areas; however, a significant gender disparity was found between regions ($\chi^2 = 8.89$, df = 3, $p = 0.028$) (Table 4). Educational levels also differed, with 34.0% and 32.6% of farmers on average who have completed secondary school and primary school, respectively. There is significant regional variation in educational levels ($\chi^2 = 39.87$, df = 9, $p < 0.001$). The average household size in the sample was 7 ± 2.5 members with significant regional differences ($H = 52.21$, df = 3, $p < 0.001$). The average number of active household members was 5 ± 2.3, with significant differences across territories ($H = 67.31$, df = 3, $p < 0.001$) (Table 4).

The distribution of farm sizes indicated that Goma city had the most significant number of small farms (<0.5 ha), whereas Kalehe had more farmers with larger farms (1–2 ha and >2 ha). The overall trend indicated significant differences in farm size distribution across urban and rural regions ($\chi^2 = 136.16$, df = 9, $p < 0.001$). Regarding cabbage cultivation, Goma had the highest proportion of farmers dedicating over 50% of their land to cabbage, whereas Masisi had the most farmers using less than 25% for cabbage. The cropping systems across the surveyed regions showed significant variations (Table 4). Monoculture was the dominant cropping system, particularly in Nyiragongo (61.1%). Intercropping ranked second, while a small portion of farmers used companion plants in all regions. Agroforestry was relatively rare, with the highest usage in Kalehe (7.9%).

Farmers' knowledge and perception of cabbage pests

Seven pests were highlighted by farmers as the most dominant, namely: *Plutella xylostella*, *Brevicoryne brassicae*, *Trichoplusia ni*, *Hellula undalis*, *Pieris brassicae*, *Myzus persicae*, and *Phyllotreta* spp. (Figure 7). The DBM (*P. xylostella*) was the most frequently cited pest overall (28.9%), with high proportions in Masisi (35.4%), Kalehe (34.9%), and Goma (27.1%). The cabbage aphid (*B. brassicae*) was also highly prevalent in Kalehe (35.7%) and Goma (23.6%), resulting in the second-highest average perception rate across all territories (27.0%). The cabbage looper (*T. ni*), the cabbage webworm (*H. undalis*), and the white butterfly (*P. brassicae*) were moderately cited, with ± average values of 12.8%, 10.4% and 9.3%, respectively. *T. ni* had a high perception rate in Goma (17.2%) and Nyiragongo (12.9%). *H. undalis* was notably mentioned in Masisi (22.9%), while *P. brassicae* had moderate perception across all zones, ranging from 6.4% in Kalehe to 12.5% in Masisi. *M.*

persicae and *Phyllotreta* spp. had lower overall frequencies (6.5 and 5.2%, respectively), though the green aphid was more prevalent in Nyiragongo (12.3%). Other cabbage pests occasionally reported include cutworms (*Agrotis* spp.) and millipedes.

Table 4. Socioeconomic profiles and farm characteristics across the four surveyed areas

		Survey Areas						
Variables	**Categories**	**Goma n = 71**	**Nyiragongo n = 145**	**Masisi n = 30**	**Kalehe n = 184**	**Overall n = 430**	**χ^2 Test**	**Kruskal–Wallis**
Gender (%)	Female	33.8	22.8	6.7	23.4	23.7	8.89 *	
	Male	66.2	77.2	93.3	76.6	76.3		
Level of studies (%)	Uneducated	8.5	19.3	26.7	34.8	24.7	39.87 ***	
	Primary school	23.9	36.6	33.3	32.6	32.6		
	Secondary school	47.9	34.5	30.0	28.8	34.0		
	University level	19.7	9.7	10.0	3.8	8.8		
Household size		5.4 ± 2.3a	6.5 ± 2.3c	8.3 ± 3.1b	7.6 ± 2.2b	6.9 ± 2.5		52.21 ***
Household active members		3.8 ± 2.1a	5.4 ± 2.3c	6.5 ± 2.3b	6.4 ± 1.9b	5.6 ± 2.3		67.31 ***
Farm size (%)	<0.5 Ha	77.5	29.7	23.3	10.3	28.8	136.16 ***	
	0.5–1 Ha	21.1	46.9	56.7	40.2	40.5		
	1–2 Ha	1.4	21.4	16.7	41.3	26.3		
	>2 Ha	0.0	2.1	3.3	8.2	4.4		
Proportion of land used for cabbage (%)	<25%	23.9	30.3	46.7	32.1	30.7	14.55 *	
	25–50%	45.1	45.5	43.3	53.3	49.1		
	>50%	31.0	24.1	10.0	14.7	20.2		
Cropping systems (%)	Monoculture	47.1	61.1	42.9	46.1	51.5	57.49 ***	
	Intercropping	37.7	15.8	40.7	40.7	31.3		
	Companion planting	14.5	15.4	5.7	5.4	11.0		
	Agroforestry	0.7	7.7	5.7	7.9	6.2		

Note: * and *** correspond to statistical significance at $p < 0.05$ and $p < 0.001$, respectively; different letters in a row indicate statistically different groups determined by a post hoc Dunn's test.

Pest incidence varied by season, with the dry mid-season reporting the highest pest pressure across all regions (59.1%). The long rainy season showed lower pest incidence, particularly in Kalehe (7.6%). These differences highlighted varying pest dynamics across the different seasons and regions ($\chi^2 = 27.52$, df = 9, $p < 0.001$). Across all sites, the post-transplanting stage was most frequently identified as the stage when pests cause damage, with 38.9% of farmers indicating this stage. This was followed by the cabbage heading stage (30.2%) and the vegetative growth stage (22.0%). The observed variation was statistically significant ($\chi^2 = 52.58$, df = 3, $p < 0.001$). Information and training sources of pest management varied significantly among territories ($\chi^2 = 83.18$, df = 12, $p < 0.001$). Non-governmental organizations (NGOs) and radio/TV were the primary sources of pest management information, with 24.0% and 23.9% average, respectively (Table 5). Other farmers rely on agricultural public services and related projects for 18.3%, and on the Food and Agriculture Organization (FAO) and its partners for 17.8%. Other sources, including peer farmers and local initiatives, also contribute to agricultural knowledge dissemination.

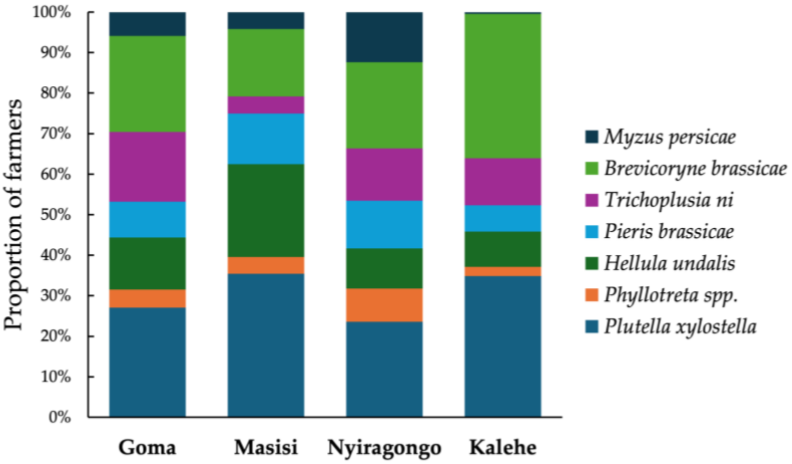

Figure 7. Farmers' perception of the main cabbage pests in the survey areas

Table 5. Farmer's perception of cabbage pest incidence and sources of information

		Survey Areas					
Variables	**Categories**	**Goma n = 71**	**Nyiragongo n = 145**	**Masisi n = 30**	**Kalehe n = 184**	**Overall n = 430**	**χ^2 Test**
Seasonal pest incidence (%)	Long rainy season	21.1	23.4	20.0	7.6	16.0	27.52 ***
	Short rainy season	33.8	26.2	23.3	20.7	24.9	
	Dry mid-season	45.1	50.3	56.7	71.7	59.1	
Crop damage stage (%)	Nursery stage	5.8	10.8	7.7	3.6	6.9	52.58 ***
	Vegetative growth stage	21.2	21.6	36.8	17.2	22.0	
	Post-transplanting stage	46.0	40.6	33.3	36.6	38.9	
	Cabbage heading	24.8	25.9	18.8	41.3	30.2	
	Post-heading stage	2.2	1.1	3.4	1.3	1.7	
Information and/or training on pest management (%)	Agriculture public services	16.8	22.6	14.3	16.7	18.3	83.18 ***
	FAO	8.8	25.9	4.8	17.7	17.8	
	NGOs	32.8	33.2	19.0	15.1	24.0	
	Radio/TV	26.4	11.5	26.2	31.4	23.9	
	Other sources	15.2	6.2	20.0	19.1	15.9	

Note: *** corresponds to statistical significance at $p < 0.001$.

Farmers' management strategies of cabbage pests

Regarding endogenous practices (Table 6), the use of available resistant varieties was relatively consistent across regions, with the highest adoption in Goma (47.9%). The differences were not statistically significant, suggesting that resistant varieties were used similarly across the regions as a potential method of pest control. Early planting was significantly more practiced in Nyiragongo (53.8%), compared to other regions, particularly Kalehe (21.7%) and Goma (21.1%). A statistically significant difference was observed, indicating that early planting was a widely adopted strategy in Nyiragongo. Crop rotation was a widely used strategy in controlling pests in all areas, but it was more frequent in Masisi (46.7%) and Goma (45.1%), compared to Nyiragongo (30.3%). Intercropping with non-Brassica crops was more practiced in Goma (22.5%), but less common in Kalehe (9.8%). Differences were statistically significant, indicating that farmers in Goma may rely more on diversifying their crops. Although intercropping with repellent or trap plants may be practiced in some regions, with the highest adoption in Nyiragongo (12.4%), this strategy was adopted by a small percentage of farmers across regions (Table 6). Regular mechanical weeding was widely practiced across all regions, with the highest adoption in Goma (62.0%) and the lowest in Kalehe (47.8%), reflecting its high adoption by farmers. Fertilization, including the use of organic and chemical fertilizers, was more widespread in certain regions, notably in Goma (26.8%). Ash or sawdust application was used similarly across most regions, with the highest usage in Nyiragongo (22.1%), suggesting that this practice was not a significant method of pest control in most regions. Pest hand picking was more frequently practiced in Goma (31.0%), while it was less common in Kalehe (16.3%) and Masisi (16.7%) (Table 6). This suggests that farmers with small-sized farms are more inclined to remove pests manually. Uprooting and burning infected plants and destroying crop residues were considered in 31.2% and 25.6% of cases, respectively, when severe plant attacks occur. The practice of replanting affected areas, which can be a labor-intensive practice, was relatively low across all regions, with the highest adoption rate in Nyiragongo (19.3%). The use of locally prepared plant extracts was a prevalent practice across all territories, though not extensively adopted (14.4% overall). The difference was not statistically significant, indicating that while some farmers rely on traditional plant-based methods, these are not the dominant strategy (Table 6).

To control pests, some farmers prepare insecticide mixtures using available pesticidal plants. The listed plants span 10 botanical families, indicating a wide range of local resources available for pest management (Table 7). The most used plant parts are leaves, followed by seeds, bulbs, and fruits. Leaf extracts are used in most cases (e.g., *Cannabis sativa* (L.), *Eucalyptus* spp., *Lantana camara* (L.), *N. tabacum*, *T.*

riparia, *T. diversifolia*). Some plants, such as *A. indica*, *Carica papaya* (L.), and *R. communis*, were reported to have multiple parts used, primarily leaves and seeds. The families Amaryllidaceae and Solanaceae were each represented by two species known for their insecticidal effects. For example, *A. cepa* and *Allium sativum* (L.), both of the family Amaryllidaceae, were used for their bulbs.

Table 6. Indigenous and agroecological approaches implemented for managing cabbage pests

	Survey Areas					
Management Practices (%)	**Goma n = 71**	**Nyiragongo n = 145**	**Masisi n = 30**	**Kalehe n = 184**	**Overall n = 430**	**χ^2 Test**
Use of resistant varieties	47.9	33.1	36.7	43.5	40.2	5.76 ns
Early planting	21.1	53.8	43.3	21.7	34.0	44.08 ***
Practice crop rotation	45.1	30.3	46.7	40.8	38.4	6.61 ns
Intercropping with non-Brassica crops	22.5	15.9	3.3	9.8	13.5	10.50 *
Intercropping with repellent/trap plants	4.2	12.4	3.3	7.1	8.1	6.21 *
Practice regular weeding	62.0	60.7	43.3	47.8	54.2	8.63 *
Practice fertilization	26.8	26.2	16.7	8.2	17.9	22.52 ***
Application of ash/sawdust	19.7	22.1	13.3	12.5	17.0	5.94 ns
Hand picking of larvae/egg clusters	31.0	32.4	16.7	16.3	24.2	14.30 **
Uproot and burn infected plants	39.4	35.2	20.0	26.6	31.2	6.86 ns
Destruct crop residues	23.9	36.6	30.0	16.8	25.6	16.95 ***
Replanting attacked areas	12.7	19.3	16.7	17.4	17.2	1.48 ns
Use self-prepared local plant extracts	18.3	16.6	13.3	11.4	14.4	2.78 ns

Note: ns corresponds to not significant; *, **, and *** correspond to statistical significance at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

Table 7. Pesticidal plants used by smallholder farmers for managing cabbage pests

Scientific name	**Common name**	**Family**	**Plant parts used**
Allium cepa (L.)	Onion	Amaryllidaceae	Bulbs
Allium sativum (L.)	Garlic	Amaryllidaceae	Bulbs
Azadirachta indica (A.Juss.)	Neem	Meliaceae	Leaves, seeds
Cannabis sativa (L.)	Hemp	Cannabaceae	Leaves
Capsicum frutescens (L.)	Pepper	Solanaceae	Fruits
Carica papaya (L.)	Papaya	Caricaceae	Leaves, seeds
Eucalyptus spp.	Eucalyptus	Myrtaceae	Leaves
Lantana camara (L.)	Wild sage	Verbenaceae	Leaves
Nicotiana tabacum (L.)	Tobacco	Solanaceae	Leaves
Ricinus communis (L.)	Castor	Euphorbiaceae	Leaves, seeds
Tetradenia riparia (Hochst.) Codd	Ginger bush	Lamiaceae	Leaves
Tithonia diversifolia (Hemsl.) A.Gray	Mexican sunflower	Asteraceae	Leaves, seeds

Chemical control was the most widely used pest management practice across all regions (69.4%), with the highest usage in Masisi (78.1%) and Kalehe (76.2%). Biological control, especially the use of plant extracts, was adopted by 14.3% of farmers, while physical control techniques (such as manual removal of insect pests) were used by 16.3% of farmers. The differences in control practices were statistically significant ($\chi^2 = 21.44$, df = 6, $p = 0.002$) (Table 8). Regarding the timing of chemical treatment, most farmers (73.3%) applied chemicals in the morning, with the highest proportions in Goma (84.5%) and Nyiragongo (82.1%). Use of chemicals in the midday and evening was less common. These differences in timing of chemical application were statistically significant ($\chi^2 = 43.50$, df = 6, $p < 0.001$) (Table 8). The time of intervention after detecting a pest infestation was at least 2 days, while the average delay between two successive chemical treatments was 12.2 ± 3.0 days. The average number of chemical applications was 3 (Table 8). In addition, some farmers reported using preventive treatments on seedlings. Significant regional differences were observed between farmers in terms of intervention time ($H = 45.97$, df = 3, $p < 0.001$), delay between two treatments ($H = 44.09$, df = 3, $p < 0.001$), and number of chemical applications ($H = 60.01$, df = 3, $p < 0.001$).

Table 8. Management methods and insecticide use patterns against cabbage pests

		Survey Areas						
Variables	**Categories**	**Goma n = 71**	**Nyiragongo n = 145**	**Masisi n = 30**	**Kalehe n = 184**	**Overall n = 430**	**χ^2 Test**	**Kruskal –Wallis**
Control methods used by farmers (%)	Physical control	23.7	19.1	15.6	10.4	16.3	21.44 **	
	Biological control	21.2	12.7	6.2	13.3	14.3		
	Chemical control	55.1	68.1	78.1	76.2	69.4		
Time of chemical treatment (%)	Morning	84.5	82.1	70.0	62.5	73.3	43.50 ***	
	Midday	5.6	10.3	10.3	32.1	18.8		
	Evening	9.9	7.6	20.0	5.4	7.9		
Intervention time after infestation detection (days)		3.8 ± 2.6a	2.4 ± 1.7b	4.3 ± 1.9a	2.0 ± 1.3b	2.6 ± 1.9		45.97 ***
Delay between two successive chemical treatments (days)		10.6 ± 3.4a	12.2 ± 3.4c	12.0 ± 2.4bc	13.0 ± 2.3b	12.2 ± 3.0		44.09 ***
Number of chemical applications		3.3 ± 1.5a	4.4 ± 2.8c	3.8 ± 2.0ac	2.8 ± 2.2b	3.5 ± 2.4		60.01 ***

Note: ** and *** correspond to statistical significance at $p < 0.01$ and $p < 0.001$, respectively; different letters in a row indicate statistically different groups determined by a post hoc Dunn's test.

The use of PPE when applying insecticides did not differ across territories, with an overall proportion of farmers using PPE of 41.9%. The type of PPE used did not vary significantly (χ^2 = 19.38, df = 12, p = 0.079). Rubber boots were the most used PPE across all regions (46.1%), with the highest usage in Masisi (51.1%) and Nyiragongo (44.6%). Secondly, long clothes were also used by a proportion of farmers (22.3%). The usage of gloves (11.3%) and glasses (0.8%) remained limited in all surveyed areas (Table 9). In terms of information sources, advice from other farmers was found to be the most common way of guidance for pesticide use (45.2%), with Kalehe (48.5%) and Masisi (46.9%) reporting the highest reliance on peer advice. Even without formal training, agrochemical retailers provided 27.5% of the information. Thirdly, farmers relied on their experience or, for those who are educated, on reading instructions (18.4%). Extension services provided information to fewer farmers, with an overall usage of 9.0%. Significant differences in the sources of pesticide information were finally determined (χ^2 = 18.17, df = 9, p = 0.033), indicating a varying reliance on formal and informal channels across areas (Table 9). Farmers in all territories reported experiencing the same health issues related to pesticide use (χ^2 = 13.64, df = 12, p = 0.322). Headache and skin irritation were the most common symptoms, affecting more than 25% of farmers overall. Eye irritation and breathing problems were less frequently reported, but still present, with eye irritation at 21.6% and breathing problems at 17.3%. Less regularly, stomach problems were reported by 9.0% of farmers (Table 9).

Table 9. Farmers' safety and attitudes when using insecticides

		Survey Areas					
Variables	**Categories**	**Goma n = 71**	**Nyiragongo n = 145**	**Masisi n = 30**	**Kalehe n = 184**	**Overall n = 430**	**χ^2 Test**
Wear PPE (%)	Yes	39.4	35.2	36.7	45.1	41.9	3.54 ns
	No	60.6	64.8	63.3	54.9	58.1	
Type of PPE used (%)	Face mask	19.1	24.7	13.3	16.2	19.5	19.38 ns
	Long clothes	25.7	17.2	24.4	24.8	22.3	
	Rubber boots	40.4	44.6	51.1	49.2	46.1	
	Gloves	12.5	12.7	11.1	9.5	11.3	
	Glasses	2.2	0.7	0.0	0.3	0.8	
Information on pesticide use (%)	Previous experience/Reading instructions	22.7	24.8	15.6	11.9	18.4	18.17 *
	Agriculture extension services	10.9	8.8	6.2	8.5	9.0	
	Other farmers	42.0	42.5	46.9	48.5	45.2	
	Agrochemical retailers	24.4	23.9	31.2	31.1	27.5	
Health problems related to pesticide use (%)	Headache/Coughing	28.0	26.6	28.0	23.5	25.5	13.64 ns
	Stomachache	13.3	6.5	6.5	9.2	9.0	
	Skin irritation	24.0	27.9	28.0	26.8	26.7	
	Eye irritation	20.4	21.1	23.7	21.9	21.6	
	Breathing problems	14.2	17.9	14.0	18.6	17.3	

Note: Ns and * correspond to not significant and statistically significant at $p < 0.05$, respectively.

A total of eleven insecticide formulations were identified, spanning several chemical families including pyrethroids, organophosphates, avermectins, neonicotinoids, and organochlorines (Table 10). Thiodan, an organochlorine insecticide classified as moderately hazardous (WHO class II), had the highest overall adoption rate (23.6%). Pyrethroid-based formulations accounted for many of the adopted products, either as single active ingredients or in combination with other insecticide classes. Roket+ (20.1%) and Dudu Alpha 3EC (17.1%) were the most frequently applied pyrethroids, indicating a farmer preference for cypermethrin-based formulations. Dudu Acelamectin 5%EC, a mixture of abamectin and acetamiprid, was adopted by 13.0%. Another cypermethrin-based insecticide, Cyperscope 5EC, recorded an adoption rate of 6.8%. Other insecticides, including Tafgor 40EC at 6.1% and Dudu Aba+ at 5.8%, showed moderate adoption levels. The use of organophosphorus compounds like dichlorvos (Lava; 2.5%) and chlorpyrifos (Kuu-Kill; 0.5%) was less common among farmers. Dudu Acelamectin 5% EC and Dudu Aba+ contain abamectin (Avermectin), a toxic substance classified as a highly hazardous compound (WHO Class Ib). Lava remains a highly hazardous compound, which raises safety concerns. The data indicate a heavy reliance on moderately and highly hazardous insecticides, with widespread adoption of restricted or risky compounds among farmers.

Table 10. List of commercial insecticides and corresponding active ingredients used in the study areas

Commercial Name	**Active Ingredient**	**Family**	**WHO Class**	**Adoption Rate (%)**
Cyperscope 5EC	Cypermethrin 5%	Pyrethroid	II	6.8
Dudu Aba+	Abamectin 2%	Avermectin	Ib	5.8
Dudu acelamectin 5%EC	Abamectin 2% + acetamiprid 3%	Avermectin + Neonicotinoid	Ib + II	13.0
Dudu alpha 3EC	Alpha-cypermethrin 3%	Pyrethroid	II	17.1
Dudu cyper 5% EC	Cypermethrin 5%	Pyrethroid	II	2.1
Lava 100% EC	Dichlorvos 100%	Organophosphorus	Ib	2.5
Kuu-Kill	Chlorpyrifos 48%	Organophosphorus	II	0.5
Roket +	Profenofos 40% + cypermethrin 4%	Organophosphorus + Pyrethroid	II + II	20.1
Simba +	Cypermethrin 5%	Pyrethroid	II	2.2
Tafgor 40EC	Dimethoate 40%	Organophosphorus	II	6.1
Thiodan	Endosulfan 50%	Organochlorine	II	23.6

Note: Based on the WHO (World Health Organization) Classification, Ib = highly hazardous, II = moderately hazardous (WHO, 2020).

3.4. Discussion

The predominance of male respondents across all study areas aligns with observations in other sub-Saharan African countries, where men often dominate commercial vegetable production. Women participate extensively in agricultural work, but men remain the decision-makers and farm managers (Doss et al., 2018; FAO, 2011). While most farmers had completed secondary school, about a quarter are reported to have no formal education. In contrast, education is a key factor influencing the adoption of improved farming techniques and sustainable pest management practices (Kassie et al., 2013). Farm size data highlight the dominance of small-scale holdings (<0.5 ha) in urban centers like Goma. In contrast, rural territories like Kalehe show higher proportions of farmers managing larger plots (1–2 ha and >2 ha). These differences likely reflect urban land scarcity and higher land competition in cities, consistent with recent urbanization trends in DRC (Wagemakers and Diki, 2011). The dominance of monocropping cabbage farms, especially in Nyiragongo, raises concerns regarding ecological sustainability. Monoculture often fosters pest and disease buildup and depletes soil nutrients (Letourneau et al., 2011). Alternative systems such as intercropping and companion planting remain underutilized, while agroforestry in cabbage production is rare, despite their potential to enhance agroecosystem resilience. These diversified systems are known to support pest regulation, biodiversity conservation, and soil fertility (Huss et al., 2022).

Results highlight two dominant pests: *P. xylostella* and *B. brassicae*, which farmers perceive as major threats. Our earlier findings (Gakuru et al., 2024), based on standard trapping methods, also highlighted the presence of these cabbage pests in eastern DRC. Our findings are in accordance with Asante et al. (2025) and Balasha and Nsele (2019), who reported that farmers are aware of these key cabbage pests. The widespread recognition of *P. xylostella* among farmers is not surprising given its status as one of the most destructive pests of cruciferous crops worldwide (Badenes-Perez and Shelton, 2006). The DBM has been associated with substantial yield losses and is notorious for its rapid development of resistance to multiple classes of insecticides, including pyrethroids (Furlong et al., 2012). Also, aphids are known for their ability to reproduce rapidly and as major sap-sucking pests that transmit plant viruses, posing serious threats to crop productivity and marketability (Iftikhar et al., 2022). The cabbage looper (*T. ni)*, cabbage webworm (*H. undalis*), and white butterfly (*P. brassicae*) were also reported to affect cabbage production. Previous studies linked the prevalence of *T. ni* to high temperatures and dense foliage, which favor larval development (Labou et al., 2017). Although *T. ni* feeds on several species, female moths prefer to lay eggs on cabbage due to its suitability for larval development, which is critical for the pest's lifecycle (Coapio et al., 2018; Li and Liu, 2015). The presence of *H. undalis* and *P. brassicae* confirmed previous studies, which showed their preference for Brassicaceae crops (Mpumi et al., 2020; Okamura et al., 2019). As with other cabbage-specialist pests like *P. xylostella*, glucosinolates, which are secondary plant compounds characteristic of the cruciferous plants, play an important role in cabbage plant selection. For example, *H. undalis* showed a preference for plants with higher concentrations of glucosinolates, enhancing attraction and egg-laying behavior (Mewis et al., 2002). The distribution of different lepidopteran pests across survey areas may indicate wide host availability, as well as farmers' familiarity with their characteristic feeding damage and presence of larvae. The green peach aphid and flea beetles were less frequently mentioned in survey areas. While both pests are known to impact crucifers, their lower perception could be due to their less conspicuous feeding symptoms when there are few, compared to leaf-eating caterpillars. Furthermore, *M. persicae* is a generalist pest that can feed on a range of host plants grown in the survey areas, including bean, cauliflower, tomato, spinach, and pepper (Ali et al., 2023; Wamonje et al., 2020).

Pest incidence was reported to be highest during the dry mid-season, compared to the short rainy season and the long rainy season, indicating that seasonal climatic conditions strongly influence pest pressure. This finding aligns with broader evidence that dry seasons typically favor the proliferation of many lepidopteran and aphid pests, especially *P. xylostella* and *B. brassicae*. In tropical regions, dry and hot microenvironments during dry seasons accelerate pest development cycles, thereby increasing infestation intensity (Furlong et al., 2012). In contrast, rainfall can act as a natural suppressant of pest populations by dislodging eggs and larvae and enhancing fungal pathogen activity (Chen et al., 2019; Embaby and Lotfy, 2015). Across all sites, farmers most frequently reported the post-transplanting stage and the heading stage as the most susceptible to pest damage. This suggests that pest infestations often begin during early foliar development, when plants are less structurally defended and many cabbage pests prefer young, tender tissues, leading to high vulnerability in early field stages (Embaby and Lotfy, 2015). The cabbage heading stage is also sensitive, as leaf mining or aphid feeding at this time can severely inhibit growth, leading to poor head formation and rendering heads unfit for marketing and consumption (Moorthy et al., 2022).

Chemical control remains the most widespread pest management practice across all areas. This finding is in line with previous studies (Andersson and Isgren, 2021; Mengistie et al., 2017), where smallholder farmers consider pesticide use to be more effective in reducing pest populations. Chemicals are considered economically viable to maintain high yields and to prevent financial losses, particularly in large-scale farms (W. Zhou et al., 2025). However, overreliance on insecticides has led to pesticide resistance, secondary pest outbreaks, and ecological disruption, particularly against cabbage pests like *P. xylostella* (Furlong et al., 2012) and *B. brassicae* (Hamedani Radja et al., 2020). Recurrent use of broad-spectrum insecticides may also impact pollinators and natural enemies (Desneux et al., 2007), undermining biological regulation within the cropping system. Most farmers apply insecticides in the morning rather than in midday and evening. This pattern is highly recommended, as morning applications avoid high solar radiation that can degrade insecticides and reduce efficacy. However, afternoon applications may coincide with peak pollinator activity and increase non-target effects, especially in open-field systems (McClure et al., 2023). Regarding the use of insecticides, the lack of training, failure to read instructions, and low rate of PPE use are quite concerning, given the risks associated with pesticide exposure. Our findings match those of Madaki et al. (2024) and Mergia et al. (2021) regarding the improper pesticide handling by farmers in the global South. Farmers across all surveyed regions reported experiencing health issues related to pesticide exposure. The reported symptoms are consistent with acute pesticide poisoning syndromes (W. Zhou et al., 2025). Indeed, all chemical insecticides used in survey areas are classified as moderately and highly hazardous pesticides with significant concerns regarding human safety, resistance development, and environmental impact (WHO, 2020). For example, the organochlorine endosulfan is widely used by farmers; however, it has been banned globally under the Stockholm Convention as a persistent organic pollutant and by many countries due to its high toxicity to humans, persistence in the environment, and bioaccumulation in food chains (Becker et al., 2011; W. Zhou et al., 2025). Organophosphates like dichlorvos and chlorpyrifos are still used unsafely, despite their known acute toxicity and neurological effects, including the inhibition of cholinesterase. This enzyme is important for healthy nerve function and remains a target for neurotoxins (A. Patel et al., 2024). In Tanzania, a survey of farmers using organophosphorus pesticides looked at acetylcholinesterase. The enzyme was found to be at significantly lower levels in exposed farmers compared to unexposed (Kapeleka et al., 2019). The use of neonicotinoids, such as acetamiprid found in Dudu acelamectin 5%EC, is also concerning given their documented adverse impact on human health and non-target organisms, particularly bees (Manzer et al., 2024; Oladosu and Flaws, 2025).

The use of botanical extracts is limited, even though plant-based pest control offers affordable and eco-friendly alternatives, particularly for resource-limited farmers (Souto et al., 2021). Botanicals from *A. indica*, *A. sativum*, and *R. communis* have demonstrated effectiveness against common Brassica pests and are compatible with ecological farming systems (Amoabeng et al., 2014; Divekar et al., 2024). Detailed lists of plants to be used against cabbage pests in DRC conditions have been provided by Mayanglambam et al. (2021) and Anjarwalla et al. (2020). Uptake remains low in survey areas, likely due to limited awareness, the lack of formulations, or the absence of processing tools. Also, smallholder cabbage farmers use a combination of indigenous knowledge, ecologically based practices, and low-input techniques to manage pest pressures. Early planting is adopted to avoid peak pest emergence windows during the early growth stages of cabbage (Mayanglambam et al., 2021). Farmers rely on available resistant varieties to prevent pest infestations, despite access difficulties. Recent breeding efforts have produced cabbage varieties resistant to a wide range of pests. However, adoption in sub-Saharan Africa is hindered by poor seed system infrastructure and limited extension services (Daniel et al., 2023). Handpicking of insects, specifically caterpillars and regular manual weeding, which are typical physical control practices in small-scale systems, are highly practiced by farmers in eastern DRC, as reported by Cokola et al. (2023b). Weed management reduces alternative pest habitats and promotes airflow, helping to suppress pest buildup (Mpumi et al., 2020). Other farmers apply crop rotation and intercropping for their benefit against insect pests. Rotating cabbage with nonhost crops contributes to disrupting pest life cycles and reducing their harmful effects (Juventia et al., 2021). While intercropping with non-Brassica species remains limited in some areas, companion planting (e.g., repellent or trap plant) is marginally adopted. However, studies have shown that several plants, including garlic (Yang et al., 2025), onion (Baidoo et al., 2012), and basil (Yarou et al., 2017a), act as repellents against cabbage pests. Others, including flowering plants, enhance the colonization of natural enemies (Hithesh et al., 2024). For example, Badenes-Pérez et al. (2017) tested the attractiveness of flowering *Barbarea vulgaris* (R. Br.) to *P. xylostella* and to its parasitoids, *Diadegma insulare* (Cresson, 1865) and *Diadromus collaris* (Gravenhorst, 1829). They found that *P. xylostella* suffered 1.7 and 4.0 times more parasitism by *D. insulare* and *D. collaris*, respectively. Such prophylactic and cultivation measures are practicable in DRC, considering that many potential companion plant species are already grown in the region for other uses. Other approaches, such as push-pull, should be considered in the IPM of cabbage, as shown by Mayanglambam et al. (2021) and da Silva et al. (2022). None of the respondents reported using natural enemies (e.g., parasitoids, predators), nor did they have access to biological control inputs (e.g., microbial biopesticides) or training. This aligns with broader regional patterns where biocontrol remains underdeveloped due to a lack of capacity, limited commercial formulations, and insufficient policy support (Grzywacz et al., 2009; Srinivasan et al., 2022).

To achieve sustainable pest management in the region, it is essential to combine the use of available resistant cultivars, local biological pesticides, and good agricultural practices, such as regulating the planting period, real-time pest monitoring, crop rotation, and habitat management (Mayanglambam et al., 2021; Srinivasan et al., 2022). Chemical pesticides should only be considered as a last resort and with great caution. In this case, it will be necessary to adopt selective pesticides and rotate the insecticide mode of action to reduce the development of resistance in target insects (Barbosa et al., 2020). Strategies for the efficient use of synthetic pesticides can include seed treatment or localized application to reduce the spread and direct contact of toxic molecules with beneficial insects (Desneux et al., 2007). New sustainable prospects would include the use of pheromone-based products and microbial agents (e.g., entomopathogenic fungi-based formulations) to control cabbage pests (Mayanglambam et al., 2021).

3.5. Conclusion

Cabbage production in Eastern DRC faces significant challenges from key insect pests, especially *P. xylostella*, *B. brassicae*, *T. ni,* and *H. undalis*, which lead to yield losses. Farmers mostly rely on chemical insecticides, often with hazardous profiles and inadequate protective measures, raising significant concerns for both human and environmental health. Although some indigenous practices, such as crop rotation and botanical extracts, are used by farmers, they require adequate design and broader adoption to be effective. The virtual absence of biological control and biopesticide use underscores critical gaps in knowledge transfer and policy support. These findings highlight the need to shift towards more sustainable and context-appropriate pest management strategies. This could involve promoting agroecological pest management strategies adapted to smallholder contexts, enhancing farmer training on safe pesticide handling, improving access to affordable and adequate biopesticides, and encouraging participatory research to co-develop locally relevant solutions. Integrating these actions within a coherent program would support the transition towards safer and more sustainable cabbage production systems in the region.

Chapter 4

Plant-based strategies against cabbage aphid

Cabbage plants associated with a lemongrass plant © P.N. Gakuru

Chapter 4. Plant-based strategies against cabbage aphid

This chapter explores the use of plant species with bioactive properties as part of an integrated pest management strategy. Three species of aromatic plants were selected, two from the Verbenaceae family, *Lantana camara* and *Lippia alba*, and one from the Poaceae family, *Cymbopogon citratus*. These plants were selected for their development in tropical environments, wild or cultivated, and are known to have medicinal, antifungal, or insecticidal properties. Two approaches were used with these plants to assess bioactive and repellent effects on cabbage aphids. In the first approach, each of the three plants was intercropped with cabbage to assess how their compounds affected aphid dispersion. The second approach focused on investigating the aphicidal and deterrent properties of essential oils from each of the three aromatic plants.

Accordingly, **Chapter 4** is subdivided into two main sections:

- Repellent effects of aromatic companion plants on cabbage aphid.
- Bioactivity of essential oils against cabbage aphid.

4.1. Repellent effects of aromatic companion plants on cabbage aphid

This section was adapted from the manuscript : **Gakuru, P.N.**, Francis, F. Dispersal response of cabbage aphid, *Brevicoryne brassicae*, to aromatic companion plants. Prepared for submission to *Ecological Entomology*

Abstract

Sustainable pest management strategies increasingly rely on plant diversification, particularly through intercropping and companion planting, to reduce dependence on synthetic pesticides. This study investigated the potential of three aromatic species, *Lantana camara*, *Lippia alba*, and *Cymbopogon citratus*, to modify the dispersal and host-selection behavior of the cabbage aphid (*Brevicoryne brassicae* L., 1758). Experiments were conducted under greenhouse conditions using two complementary approaches: (i) a dispersal assay measuring aphid movement at different distances (0, 8, and 12 cm) from aromatic plants, and (ii) dual-choice tests assessing aphid preference between a cabbage plant alone and one associated with an aromatic plant. Results showed that treatment, distance, and their interaction significantly affected aphid dispersal. In the associated treatments, aphids tended to move away from aromatic plants, yielding positive repellent indices (RI = 0.91–1.00), with *C. citratus* showing the highest values. Control treatment exhibited negative RI values (−0.22 to −0.55), indicating an attractant effect of cabbage. In the dual-choice assays, the strongest avoidance was recorded with *L. camara*, while *L. alba* and *C. citratus* induced moderate to weak repellence. These findings confirm that certain companion plants, particularly *C. citratus*, can influence host avoidance behavior and reduce cabbage aphid colonization. Integrating aromatic plant species into intercropping systems therefore represents a promising, eco-friendly strategy for sustainable vegetable protection.

Key words: Companion planting, *Brevicoryne brassicae*, volatiles, intercropping, pest management.

4.1.1. Introduction

Cabbage aphid *Brevicoryne brassicae* L., 1758 (Hemiptera: Aphididae) is an oligophagous species which significantly affect cruciferous plants (Brassicaceae Family). This aphid directly feed on sap from host plants, leading to yellowing and curling of the leaves, and can transmit phytoviruses (Mandavi and Rajendra, 2013; Zanardi et al., 2024). To face these threats, farmers often apply chemical insecticides as the main management strategy. Although these products can provide rapid suppression of pests, their excessive use leads to major issues such as the development of pest resistance, environmental contamination and human health hazards (Galani et al., 2021; Oladosu and Flaws, 2025). Moreover, they can adversely affect non-target organisms, including pollinators and natural enemies that contribute to natural pest regulation (Manzer et al., 2024).

In the search of more sustainable pest management, polyculture and intercropping systems such as companion planting have emerged as promising agroecological alternatives to reduce pesticide dependence while maintaining effective crop protection (Ratnadass et al., 2012; Wang et al., 2026). Companion plants are grown next to the target crop to ensure ecosystem services such as pest regulation, by discouraging pest establishment or hosting natural enemies (Ben-Issa et al., 2017b; Zuma et al., 2023). This approach not only contribute to control crop pests but also promotes biodiversity and aligns with the growing emphasis on sustainable agriculture, where the plant diversification can contribute to healthier ecosystems (Ratnadass et al., 2012). The use of aromatic plant species as companion plants is an efficient way of managing interactions between insects and their hosts. Aromatic plants, also called herb plants, are wild or cultivated plants with protective properties against crop pests through various mechanisms involving volatile organic compounds (VOCs) they emit (Wang et al., 2026). These VOCs can have a lethal effect or directly affect the behavior of phytophagous insects due to their repellent or deterrent properties (Saldanha et al., 2019). Companion plant VOCs can also mask host plant odor or modify host plant acceptance by phytophagous insects, thereby enhancing pest control (Ben-Issa et al., 2017a, 2017b). Research indicate that certain companion plants, such as basil, mint, onion, and rosemary, can effectively alter aphids, thus protecting crops from infestation (Dardouri et al., 2019; Dieudonné et al., 2022). For example, the introduction of basil was shown to significantly reduice the number and spatial expansion of rosy apple aphid (*Dysaphis plantaginea* Passerini, 1860) colonies in apple orchards, enhancing biocontrol efforts (Rizzi et al., 2024). Similarly, intercropping *Brassica rapa* L. with *Allium sativum* L. (garlic) delayed the green peach aphid (*Myzus persicae* Sulzer, 1776) development and largely reduiced aphid infestations (Yang et al., 2025).

Companion plants may belong to various botanical families, each characterized by distinctive ecological or biochemical traits that influence pest behavior (Ben-Issa et al., 2017b). Species of the Verbenaceae family, such as *Lantana camara* (L.) and *Lippia alba* (Mill.) N.E. Brown, are well known for their pharmacological and biocontrol properties (Kumar et al., 2024). These plants produce essential oils rich in terpenoids, flavonoids, and phenolic compounds with demonstrated insecticidal, repellent, and anti-feeding effects against various crop pests (Nea et al., 2020; Caballero-Gallardo et al., 2023). Similarly, *Cymbopogon citratus* (DC.) Stapf (Poaceae), commonly known as lemongrass, is highly valued for its aromatic properties and is a major source of citral and geraniol, volatiles widely reported for their repellent effects on several insect species (Loko et al., 2021; Koijam et al., 2025). The biochemical attributes make these aromatic species promising candidates for integration into intercropping systems as functional companion plants to reduce aphid colonization on cabbage crops.

The aim of this study was to assess the potential of three aromatic plant species (*Lantana camara*, *Lippia alba*, and *Cymbopogon citratus*) used as repellent plants for managing cabbage aphid *B. brassicae*. It involved evaluating the dispersion of aphids at different distances from the aromatic plants and observing their preference to move toward or away from cabbage plants associated with aromatic plants in the dual-choice tests.

4.1.2. Materials and methods

Growing plants and aphid rearing

These experiments were carried out in eastern RDC, Goma city, in the peri-urban area known as Lac vert (1°37'01"S; 29°08'37"E), over the period June to September 2024. Cabbage seedlings were grown under an aerated greenhouse (indoor temperature, 20 ± 5°C; relative humidity, 55 - 80%) in 10 cm diameter plastic pots filled with potting soil. The greenhouse was a plastic-covered tunnel structure that provided protection from external weather conditions while ensuring optimal levels of temperature, humidity, and light for plant growth. Seedlings were held in insect-proof cages (90 × 45 × 45 cm) (BugDorm, MegaView Science Co. Ltd., Taichung, Taiwan), watered every two days and used in the experiments after the six-leaf stage. The seedlings of the three pesticidal plants (*L. camara, L. alba,* and *C. citratus*) were grown separately in different cages until they reached a similar growth heigh to cabbage plants. Aphids were collected from infested plants in nearby vegetable gardens, then reared on cabbage seedlings in closed cages. Several generations were monitored to ensure aphid colonies were parasitoid-free.

Impact of companion plants on aphid dispersion

Four treatments were tested to assess the aphid dispersion: (1) *Brassica oleracea* alone (control), (2) *B. oleracea* + *Lantana camara* (Bo + Lc), (3) *B. oleracea* + *Cymbopogon citratus* (Bo + Cc) and (4) *B. oleracea* + *Lippia alba* (Bo + La).

Each treatment included nine plants in three equal rows. The central plant was either *L. camara*, *C. citratus* or *L. alba* for the companion plant treatments, and *B. oleracea* for the control treatment. The cabbage border plants were distributed as follows: 4 were 8 cm and 4 others were 12 cm from the central plant (Figure 8). The central plant was considered as a dissemination plant and received 20 unwinged adult aphids. Plants located at 8 and 12cm from the central plant were considered as potential aphid-selected plants. Separate trials were individually placed in 45×45×45 cm net cages, with all cages housed in an aerated greenhouse. Five replicates were assessed per treatment. Twelve days later, the number of aphids on the central and selected plants at 8 cm and 12 cm was recorded. The repellent index (RI) was calculated using the following equation adapted from Yarou et al. (2020) and Stefanazzi et al. (2011).

$$\mathrm{RI} = \frac{\%\ \mathrm{ASP}\ - \%\ \mathrm{ACP}}{\%\ \mathrm{ASP}\ + \%\ \mathrm{ACP}}$$

Where % ASP=percentage of aphids on selected plant (cabbage plants at 8 cm or 12 cm) and % ACP=percentage of aphids on central plant (aromatic plants for associated treatments and cabbage for the control). RI ranges from -1.00 to 1.00. RI values ranging from −1.00 to −0.10 indicate attractant effects toward the central plant, RIs ranging from −0.10 to +0.10 indicate neutral effects, and RIs ranging +0.10 to +1.00 indicate repellent effects to the central plant.

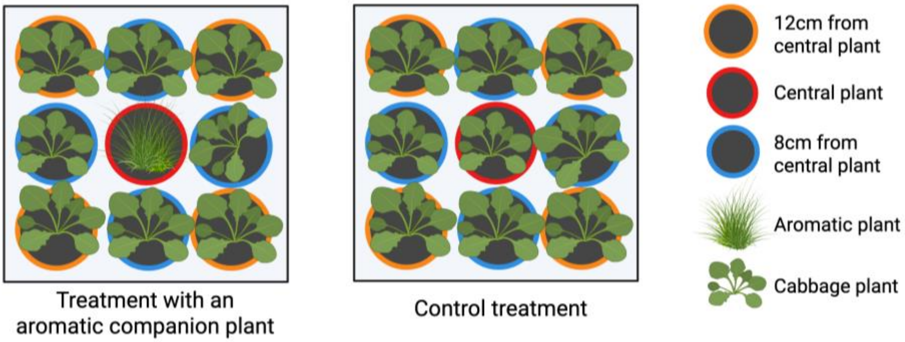

Figure 8. Illustration of the experimental setup of the aphid dispersion test.
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Dual-choice test involving companion plants

The aim was to assess the impact of companion plants placed near cabbage plants on aphid behavior. Four treatments were studied. On one hand, a cabbage plant associated with each of three aromatic plant species (*L. camara*, *C. citratus*, *L. alba*) versus a cabbage plant alone (Figure 9). On the other, a control treatment comparing a cabbage plant alone versus a cabbage plant. Each replicate was housed in a 90 × 45 × 45 cm cage. Twenty-winged adult aphids were released in the central area of the flight cage and after 24 h, we recorded the number of insects on *B. oleracea* alone or next to a pesticidal plant (Yarou et al., 2020).

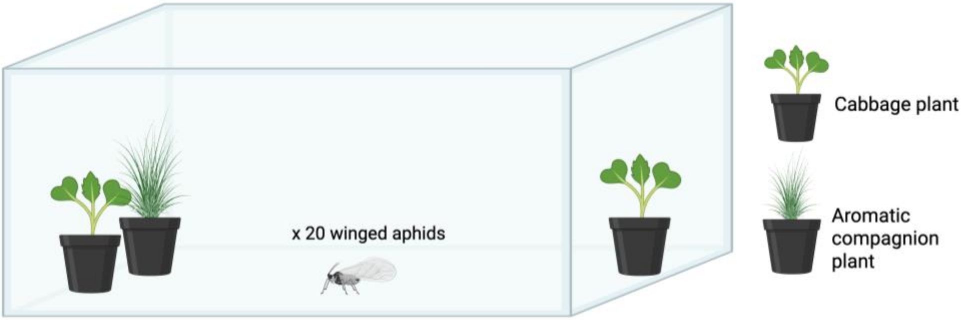

Figure 9. Illustration of the experimental setup of the dual-choice test.
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Data analysis

Data were analysed to determine whether the plant associations and distances influenced aphid dispersal behavior. A generalized linear model (GLM) with a binomial distribution and logit link function was fitted to the proportion of moved aphids as a function of *Treatment* (plant association), *Distance* (0, 8, and 12 cm), and their interaction. Model significance was evaluated by analysis of deviance with likelihood ratio chi-square tests, and pairwise comparisons among distances within each treatment performed using Tukey's post hoc test. In a separate analysis, a generalized linear mixed model (GLMM) with a binomial error distribution and logit link was used to test the effect of treatments on aphid choice. Significance of fixed effects was assessed by likelihood ratio tests (χ^2) using the glmer function of the "lme4" package (Bates et al., 2015). All statistical analyses were made in R version 4.4.1 (R Core Team, 2024).

4.1.3. Results

Effects of aromatic plants on dispersion of unwinged aphids

The analysis revealed significant effects of treatment, distance, and their interaction on aphid dispersal behavior, indicating that the effect of distance varied depending on the plant association (Table 11).

Aphid dispersal in response to plant associations and distance is shown in Figure 10. Overall, the proportion of aphids moving away from the central plant (0 cm) increased with distance (8 and 12 cm) in the association treatments (Bo + Cc, Bo + Lc, and Bo + La). In the treatments where *C. citratus* was the release plant, similar proportions of aphids were recorded at 8 and 12 cm. When the release plants were *L. camara* or *L. alba*, the number of moved aphids increased progressively with distance, with significantly more individuals observed at 12 cm than at 8 cm. In contrast, the opposite trend was observed in the control treatment (without aromatic plants) where the most aphids remained on the central plant, and their numbers decreased sharply with increasing distance.

Table 11. Synthesis of analysis of deviance for fixed factors, treatment and distance, and their interaction on aphid's abundance on cabbage plants

Factors	**Df**	χ^2	***p*-value**
Treatment	3	151.04	<0.001 ***
Distance	2	103.44	<0.001 ***
Treatment x distance	6	216.28	<0.001 ***

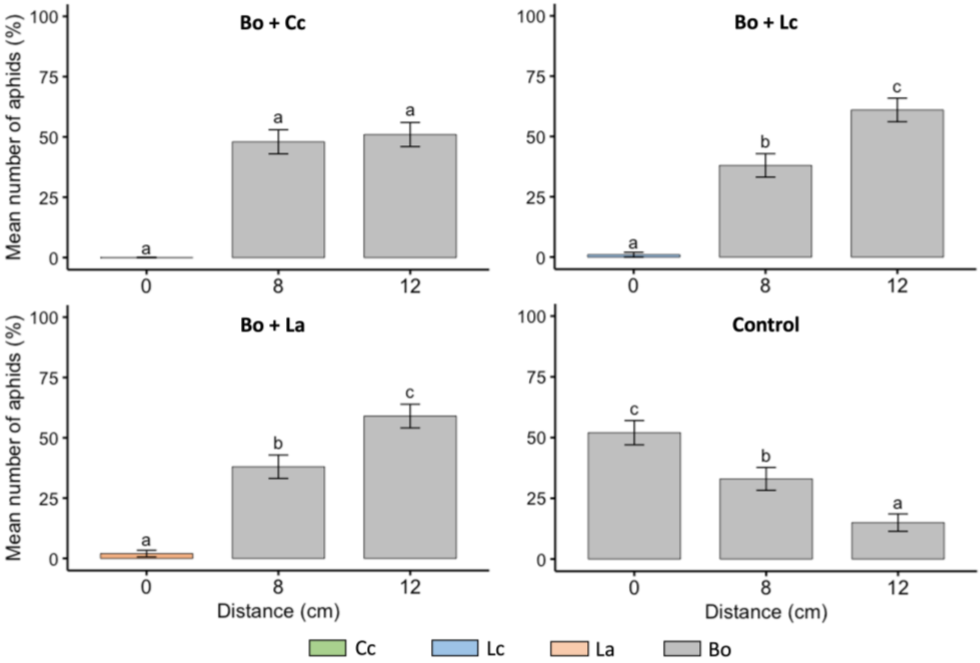

Figure 10. Aphid number (Mean ± SE, five replicates) on the central plant (0 cm) and selected plants (8 and 12cm) according to the treatments, twelve days after aphid release. Bo = *Brassica oleracea*, Lc = *Lantana camara,* Cc = *Cymbopogon citratus*, and La = *Lippia alba*. Within each treatment (Control, Bo+Cc, Bo+La, and Bo+Lc), the bars sharing the same letter are not significantly different ($p > 0.05$).

The RI varied among treatments and between distances (Table 12). Positive RI values were obtained in all association treatments, indicating a repellent effect of the aromatic plants on *B. brassicae*. Treatments with aromatic plants also showed positive RI values ranging from 0.91 to 1, confirming their repellent effect, which tended to be slightly stronger at 12 cm than at 8 cm. The overall trend of higher RI values at 12 cm than at 8 cm suggests that aphids preferred to remain further away from the central aromatic plants. In contrast, negative RI values were recorded in the control treatment, indicating that cabbage alone was not repellent to aphids. The selected plants in the control exhibited an attractant effect that was slightly stronger on plants located closer to the release point (8 cm, RI = – 0.22 ± 0.13) than on those farther away (12 cm, RI = – 0.55 ± 0.13).

Table 12. Repellent index towards *Brevicoryne brassicae* according to the position of selected *Brassica oleracea* plants (8 and 12cm) compared to the central plant (0 cm) in each treatment

Treatment	Central plant	Selected plants	Distance from the central plant	Repellent Index ± SE
Bo + Cc	Cc	Bo	8cm	1.00 ± 0.00
			12cm	1.00 ± 0.00
Bo + Lc	Lc	Bo	8cm	0.95 ± 0.11
			12cm	0.97 ± 0.06
Bo + La	La	Bo	8cm	0.91 ± 0.12
			12cm	0.94 ± 0.08
Control	Bo	Bo	8cm	– 0.22 ± 0.13
			12cm	– 0.55 ± 0.13

where RI values ranging from −1.00 to −0.10 indicate attractant effect, and RI values ranging +0.10 to +1.00 indicate repellent effect. Bo = *Brassica oleracea*, Lc = *Lantana camara,* Cc = *Cymbopogon citratus*, and La = *Lippia alba*.

Repellent effects of aromatic plants on winged aphids

The two-choice tests revealed that aphid behavior varied among treatments. In associations with aromatic plants, aphids tended to move preferentially toward the non-associated cabbage plants, indicating a repellent effect of the companion plant species. The association with *L. camara* showed the strongest response, with significantly fewer aphids found on associated cabbage plants compared to cabbage plants alone (χ^2 = 13.19, $p < 0.001$). Associations with *C. citratus* (χ^2 = 1.88, p = 0.170) and *L. alba* (χ^2 = 3.80, p = 0.051) did not significantly affect aphid distribution, suggesting weaker or negligible repellence under the tested conditions. In the control treatment comparing two cabbage plants, aphid numbers did not differ significantly (χ^2 = 0.47, p = 0.49), confirming the absence of positional bias (Figure 11).

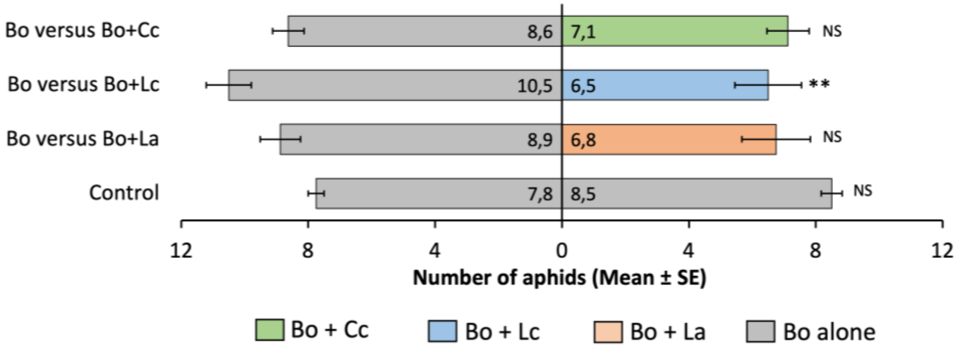

Figure 11. Selection of plants by aphids between a cabbage plant alone and another cabbage plant associated (or not) with an aromatic plant. Bo = *Brassica oleracea*, Lc = *Lantana camara*, Cc = *Cymbopogon citratus*, and La = *Lippia alba*.

4.1.4. Discussion

The present study demonstrated that the presence of pesticidal plants significantly affected the dispersal behavior of *B. brassicae* on cabbage. Overall, aphids tended to move away from the central aromatic plants, resulting in higher numbers on selected cabbage plants located 8 and 12 cm away, whereas the opposite pattern was observed in the control treatment. This indicates that the aromatic species tested are not suitable for *B. brassicae* and their emitted volatile compounds acted as repellents, altering the settling and distribution of aphids within the experimental setup.

A likely mechanism underlying these patterns is the emission of VOCs by the aromatic plants used. These VOCs can confuse aphids or repel them away from host plant (Ben-Issa et al., 2017b). Regarding the repellent effect of *C. citratus*, we observed a slight effect on winged aphids in the dual choice assays and aphid avoidance of this plant in the dispersion tests, as reflected by positive RI values. However, there was no significant difference in the number of aphids at 8cm and 12cm, indicating the plant's weak repellency against aphids, likely due to low volatile emission. Nevertheless, previous reports highlight the insect-repellent properties of the lemongrass volatiles (Koijam et al., 2025). Major constituents such as citral (neral + geranial), and geraniol are known to deter aphid landing and feeding by interfering with their olfactory orientation (Deletre et al., 2016a; Diabate et al., 2019). For example, Deletre et al. (2016) demonstrated that bioactive volatiles such as geraniol and citronellol, identified in lemongrass and other herbs, exhibited strong repellent effects against the whitefly *Bemisia tabaci* (Gennadius, 1889) when applied as net coatings, supporting the potential use of companion plants naturally emitting these compounds. Similarly, Diabate et al. (2019) reported that volatiles released from lemongrass leaves repelled the bean flower thrips *Megalurothrips sjostedti* (Trybom, 1908), particularly females, thereby reducing their infestation in cowpea crops.

Similar repellence patterns were observed with *L. camara* and *L. alba*, both of which also produced positive RI values between 0.91 and 0.97. The rise in the RI at 12 cm compared to 8 cm in these treatments further suggests a spatial gradient of VOC concentration or behavioral avoidance, with unwinged aphids selecting more distant plants to avoid the volatile plume of the central aromatic species. These Verbenaceae species contain terpenoids and other compounds with proven insecticidal or repellent activities (Nea et al., 2020; Sá Filho et al., 2022). Regarding the highest repellent activity of *L. camara* on winged aphids in the dual-choice tests, it is known to contain sesquiterpenes (e.g., β-caryophyllene, α-humulene) and other terpenoid constituents that may contribute to its insect-repellent properties (Kumar et al., 2024). Our findings align with earlier reports showing that VOCs emitted by *L. camara* are effective in repelling insect pests when used in plant association with crops, or as an extract treatment (Mvumi and Maunga, 2018) or in essential oil based formulations (Aisha et al., 2024). *L. alba*, commonly known for its essential oil, showed potential in influencing the aphid behavior, likely due also to its rich composition in volatile terpenoids (Sá Filho et al., 2022). For example, the *L. alba* EOs contain major compounds such as citral, which have been reported to have repellent potential in several insect species (Caballero-Gallardo et al., 2023). These compounds may interfere with host-plant recognition, making cabbage plants associated with *L. alba* less attractive to *B. brassicae*.

Conversely, the negative RI values obtained in the control treatment indicate that *B. oleracea* alone was attractive to unwinged aphids. This attraction may be attributed to host-specific cues such as glucosinolate-derived volatiles and green leaf odors, which are known to mediate host finding and colonization by *B. brassicae* (Malik et al., 2025). The stronger attractant effect at 8 cm compared with 12 cm in control treatments further supports the hypothesis that aphids preferentially settle near suitable host plants and avoid exposure to repellent stimuli from nearby aromatic species.

The present findings are in agreement with previous studies showing that intercropping with aromatic plants can reduce pest infestations through volatile emissions (Wang et al., 2026). Mutiga et al. (2010) compared the *B. brassicae* infestations in collard *Brassica oleracea* L. var. *acephala* associated with onion *Allium cepa* L. and with chili *Capsicum frutescens* L. They found that the onion-collard association had the lowest *B. brassicae* densities compared to the collard monoculture. Moreover, Ben-Issa et al. (2017) reported that herbs such as basil, coriander, thyme, and rosemary significantly decreased aphid populations on different vegetables. Dieudonné et al. (2022) also observed strong avoidance behavior of the rosy apple aphid (*D. plantaginea*) in response to volatiles from aromatic species. Similarly, Yarou et al. (2020) observed reduced aphid abundance in *Amaranthus hybridus* L. associated with *Ocimum* species. Overall, these results support the use of aromatic plants with pesticidal properties as effective companion species for cabbage. They should be incorporated into habitat management strategies, such as push-pull frameworks, or other systems involving insect behavior manipulation.

4.1.5. Conclusion

Overall, this study highlights the potential of the tested aromatic plants as valuable components of integrated pest management strategies. By releasing volatile organic compounds that interfere with aphid host-location cues, these species, can effectively alter aphid dispersal and reduce colonization on cabbage plants. Their integration into agroecological systems offers a sustainable alternative to chemical control. However, most experiments to date, including the present one, have been conducted under controlled conditions. Further investigations under field environments are needed to assess the stability of repellence across varying climatic factors and optimize spatial configurations. In addition, future work should focus on the chemical characterization and quantification of VOCs released by these aromatic species in field to better understand their mechanisms of action for practical use in sustainable vegetable production.

To further explore the potential of the tested aromatic plant species, the following section evaluates the insecticidal and repellent effects of their essential oils, allowing a complementary assessment of their bioactivity under controlled conditions.

4.2. Bioactivity of essential oils against cabbage aphid

This section was adapted from the manuscript : **Gakuru, P.N.**, Segers, A., Fauconnier, M-L., Francis, F. Bioactivity of essential oils from *Cymbopogon citratus*, *Lantana camara*, and *Lippia alba* against *Brevicoryne brassicae* (Hemiptera: Aphididae). Prepared for submission to *Pest Management Science.*

Abstract

The cabbage aphid (*Brevicoryne brassicae* L., 1758) is a significant pest threatening *Brassica* crops worldwide. Given the adverse effects of chemical control, the use of essential oils (EOs) represents a promising, eco-friendly alternative for pest management due to their bioactivity and biodegradability. This study aimed to assess the chemical composition of EOs from *Cymbopogon citratus, Lantana camara*, and *Lippia alba* and test their contact toxicity and repellent effects against *B. brassicae*, as well as their phytotoxic effects on cabbage (*Brassica oleracea*) leaves. Chemical analyses using the GC-MS technique revealed diverse chemical profiles dominated by monoterpenes and sesquiterpenes. Bioassays demonstrated strong, concentration-dependent insecticidal activity in all three EOs, with *C. citratus* exhibiting the lowest LC_{50} (1.26 µL/mL) after 24h and the highest efficacy after 48h of aphid exposure. *L. alba* displayed intermediate toxicity (LC_{50} = 2.45 µL/mL) with moderate leaf damage, whereas *L. camara* was the least toxic (LC_{50} = 3.45 µL/mL) but induced the strongest and most dose-dependent repellency (77.22% at 10 µL/mL) with minimal phytotoxicity. Conversely, *C. citratus* caused significant phytotoxic effects at higher concentrations. These findings underline the species-specific bioactivity of EOs, emphasizing the dual need to balance insecticidal efficacy and crop safety. Overall, essential oils offer an eco-friendly alternative to conventional pesticides, with *L. camara* EO emerging as a particularly sustainable option for Integrated Pest Management (IPM) programs, combining effective control of *B. brassicae* with low crop and environmental risks.

Key words: *Brevicoryne brassicae*, biopesticide, repellency, phytotoxicity, essential oils, IPM

4.2.1. Introduction

Brassica crops are among the most popular for human consumption worldwide (Moreb et al., 2020). Others are used for animal feeding, medicinal applications, or byproducts (Shinali et al., 2024). *Brassica* crops include common vegetables, such as cabbage, cauliflower, broccoli, and Chinese cabbage, widely consumed for their high vitamin and mineral content (Šamec et al., 2017). However, these crops are subject to damage from insect pests, including the cabbage aphid (*Brevicoryne brassicae* L., 1758) (Zanardi et al., 2024). *B. brassicae* affects cruciferous crops by sucking sap from host plants and by transmitting plant viruses (Munthali and Tshegofatso, 2014; Zanardi et al., 2024). To manage pest threats, farmers mainly rely on chemical control. While its application may yield short-term benefits, chemical control comes with adverse effects, including pest resistance, environmental pollution, and risks to human health (Galani et al., 2021). Synthetic pesticides can also hurt nontarget species, particularly beneficial insects (Cheng et al., 2022).

In the search for more environmentally friendly solutions, the use of essential oils (EOs) is attracting increasing interest as a promising ecological alternative to synthetic pesticides. Many of these phytochemical extracts possess biopesticidal potential, exhibiting inherent insecticidal, acaricidal, and repellent properties (Werrie et al., 2020). They offer a natural, biodegradable solution with a low impact on the ecosystem while being effective against insect pests (Benelli et al., 2019). Recent studies have highlighted the direct and indirect efficacy of EOs from various plant species in controlling sucking aphids, providing a sustainable approach to pest management (Khaled-Gasmi et al., 2021; Saıfı et al., 2023; X. Wang et al., 2024). For instance, Ahmed et al. (2021) evaluated the insecticidal effects of black pepper, eucalyptus, rosemary, and tea tree EOs against the green peach aphid *Myzus persicae* Sulzer, 1776 (Hemiptera: Aphididae). They found that all EOs significantly controlled the aphid population, with the most efficient being black pepper and tea tree. Essential oils from green anise and fennel, both from the Apiaceae family, showed promising fumigation results against the potato aphid, *Macrosiphum euphorbiae* (Thomas, 1878) (Dunan et al., 2021).

Previous research has indicated the remarkably antimicrobial activity and insecticidal effects of extracts from Verbenaceae family plants, including *Lantana camara* (L.) and *Lippia alba* (Mill.) N.E. Brown (Hennebelle et al., 2008; Kumar et al., 2024; Pérez Zamora et al., 2018). *L. camara* extract has shown effectiveness against *B. brassicae* with the highest dosage which induced the highest mortality, leading over 90% mortality after 24h exposure (Mvumi and Maunga, 2018). Additionally, *Lippia alba* (Mill.) N.E. Brown was tested against the potato aphid, *Aulacorthum solani* (Kaltenbach, 1843) (Tacaliti et al., 2024), and against *M. euphorbiae* (Pierquin et al., 2025), yielding promising results. Moreover, EOs derived from Poaceae herbs of the *Cymbopogon* genus, including *Cymbopogon citratus* (DC.) Stapf and *Cymbopogon winterianus* (Jowitt), have demonstrated significant insecticidal and repellent effects against various aphids, such *M. persicae* and *B. brassicae* (Costa et al., 2013; da Silva et al., 2023). While essential oils show promise for aphid management, studies have shown that the properties and compounds of EOs can vary depending on the plant species, the part of the plant used, and the geographical area where the plant is grown (Nea et al., 2020; Sá Filho et al., 2022). In this context, EOs from three plant species, *L. camara*, *L. alba*, and *C. citratus*, from different climatic zones were tested against the cabbage aphid *B. brassicae*.

The study focused on characterizing and assessing the insecticidal properties of these compounds by considering their contact toxicity and repellent effects against *B. brassicae*. The phytotoxicity of these EOs was also evaluated to determine the potential side effects on the host plant *Brassica oleracea* L.

4.2.2. Materials and methods

Materials

Cabbage plants (*Brassica oleracea* L.) were sown in soil-filled trays (TERS50, La Plaine Chassart, Belgium) and placed in insect-proof cages to ensure healthy seedlings. Two weeks after sowing, seedlings were transplanted into plastic pots (8 × 8 × 9 cm) and grown in cages up to trials in conditions of temperature, 23 ± 2°C; relative humidity, 60%; and a photoperiod of 18h of light, six hours of darkness.

B. brassicae individuals were collected from field infested cabbage crops in the surrounding of Gembloux (Belgium). Insects were transferred by touching leaves recovered with aphids to leaves of healthy cabbage potted plants at the 6-leaf stage in cages (BugDorm 4M4545, 90 MegaView Science Co. Ltd., Taichung, Taiwan). Several generations were monitored to ensure aphid colonies were parasitoid-free. The adult insects obtained were selected to be used in the bioassays.

Three EOs were tested in the laboratory bioassays. *C. citratus* EO was purchased from *Pranarôm International* S.A., Belgium (Reference 2801017, EAN 5420008502170, plant origin: India). *L. camara* EO was purchased from *Le Comptoir d'Essences*, Belgium (Reference 115316HELANT, plant origin: Madagascar). *L. alba* EO was obtained from the Dakar Technological Institute (Senegal), from plants cultivated at that site, and extracted after a 2-hour distillation using a Clevenger apparatus.

Essential Oil chemical characterization

The analysis of EOs was performed at the Laboratory of Chemistry of Natural Molecules, Gembloux Agro-Bio-Tech, University of Liège (Belgium). The EO's chemical composition was determined by gas chromatography–mass spectrometry (GC–MS) using an Agilent 6890 gas chromatograph coupled to a mass-spectrometer. Prior to GC–MS analysis, each essential oil sample was diluted 1:100 (v/v) in n-hexane. Analyses were performed in splitless mode, using helium as the carrier gas at a constant pressure of 13.9 psi. A capillary HP-5 column (30 m × 0.25 mm, film thickness 0.25 µm) was employed for compound separation. The oven temperature was initially set at 50 °C (held for 1 min) and then increased at a rate of 5 °C/min to 300 °C, followed by an isothermal hold for 5 min, resulting in a total run time of 56 min. The injector and transfer line temperatures were maintained at 320 °C and 300 °C, respectively. The mass spectrometer operated in scan mode over a mass range of m/z 40–400, with an electron ionization source set at 230 °C. The quadrupole temperature was maintained at 150 °C, and the solvent delay was set to 3 minutes. Electron energy was 69.9 eV, with an emission current of 34.6 µA. A 1.0 µL volume of each essential oil sample was injected using an automated liquid sampler. Compound identification was performed by comparison of the obtained mass spectra with those of the NIST mass spectral library. The relative abundance of individual components was calculated using the peak area normalization method and expressed as a percentage of the total chromatographic area.

Contact toxicity

The assay measured the contact toxicity of *C. citratus*, *L. camara*, and *L. alba* EOs against *B. brassicae*. Five EO concentrations were tested separately: 1.0, 2.5, 5.0, 7.5, and 10.0 µL/mL, with five replicates per treatment. For each test, a 9-cm Petri dish lined with a filter paper disc (VWR Grade 424, 90 mm) was prepared. The filter paper was treated with 1 mL of a 0.01% Tween 80 solution containing the EO; control dishes received 1 mL of 0.01% Tween 80 only. A fresh leaf piece was added to each dish as a food source. Ten adult aphids were gently placed in the center of the dish using a fine brush. All dishes were incubated at 25 ± 2 °C, 65 ± 5% RH, under a 18:6 h light: dark photoperiod. Dead insects were counted at 12, 24, and 48 hours after application. Aphids were considered dead when no movement was detected using a fine brush under a magnifying glass (Ahmed et al., 2021; Alghamdi, 2018).

Phytotoxicity assessment

The phytotoxicity of each EO was determined by exposing fresh leaf tissues of cabbage to different concentrations of each EO in the Petri dishes for 48h. Visual observations of the leaf tissues allowed to assess the differences in appearance among the EO treatments and the healthy controls (percentage of leaf surface affected by discoloration, yellowing, or necrotic spots). The phytotoxicity rating scale was adapted from Cloyd et al. (2009) and Pavela (2006). The following indices were considered: **0** suggests zero damage, **1** indicates 1-25 % damage, **2** indicates 25-50 % damage, **3** indicates 50-75% and **4** indicates more than 75% damage to the leaf tissue surface.

Repellent activity with the dual-choice test

The repellent effect of the *C. citratus*, *L. camara*, and *L. alba* EOs was assessed using the area preference method adapted from Al-Harbi et al. (2021) and Saıfı et al. (2023). The following concentrations were tested: 1.0, 2.5, 5.0, 7.5, and 10.0 µL/mL, with each concentration having three replicates. A filter paper disc (VWR Grade 424, 90mm diameter) was divided into two equal parts; one half was treated with 0.5 mL of each EO solution, while the control half was treated with an equal volume of 0.01% Tween 80. After about a quarter of an hour of air-drying, the treated and control filter paper halves were joined and fixed at the bottom of a Petri dish. Then, 10 apterous aphids were released in the center of the two halves. Petri dishes were placed in an incubator chamber (25 ± 2°C, 65 ± 5% RH). The repellency effect was recorded after 1, 3, and 5 hours, depending on the number of insects found on each treated and untreated half. The average percentage of repellency was calculated for each concentration as follows (Al-Harbi et al., 2021):

$$Pr\ (\%) = \frac{(Nc - Nt)}{(Nc + Nt)}\ X\ 100$$

Where *Nc* = The number of insects on the control half, *Nt* = The number of insects on the treated half, and *Pr* (%) = Percentage of repellency.

Statistical analysis

All analyses were performed using R 4.4.1 software, with statistical significance set at $p < 0.05$. Median lethal concentrations (LC_{50}) were estimated using probit regression (Finney, 1971). The sigmoidal relationship between dose and cumulative mortality (**P**) was linearized by applying a probit transformation to mortality proportions and a base-10 logarithmic transformation to EO concentrations. This relationship was modeled using a simple linear regression of the form: probit(P) =

slope × $\log_{10}$(concentration) + intercept. LC_{50} values were obtained from these models by identifying the dose corresponding to 50% mortality. The reliability of the regression coefficients was assessed with Student's *t*-tests, and overall model fit was evaluated using the associated *p*-value. A two-way ANOVA with fixed factors concentration and time, including their interaction, was fitted to aphid mortality. Significative results were further analyzed using a post hoc test of Tukey's HSD. Similarly, an ANOVA was applied to assess the effect of concentration on repellence of each EO. To visualize the repellent effect of concentrations over time for each EO, results were displayed as heatmaps across time and concentration. Figures were generated with *ggplot2* (Wickham, 2016).

4.2.3. Results

Chemical composition of essential oils

The *C. citratus* EO contains 19 compounds with predominance of oxygenated monoterpenes, with geraniol (37.51%), neral (32.21%), geranial (6.77%), and geranyl acetate (5.68%) as the major constituents (Table 13). The *C. citratus* EO was found to be rich in bioactive constituents with known insecticidal potential.

Table 13. Chemical composition of *Cymbopogon citratus* essential oil

N°	Name	Retention time	Retention index	Area %
1	Camphene	6,68	947	2,32
2	5-Hepten-2-one, 6-methyl-	7,69	986	0,70
3	trans-β-Ocimene	9,12	1037	0,53
4	4-Nonanone	10,14	1073	1,42
5	Linalool	10,94	1100	1,42
6	Isoneral	12,87	1165	1,32
7	Rose furan oxide	13,20	1176	0,48
8	3,6-Octadienal, 3,7-dimethyl-	13,41	1183	2,10
9	L-α-Terpineol	13,66	1192	0,42
10	Neral	15,19	1245	32,21
11	Geranial	15,53	1256	6,77
12	Geraniol	16,07	1275	37,51
13	Geranyl acetate	19,13	1384	5,68
14	Caryophyllene	20,20	1424	3,18
15	trans-Isoeugenol	20,96	1453	0,40
16	Humulene	21,83	1486	0,43
17	Ethanone, 1-[4-(1-hydroxy-1-methylethyl)phenyl]-	22,47	1511	0,41
18	Gamma-cadinene	22,67	1519	2,25
19	Delta-cadinene	22,88	1528	0,44

The chemical analysis revealed 31 compounds in the *L. camara* EO. The major components identified were cis-Davanone (16.92%), Caryophyllene (15.85%), Humulene (8.96%), Sabinene (10.36%), Eucalyptol (6.04%), γ-Guaiene (5.75%), and α-Pinene (5.19%) (Table 14). Many of these compounds belong to the sesquiterpene and monoterpene classes, which are frequently associated with insecticidal or repellent effects in arthropods.

Table 14. Chemical composition of *Lantana camara* essential oil

N°	Name	Retention time	Retention index	Area%
1	α-Thujene	6,18	927	0,63
2	α-Pinene	6,36	934	5,19
3	Camphene	6,72	948	1,72
4	Sabinene	7,42	975	10,36
5	β-Pinene	7,49	978	4,43
6	α-Phellandrene	8,19	1005	0,70
7	3-Carene	8,36	1011	2,45
8	p-Cymene	8,77	1025	1,25
9	α-Terpinene	8,90	1030	2,40
10	Eucalyptol	8,98	1033	6,04
11	1,3,6-Octatriene, 3,7-dimethyl-, (Z)-	9,43	1048	0,53
12	γ-Terpinene	9,75	1059	0,88
13	Terpinolene	10,61	1089	0,44
14	Linalool	10,97	1101	3,24
15	Camphor	12,29	1146	0,85
16	endo-Borneol	12,92	1167	0,65
17	Terpinen-4-ol	13,27	1179	1,05
18	L-α-Terpineol	13,66	1192	0,35
19	Copaene	18,99	1379	1,36
20	Beta-elemene	19,24	1388	0,25
21	Beta-copaene	19,38	1393	1,25
22	Caryophyllene	20,28	1427	15,85
23	Humulene	21,15	1461	8,96
24	Aromadendrene	21,31	1466	0,23
25	γ-Muurolene	21,70	1481	0,89
26	Gamma-guaiene	22,26	1503	5,75
27	Germacrene D	22,49	1512	1,85
28	Gamma-cadinene	22,72	1521	1,41
29	Delta-cadinene	22,89	1528	1,42
30	Spatulenol	24,30	1585	0,71
31	cis-Davanone	24,55	1594	16,92

The chemical profile of *L. alba* EO revealed 28 compounds and a dominance of monoterpenes and oxygenated monoterpenes, with m-mentha-6,8-diene (26.82%), geraniol (19.09%), neral (16.02%), α-Phellandrene (8.55%), and Caryophyllene (6,05%) as the major constituents (Table 15). These compounds are well known for their insecticidal, repellent, and oviposition deterrent properties.

Table 15. Chemical composition of *Lippia alba* essential oil

N°	Name	Retention time	Retention Index	Area %
1	α-Pinene	6,34	933	0,61
2	5-Hepten-2-one, 6-methyl-	7,72	987	1,17
3	β-Myrcene	7,82	992	1,33
4	α-Phellandrene	8,23	1006	8,55
5	o-Cymene	8,79	1026	4,92
6	m-Mentha-6,8-diene	9,02	1034	26,82
7	1,3,6-Octatriene, 3,7-dimethyl-, (Z)-	9,46	1049	3,29
8	m-Cymenene	10,70	1092	0,39
9	Linalool	10,95	1101	1,33
10	trans-2-Menthenol	11,59	1122	0,40
11	Isoneral	12,88	1166	0,55
12	Rose furan oxide	13,20	1176	0,34
13	3,6-Octadienal, 3,7-dimethyl-	13,42	1184	1,23
14	(Z)-Carveol	14,51	1221	0,37
15	Citronellol	14,80	1231	0,42
16	Neral	15,26	1247	16,02
17	Geranial	15,56	1258	0,47
18	Geraniol	16,15	1278	19,09
19	3-Methoxy-5-propylphenol	18,74	1370	1,74
20	Geranyl acetate	19,14	1385	0,30
21	Caryophyllene	20,23	1425	6,05
22	cis-β-Farnesene	21,11	1459	1,30
23	bicyclogermacrène	21,30	1466	0,77
24	Cubebol	22,71	1521	0,64
25	Calamene	22,89	1528	0,88
26	trans-α-Bisabolene	23,33	1546	0,31
27	Spatulenol	24,30	1584	0,34
28	(1R,7S)-Germacra-4(15),5,10(14)-trien-1β-ol	26,14	1661	0,37

Contact toxicity of essential oils

The cumulative mortality of *B. brassicae* adults significantly increased with both exposure time for *C. citratus* (*F* = 22.60, df = 2, *p* < 0.001), *L. camara* (*F* = 47.32, df = 2, *p* < 0.001), and *L. alba* (*F* = 23.61, df = 2, *p* < 0.001) EOs, and concentration (p < 0.001) for all three plant-derived treatments, *C. citratus* (*F* = 56.61, df = 4, *p* < 0.001), *L. camara* (F = 65.74, df = 4, p < 0.001), and *L. alba* (*F* = 68.61, df = 4, *p* < 0.001). *L. camara* and *L. alba* EOs showed a slightly steeper increase in mortality between early (12h) and mid-exposure (24h) times compared to *C. citratus*, which showed increases from the first 12 hours of exposure. The highest mortality rates recorded after 48 hours of Eos exposure were 100%, 92%, and 96% for *C. citratus* (7.5 and 10μL/mL concentrations), *L. camara* (7.5μL/mL concentration), and *L. alba* (7.5 and 10μL/mL concentrations), respectively (Figure 12).

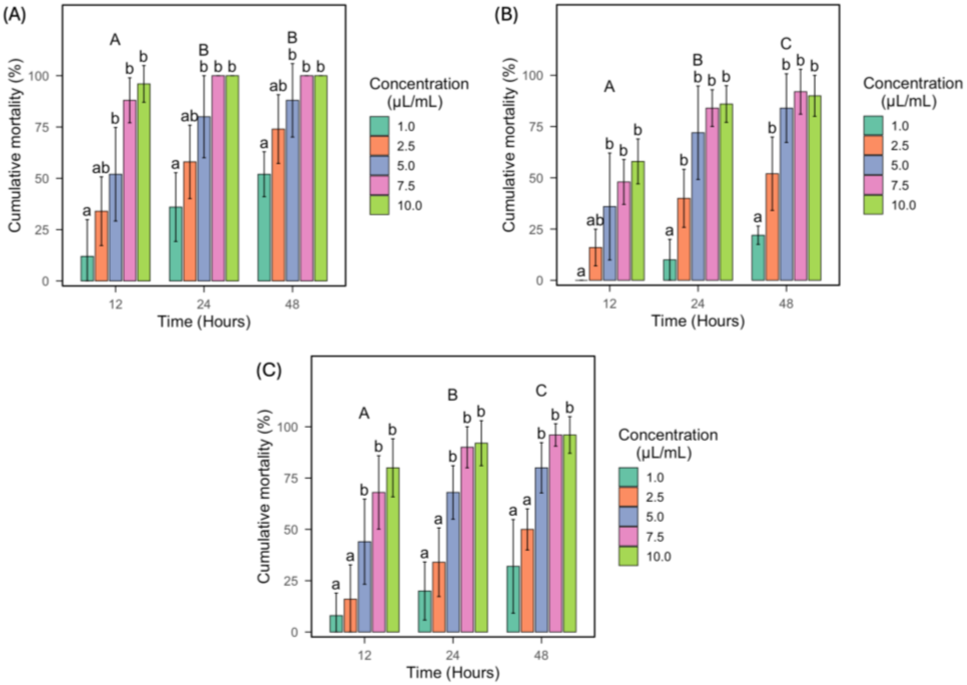

Figure 12. Mean cumulative mortality rates of *Brevicoryne brassicae* adults following the treatments with essential oils. **A**: Effect of different concentrations of *Cymbopogon citratus* EO on the mortality of *B. brassicae*; **B**: Effect of different concentrations of *Lantana camara* EO on the mortality of *B. brassicae*; **C**: Effect of different concentrations of *Lippia alba* EO on the mortality of *B. brassicae*. The same upper and lower-case letters above the bars indicate no significant difference between the time and the concentrations, respectively. The bar represents standard deviations (SD).

Probit analysis revealed significant differences in the toxicity of the three EOs against *B. brassicae* adults after 24 h of exposure (Table 16). *C. citratus* EO presented the highest potency, with the lowest LC_{50} (1.26 µL/mL) and LC_{90} (4.52 µL/mL), indicating strong activity at relatively low concentrations. *L. alba* EO showed intermediate toxicity (LC_{50} = 2.45 µL/mL; LC_{90} = 6.47 µL/mL), while *L. The camara* EO was the least toxic, with an LC_{50} of 3.45 µL/mL and an LC_{90} of 11.82 µL/mL. Overall, the three EOs exhibited strong aphicidal activity, with *C. citratus* being the most effective against *B. brassicae*.

Table 16. Lethal concentrations of essential oils against the cabbage aphid at 24 and 48 hours

Essential oil	**Exposure Time (hours)**	**LC_{50} (µL/mL)**	**LC_{90} (µL/mL)**	**Slope**	**R^2**
Cymbopogon citratus	24	1.26 (0.89−2.20)	4.52 (2.04 – 8.44)	2.87	0.77
Lantana camara	24	3.45 (2.29 – 6.13)	11.82 (6.57 – 26.95)	2.39	0.87
Lippia alba	24	2.45 (1.39−6.94)	6.47 2.94−27.46)	3.04	0.68

Repellent effects of essential oils

Repellent bioassays revealed apparent differences among the three EOs tested against *B. brassicae* (Table 17, Figure 13). *L. camara* EO exhibited the most potent and most concentration-dependent effect, with repellency increasing from 43.95% at 1 µL/mL to 77.22% at 10 µL/mL. Statistical analysis showed significant differences among concentrations ($F = 3.04$, df = 4, $p = 0.028$). *C. citratus* EO produced moderate repellency (33.58–68.14%) but showed no significant variation among concentrations ($F = 1.97$, df = 4, $p = 0.118$), indicating a relatively stable response. In contrast, *L. alba* EO displayed the lowest repellency (29.44), with significant increases observed (60.98%) at the highest concentrations. Significant differences were observed between the lowest and highest concentrations ($F = 3.796$, df = 4, $p = 0.010$). Overall, the repellency increased progressively with concentration (Table 17). The temporal dynamics illustrated in Figure 13 confirm these trends: *C. citratus* exhibited a more gradual repellent response, one that depended on time rather than concentration. In contrast, repellency of *L. camara* increased sharply with both concentration and time, reaching the highest levels after 5 hours, while *L. alba* produced weaker but still progressive effects over time.

Phytotoxicity of essential oils

Phytotoxicity results revealed apparent differences among the tested EOs (Table 18). *L. camara* EO caused no visible damage at concentrations up to 5.0 µL/mL and only slight injury (1–25%) at 7.5 and 10 µL/mL, indicating low phytotoxicity. *L. alba* EO induced minor to moderate leaf damage, with no effects at lower concentrations, but damage reaching 25–50% of the leaf surface at 10 µL/mL. In contrast, *C. citratus* EO exhibited the highest phytotoxic effect, producing visible injury at 2.5 µL/mL and increasing in severity with the dose, reaching 50–75% damage at 10 µL/mL.

Table 17. Repellent effects of different essential oil concentrations against *Brevicoryne brassicae*

Essential oil	**Concentration (µL/mL)**	**Mean (%) ± SD**
Cymbopogon citratus	1.0	33.58 ± 12.04 ^{a}
	2.5	51.48 ± 18.42 ^{a}
	5.0	54.87 ± 24.85 ^{a}
	7.5	60.18 ± 23.37 ^{a}
	10.0	68.14 ± 20.42 ^{a}
Lantana camara	1.0	43.95 ± 16.20 ^{b}
	2.5	56.17 ± 22.69 ^{ab}
	5.0	63.39 ± 18.55 ^{ab}
	7.5	71.85 ± 20.63 ^{a}
	10.0	77.22 ± 18.55 ^{a}
Lippia alba	1.0	29.44 ± 15.63 ^{b}
	2.5	45.92 ± 17.67 ^{ab}
	5.0	49.52 ± 15.79 ^{ab}
	7.5	55.74 ± 15.44 ^{a}
	10.0	60.98 ± 15.59 ^{a}

Means ± standard deviation (SD) followed by the same letter do not differ statistically at 5% significance.

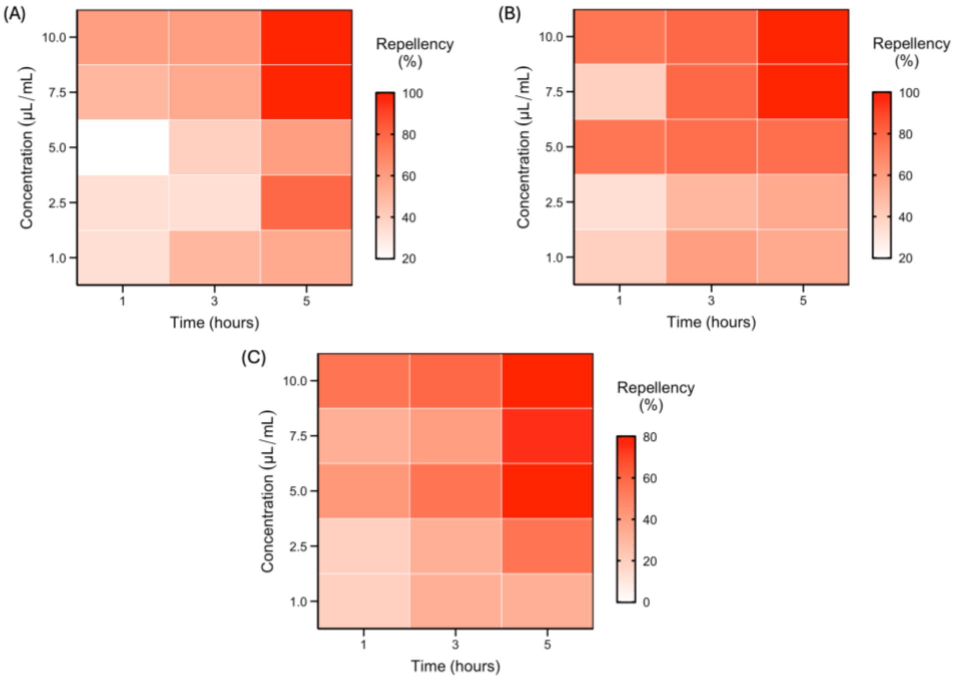

Figure 13. Evolution over time of the repellent effects of different essential oils against *Brevicoryne brassicae*. **A**: Effect of different concentrations of *Cymbopogon citratus* EO on the repellency of *B. brassicae*; **B**: Effect of different concentrations of *Lantana camara* EO on the repellency of *B. brassicae*; and **C**: Effect of different concentrations of *Lippia alba* EO on the repellency of *B. brassicae.*

Table 18. Evaluation of phytotoxicity from essential oil solutions on cabbage leaf tissues

Essential oil	**Concentrations (µL/mL)**				
	1.0	**2.5**	**5.0**	**7.5**	**10.0**
Cymbopogon citratus	0	1	1	2	2
Lantana camara	0	0	0	1	1
Lippia alba	0	0	1	1	1

With 0 = zero damage, 1 = 1-25 % damage, and 2 = 25-50 % damage.

4.2.4. Discussion

The EOs analyzed in this study revealed rich and diverse chemical profiles dominated by monoterpenes and sesquiterpenes, many of which are known for their insecticidal properties. Caryophyllene, a shared constituent between the *L. camara* (15.85%), *L. alba* (6.05%), and *C. citratus* (3.18%), is a sesquiterpene hydrocarbon known for larvicidal and neurotoxic activities (Lima et al., 2024). The combined abundance of geraniol, neral, and geranial (>75%) in the lemongrass EO suggests a synergistic contribution to the oil's bioactivity. This is consistent with previous findings (Brügger et al., 2019; Plata-Rueda et al., 2020) regarding major components of the *C. citratus* EO. In *L. camara* EO, the dominant constituents were cis-davanone and Caryophyllene. Our findings are consistent with previous studies (Aisha et al., 2024; Nea et al., 2020) that confirmed Caryophyllene among the main compounds of *L. camara* EO. Besides m-Mentha-6,8-diene*, L. alba* EO mainly included geraniol and neral. Our results support previous findings (Sá Filho et al., 2022) that identified similar chemical compounds. Globally, variations exist in the richness of components, depending on the geographical area of collection, seasonal variability, the extracted organ, and the extraction method (Nea et al., 2020; Sá Filho et al., 2022). The varying composition among species highlights the importance of chemical profiling to guide targeted applications in pest control programs.

Our study demonstrated that the contact toxicity of *C. citratus*, *L. camara*, and *L. alba* EOs against *B. brassicae* adults was both time- and concentration-dependent. Mortality significantly increased with higher doses and longer exposure times, confirming the potent bioactivity of these plant-derived products. Similar findings were reported for other insect pests, where EOs rich in oxygenated monoterpenes and sesquiterpenes, such as β-caryophyllene and linalool (Kumar et al., 2024), disrupt the insect nervous system and physiological processes, leading to rapid mortality (Gad et al., 2022). For *C. citratus*, the rapid and concentration-dependent mortality observed is in agreement with previous studies showing strong larvicidal and contact toxicity against *Agrotis ipsilon* (Hufn., 1766) (Moustafa et al., 2021) and *Ulomoides dermestoides* (Fairmaire, 1893) (Plata-Rueda et al., 2020), which is attributed mainly to citral compounds (neral and geranial), one of the major constituents. Similar activity was reported against *Spodoptera littoralis* (Boisd., 1833) (Moustafa et al., 2023), while lower toxicity was noted on the beneficial predatory bug *Podisus nigrispinus* (Dallas, 1851) (Brügger et al., 2019), suggesting species-specific selectivity. Enhanced performance through nanoformulations was also documented against *Spodoptera frugiperda* (J.E. Smith, 1797) (Ibrahim et al., 2025), highlighting formulation effects on efficacy. For *L. camara*, our results showing high aphid mortality align with previous studies on other insect pests where *L. camara* extracted oil reduced *Sitophilus zeamais* (Mots., 1855) populations (Ayalew, 2020) and with other assays reporting its insecticidal and repellent activities against *Callosobruchus maculatus* (Fabricius, 1775) (Zandi-Sohani et al., 2012). Larvicidal effects of *L. camara* EO and nanoemulsion were also reported against *Anopheles culicifacies* (Giles, 1901) (Sonter et al., 2024). The potential of *L. camara* is often linked to β-caryophyllene and α-Pinene, two major compounds that demonstrated strong insecticidal activities against many other pests, including *Megoura japonica* (Matsumura, 1927) and *Plutella xylostella* (L., 1758) (Ma et al., 2020). However, some studies noted comparatively lower performance of *L. camara* extracts against aphids when contrasted with *Tagetes minuta* (L.) or *Carica papaya* (L.) extracts (Murovhi et al., 2020), suggesting that efficacy may vary with crop, phytocompounds used, and environmental context. Regarding *L. alba*, the moderate but significant aphid mortality observed was consistent with previous reports of strong insecticidal effects of *L. alba* chemotypes against the storage pests *S. zeamais*, *Tribolium castaneum* (Herbst, 1797) (Peixoto et al., 2015), and *C. chinensis* (Shukla et al., 2011). Nevertheless, inter-chemotype variability in composition has been shown to affect biocontrol potential (Peixoto et al., 2015). This may explain the somewhat lower efficacy observed in our assays compared to *C. citratus*, despite the presence of some shared compounds. Collectively, these comparisons confirmed that while all three oils possess insecticidal potential, their relative effectiveness varies depending on the target pest, chemical profile, and formulation (Heidary et al., 2022). Beyond the EOs assessed in our study, several other botanical extracts have demonstrated significant efficacy against *B. brassicae* (Pavela, 2006). For instance, investigations by da Silva et al. (2023) revealed that *Schinus terebinthifolius* (Raddi), *Eucalyptus citriodora* (Hook), and *C. winterianus* EOs markedly increased aphid mortality. The *C. winterianus* EO achieved a higher mortality rate after 48 hours for both *B. brassicae* (100% with a 0.5% concentration) and *M. persicae* (98.99% with a 1% concentration) (da Silva et al., 2023), paralleling our results with high-dose *C. citratus*.

The Lethal concentration values obtained in our study further reinforce the mortality patterns, highlighting differences in potency among the three tested oils. *C. citratus* exhibited the lowest LC_{50} (1.26 µL/mL), confirming its higher toxicity compared to *L. alba* and *L. camara*. Comparable findings were reported with other EOs against *B. brassicae*. Pavela (2006) showed that *Nepeta cataria* (L.) and *Lavandula augustifola* (Mill.) EOs achieved lethal dose 50 (LD_{50}) values of 1.42 µL/L and 3.35µL/L after 24 hours, respectively. Studies of Heidary et al. (2022) on *Thymus daenensis* oil, particularly when nano-encapsulated, also corroborate the benefits of formulation improvements. The authors demonstrated that *T. daenensis* oil exhibited strong aphicidal activity on *B. brassicae* under contact exposure, with LC_{50} values of 80 ppm for the raw oil and 30 ppm for the nano-encapsulated formulation, while LC_{90} values decreased from 230 ppm to 90 ppm, respectively. These comparisons confirm that *B. brassicae* is highly susceptible to a broad range of EOs, although relative toxicity depends on chemical composition, application method, and formulation.

The repellency bioassays revealed significant differences in the behavioral responses of *B. brassicae* to the three tested EOs. *L. camara* EO exhibited the highest and most concentration-dependent effect, with repellency ranging from 43.95% at 1 µL/mL to 77.22% at 10 µL/mL. This result is consistent with earlier studies showing that *L. camara* compounds exerts strong deterrent effects against various insect pests, including aphids (Mvumi and Maunga, 2018), storage beetles (Aisha et al., 2024), and mosquitoes (Sharma et al., 2021), attributed mainly to sesquiterpenes such as β-caryophyllene and germacrene D, which disrupt insect host-seeking behavior (Kumar et al., 2024). By contrast, *C. citratus* EO induced moderate repellency (33.58–68.14%) without significant variation among concentrations, indicating a relatively stable effect across doses. Such a pattern agrees with previous observations that lemongrass oil, rich in geraniol and citral isomers, provided sustained repellent effects against four aphid species – *M. persicae, Aphis gossypii* (Glover, 1877)*, Aphis spiraecola* (Patch, 1914), and *Aphis fabae* (Scopoli, 1763) – at 5µL doses, and findings show that the repellent effect did not decrease over the surveyed period (Khaled-Gasmi et al., 2021). The prolonged action observed in our assays (e.g., time-dependent increase in repellency) supports the hypothesis of progressive release and persistence of the EO over time (Khaled-Gasmi et al., 2021). *L. alba* EO exhibited the lowest repellency (29.44–60.98%), albeit with a clear positive dose-response trend. This pattern mirrors findings in *Lippia* chemotypes, where oils rich in oxygenated monoterpenes, such as citral and carvone, displayed enhanced repellency against adults of *U. dermestoides*. Carvone-rich *L. alba* and citral-rich *L. alba* performed 100% repellency at a concentration of 16 µL/mL (Caballero-Gallardo et al., 2023). Further studies highlighted the repellent properties of *L. alba* against various insect pests, including the pulse beetle, *C. chinensis* (Shukla et al., 2011), and the mosquitoes *Culex quinquefasciatus* (Say, 1823) and *Aedes aegypti* (L., 1762) (Mahanta et al., 2019).

The phytotoxicity assays showed that the tested EOs differed considerably in their impact on cabbage leaves, with *C. citratus* being the most phytotoxic. Such variability is strongly associated with their chemical profiles. In particular, aldehydic monoterpenes, such as neral and geranial, which are abundant in *C. citratus*, are known to be highly reactive, leading to oxidative stress, membrane disruption, and visible leaf injury at higher doses (Graña et al., 2016; Werrie et al., 2020). For instance, Poonpaiboonpipat et al. (2013) tested *C. citratus* EO on the seedlings of *Echinochloa crus-galli* (L.) Beauv. They observed leaf wilting and desiccation symptoms one hour after treatment, as well as a decrease in chlorophyll and carotenoid content six hours after spraying. Similarly, *L. alba* exhibited moderate phytotoxic effects, which may be attributed to variations in its chemotype composition. Globally, oils richer in oxygenated monoterpenes such as neral and linalool tend to be phytotoxic in several crops, where they act by altering cell permeability and impairing germination and growth processes (Graña et al., 2013; Abd-ElGawad et al., 2020). In contrast, *L. camara* EO appears much safer for cabbage leaf tissue, likely due to its higher proportion of sesquiterpene hydrocarbons such as Caryophyllene with less reactivity compared to highly reactive oxygenated monoterpenes (Abd-ElGawad et al., 2020).

4.2.5. Conclusion

Overall, the three EOs assessed in this study demonstrated significant bioactivity against *B. brassicae*, with *C. citratus* showing the highest toxicity but also greater phytotoxicity. *L. camara* combined strong repellent and insecticidal effects with minimal leaf damage, and *L. alba* displayed intermediate efficacy and moderate phytotoxicity. These findings confirm that EOs, particularly *L. camara*, represent valuable eco-friendly alternatives to synthetic pesticides, providing effective aphid management while mitigating environmental risks. Field trials are necessary to evaluate the effectiveness of EOs under outdoor conditions.

To develop a global framework of complementary methods, it is necessary to evaluate the effectiveness of other components of biological control, including microbial agents. The next chapter assesses the potential of entomopathogenic fungi against *B. brassicae*.

Chapter 5

Microbe-based biocontrol of cabbage aphid

A cabbage aphid cadaver infected by *Beauveria bassiana*, showing external fungal sporulation. © P.N. Gakuru

Chapter 5. Microbe-based biocontrol of cabbage aphid

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Abstract

Entomopathogenic fungi (EPF) are promising eco-friendly alternatives to chemical insecticides against various insect pests. We assessed the efficacy of different *Beauveria bassiana* (Bals.-Criv.) Vuill. strains against *Brevicoryne brassicae* (L., 1758), a major pest of Brassica crops under both laboratory and field conditions. The virulence and endophytic properties of three strains of *B. bassiana*, the commercial strain GHA, the endemic one KA14 from the Democratic Republic of Congo (DRC), and GxABT-1 from Belgium, were assessed under laboratory conditions. Under field conditions in eastern DRC, the efficacy of the *B. bassiana* GHA was evaluated compared to that of a conventional insecticide. In vitro, the lethal time 50 (LT_{50}) values ranged from 3 days for *B. bassiana* GxABT-1 to 4 days for both GHA and KA14 following direct application of fungi. Aphid exposure to fungus-treated leaves at 7 and 14 days after inoculation (DAI) resulted in LT_{50} values of 4 to 5 days for GHA and KA14. Regarding the endophytic potential, B. *bassiana* KA14 showed higher performance in colonizing plant tissues and improving plant height growth. In the dual-choice tests, aphids were initially attracted to fungus-treated plants at 7 DAI but showed no preference between treated and control plants at 14 DAI. Field trials indicated no significant difference between fungal and chemical insecticide treatments, but both significantly reduced *B. brassicae* infestation compared to the Control. Our results support the interest of exploring the potential of the endemic *B. bassiana* KA14 strain against various insect pests and applying different methods.

Keywords: cabbage aphid, entomopathogenic fungi, Hypocreales, endophyte, microbial Control.

5.1. Introduction

The cabbage aphid, *Brevicoryne brassicae* (L., 1758) (Hemiptera: Aphididae), is an important limiting pest causing severe losses in *Brassica* crops (Gakuru et al., 2024; Mpumi et al., 2020; Pereira et al., 2019). The highly damaging potential of *B. brassicae* is mainly due to its reproductive capacity, especially in warm weather (Munthali and Tshegofatso, 2014), and its feeding activity, which causes the wilt of leaves and, therefore a poor head formation (Hao et al., 2019; Saha et al., 2018).

Chemical insecticides are the main practice used by vegetable growers to protect cabbage from insects (Tudi et al., 2021). In the southeastern Democratic Republic of Congo (DRC), 86% of smallholder farmers in Lubumbashi rely on chemical control practices to protect the Chinese cabbage crops (Balasha and Nsele, 2019). Such practices cause adverse effects on the environment and human health. They can also be toxic to non-target organisms, including beneficial insects, besides the emergence of resistance within treated insect pests (Ntow et al., 2006; Tudi et al., 2021). As an alternative control method, biological control agents are being encouraged in Integrated Pest Management (IPM) (Prabha et al., 2016; Vu et al., 2007). Beneficial microbes such as fungi are among the sustainable and eco-friendly alternative to chemical pesticides within IPM systems, enhancing crop protection while minimizing environmental impact. Due to their specificity and ability to infect through the cuticle, hypocrealean entomopathogenic fungi (EPF) exhibit a broad host range of arthropods, including sap-sucking insects such as aphids (Biryol et al., 2022; Shang et al., 2024). For example, *Beauveria bassiana* (Bals.-Criv.) Vuill. (Hypocreales: Cordycipitaceae) is one of the most studied species within EPF. They exhibit a cosmopolitan distribution and affect many insect hosts (Mascarin and Jaronski, 2016). Besides its entomopathogenic potential, *B. bassiana* is also recognized as an endophytic fungus that can colonize plant tissues without harming its host (Francis et al., 2022). In this form, a mutualistic relationship is established in which the fungus benefits from plant-derived nutrients and, in response, promotes plant growth and enhances the host immune system against biotic stressors, including pathogens and insect pests (Torkaman et al., 2023).

Many strains of *B. bassiana* have been used to control vegetable insect pests, including the diamondback moth *Plutella xylostella* (L., 1758) (Shehzad et al., 2022), the cabbage looper *Trichoplusia ni* (Hübner, 1803) (Mireles-Valdez et al., 2023), the green peach aphid *Myzus persicae* (Sulzer, 1776) (Arinanto et al., 2024; Yogananda et al., 2023) and the tomato fruit worm *Helicoverpa armigera* (Hübner, 1808) (Qayyum et al., 2015). However, little attention has been paid to their performance against *B. brassicae*. Different studies have evaluated the biocontrol properties of the

B. bassiana GHA strain in different agroclimatic areas around the world (Mantzoukas et al., 2022a; Świergiel et al., 2016), but to our knowledge, no study has been conducted to evaluate their potential against the cabbage aphid under the environmental conditions of eastern DRC.

This study aims to evaluate the virulence of different strains of *B. bassiana* against *B. brassicae*. Specifically, the study investigated (a) the pathogenicity of different *B. bassiana* strains on *B. brassicae* as well as their endophytic potential under laboratory conditions, and (b) the performance of the commercial *B. bassiana* GHA against the same aphid species under natural conditions of eastern DRC. The outcome of such a study will help to establish more sustainable practices to control *B. brassicae*.

5.2. Materials and methods

5.2.1. Evaluation of the pathogenicity and endophytic behavior of entomopathogenic fungi under laboratory conditions

Cabbage plants and aphids rearing

Healthy cabbage plants were obtained by direct seeding onto seed trays filled with a universal potting soil (TERS50, La Plaine Chassart, Belgium). Two weeks after sowing, the seedlings were transplanted into 10 cm diameter pots. Both trays and pots were placed in closed cages in a growth chamber under controlled conditions at 23±1°C, 60% RH, and LD 16:8h. Colonies of *B. brassicae* were collected from infested cabbage crops and transferred to the Functional and Evolutionary Entomology Laboratory at Gembloux Agro-Bio Tech (University of Liège, Belgium). Healthy aphids were then picked up from the samples and used to infest six-leaf stage healthy cabbage plants and kept in rearing cages (BugDorm 4M4545, 90 MegaView Science Co. Ltd., Taichung, Taiwan) under the same controlled conditions as mentioned above. Several successive generations were monitored to ensure the aphid colonies were free of parasitoids or natural fungal infection.

Beauveria bassiana: origin and inoculum preparation

A total of three strains of *B. bassiana* were used in this study, including the GHA strain isolated from the commercial formulation Botanigard 22WP, GxABT-1 isolate obtained from soil samples as described in Dessauvages et al. (2024), while KA14 is an endemic strain isolated from earwig (Dermaptera: Forficulidae) cadavers collected in eastern DRC (Cokola et al., 2023a). The different *B. bassiana* strains were maintained on Sabouraud Dextrose Agar (SDA) media and incubated for 2 weeks at 23 ± 1°C to allow mycelia and spore production. The fungal inoculum was prepared by harvesting the different fungal structures (conidia and mycelia) into 10 ml of 0.03% Tween 80 using a sterilized spatula. The fungal suspension was then filtered using sterile cheesecloth. Conidia count was determined using a Bürker hemocytometer (Marienfeld, Germany), and concentration was adjusted to 1×10^8 mL^{-1}. To test the viability of the conidia of each strain, a 100µL of 1×10^5 conidia mL^{-1} dilution was spread on an SDA plate and incubated at 23 ± 1°C. After 24 hours, germinated and non-germinated conidia were counted, and only when we reported >90% viability were used for the bioassays.

Efficacy of fungal treatments against Brevicoryne brassicae

Ten aphids were placed in a Petri dish and sprayed, from a 10 cm distance, with 1 ml of a 1×10^8 conidia mL^{-1} of fungal inoculum (*B. bassiana* GHA, KA14 and GxABT-1). The same number of aphids were sprayed with 0.03% Tween 80 for the negative control. Each group of treated aphids was then transferred to a young cabbage leaf taken from non-infested 3-week-old seedlings secured in a plastic cup containing 1.5% agar solution, following the method by Ben Fekih et al. (2019). The agar served as a micro-humid environment to keep the aphid's food resource fresh during the bioassay. Five replicates were performed for each treatment. The different cups were kept in controlled conditions at 23°C for LD 16:8h. Aphid mortality was checked daily over 8 days. Aphid cadavers were collected daily and prepared for surface sterilization by immersion in 70% ethanol for 10 seconds and then washed three times for 10 seconds in sterile water, as described in Dessauvages et al. (2024). Then, the cadavers were placed on sterile wet filter paper in a Petri dish and incubated at 23°C to allow fungal outgrowth if it existed.

Endophytic potential and indirect impact on Brevicoryne brassicae

Both *B. bassiana* GHA and the endemic strain KA14 conidia suspensions were prepared as described above. 5 ml of 1×10^8 conidia mL^{-1} of each of the fungal suspensions was used to spray the leaves and stem of three-week-old cabbage plants following the method of Jaber and Enkerli (2017) and Jaber et al. (2018). The control plants were sprayed with an equal volume of 0.03% Tween 80. Twenty plants were used to assess the indirect impact of the *B. bassiana* treatments against the aphid. The endophytic impact of *B. bassiana* GHA and KA14 in treated plants on *B. brassicae* were tested at 7 and 14 days after inoculation. Cabbage leaves harvested at 7 and 14 days after inoculation (DAI) were individually secured in 1.5% agar solution in cups of 30 ml. Then, 10 aphids were transferred onto each of the leaves. The mortality of aphids was checked daily for a period of 8 days, as above. Five replicates were adopted for each treatment and sampling time.

Impact of fungal treatments on plant growth and endophytic colonization

Growth parameters such as plant height (the distance from the base of the plant to the tip of the longest leaf) were measured at 7 and 14 DAI for both inoculated and control plants. The experiment followed a three-treatment design (two strains of *B. bassiana* GHA and KA14 and the Control); each treatment included a total of 20 plants, 10 of which were sampled at 7DAI and then at 14 DAI for plant growth parameters assessment and endophytic plant colonization assays. The net height increase parameter, adapted from Torkaman et al. (2023), was estimated as the difference between the height at 7 or 14 DAI and the initial height before inoculation. The endophytic potential of both strains of *B. bassiana* on treated plants was assessed at 7 and 14 DAI following Parsa et al. (2013). The same plants chosen randomly for assessing the growth parameters were used to perform the endophytic colonization assessment. Leaves, stems, and roots from five plants per treatment were processed on each sampling day. Plant material was surface sterilized by immersion in 70% ethanol for 2 minutes, followed by 2 minutes in sodium hypochlorite and three rinses in sterile distilled water. The plant material was surface-dried in a laminar flow hood. Six leaf discs (approximately 1 cm^2) were cut from surface-sterilized leaves, and a similar number of stem tissue pieces (approximately 1 cm long) were obtained using a sterile scalpel. Six selected segments (approximately 1 cm long) of the different root parts (primary roots and lateral roots) were cut using a sterile scalpel. The six sampled plant tissues from each plant segment were then evenly distributed on separate Petri dishes containing SDA culture medium supplemented with streptomycin (0.5 ml of 0.6 $g\ ml^{-1}$), tetracycline (0.5 ml of 0.05 $g\ ml^{-1}$) and cyclohexamide (1 ml of 0.05 $g\ ml^{-1}$). The dishes were incubated at 25 ± 1°C and inspected for fungal outgrowth after 14 days. The percent colonization frequency (CF) was calculated using the following formula from Altaf et al. (2023).

$$\mathrm{CF} = \frac{\text{Number of plant segments showing fungal growth}}{\text{Total number of plated plant segments}}\ x\ 100$$

Dual-choice tests

To assess the impact of endophytic colonization of the tested fungal strains on aphid behavior, leaf discs from cabbage plants were used for dual-choice assays, as described in Fingu-Mabola et al. (2020). Leaf discs of 1 cm diameter were collected from inoculated cabbage plants at 7 and 14 DAI and control plants. The different leaf discs were taken from leaves of the same age per plant at both sampling times and placed in 9 cm diameter Petri dishes containing moist filter paper (VWR Grade 424, 90mm diameter, Germany). The Petri dishes were kept in the dark for 10 minutes to reduce the emission of volatiles from the cutting procedure as explained in Fingu-Mabola et al. (2020). Then, two leaf discs of two combinations (fungal-treated at 7 DAI or 14 DAI and Control) were placed about 6 cm apart. A total of 10 adult aphids kept for two hours without food were placed in the center of the Petri dish to assess feeding preference. The dishes were kept in a ventilated incubator at 23°C. The number of aphids on each of the two discs was recorded after 2, 4, and 6 hours. Each combination of choice was repeated five times.

5.2.2. Field experiments

Field experiments were conducted in eastern DRC, North-Kivu province, Nyiragongo Territory, about 15 km north of Goma city during two consecutive seasons: an experiment conducted in the long rainy season (October 2022 – January 2023) named "LRS" and an experiment conducted in the short rainy season (March – July 2023) named "SRS". Geographic coordinates are 1° 36.4544'S; 29° 15.4049'E; Altitude 1801m and 01° 36.3277'S; 29° 15.4736'E; Altitude 1815m, respectively. For each experimental season, a completely randomized block design was used with three replicates for each treatment: a commercial formulation of *B. bassiana* GHA (Botanigard 22WP), the pyrethroid chemical insecticide (Cyperscope 5EC) as a positive control, and the negative control plots treated only with water (Table 19). The wettable powder of Botanigard 22WP was applied according to EU standards (Certis, 2021). The first insecticide application was made at the vulnerable stage of the crop to insect attacks, i.e., 15 days after transplanting (DAT). Two further applications were made at about ten-day intervals, depending on weather conditions, particularly rainfall. All insecticide applications were made with a backpack sprayer. The plots selected for each treatment were 4 × 2 m and contained 24 plants each. Transplanting was carried out on raised beds with a 0.5m x 0.4m spacing. No agrochemical product was applied, but all plots received an organic fertilizer. Other recommended cultural operations, such as weeding and ridging, were applied evenly using hand tools. Cabbage plants within plots were monitored every 7 days from 7 DAT, and aphids were counted using a 10x magnifying glass. Spaces between leaves and their upper and lower surfaces were also checked for hidden insects (Mantzoukas et al., 2022a; Shonga and Getu, 2021). General plant health was assessed by counting the number of plants showing signs of aphids. The following formula was used to determine the infestation rate:

$$\textbf{Infestation rate} = \frac{\text{Number of infested plants}}{\text{Total number of plants}} x\,100$$

Table 19. Description of insecticides used in the field experiments

Commercial name	Description/ active ingredient	Dose
Botanigard 22WP	Entomopathogenic fungus, *Beauveria bassiana*, GHA strain; 4.4 x 10^{10} viable conidia/kg	0.0625%, i.e., 62,5g/100 liters of water
Cyperscope 5EC	Pyrethroid insecticide, Cypermethrin 5%	100 mL/100 liters of water
Control	Water	-

5.2.3. Statistical analysis

Statistical analyses were performed using R 4.4.1 software. First, a descriptive analysis was carried out to identify general pattens in the data. Using the Kaplan-Meier method, survival probabilities at each event time were estimated using the *survival* and *survminer* packages (Kassambara et al., 2016; R Core Team, 2024; Therneau, 2024), considering the number of aphids exposed to entomopathogenic fungi and the number of observed events to generate the survival curve. The log-rank test was then used to assess differences in survival between treatments. The effect of entomopathogenic fungal treatments on endophytism rate was assessed using the Kruskal-Wallis test. When a significant difference between treatments was observed, post-hoc comparisons were made using Dunn's test with Bonferroni correction. Medians, quartiles, and interquartiles of the endophytic rate were calculated, and box plots were used to visualize the distributions. The endophytic potential was analyzed using a beta regression generalized linear model (GLM) with a logit link function, with segment, treatment, and day after inoculation as explanatory variables. Since the endophytic rate is expressed as a percentage (bounded between 0 and 100), a transformation was made using the Smithson-Verkuilen adjustment (Smithson and Verkuilen, 2006) to meet the requirements of the model. Analysis of variance (ANOVA) was performed to test the overall effect of each explanatory variable on the response variable. For significant main effects, pairwise comparisons were conducted using estimated marginal means with Tukey's honest significant difference (HSD) adjustment for multiple testing. A similar ANOVA was applied to assess the effect of treatment on plant height. A generalized linear mixed model (GLMM) was applied for the choice test using the *glmer* function. The model assessed the effects of treatment and time on the response variable (Number of insects per treatment), assuming a Poisson distribution with a log-link function. In the field experiments, A two-way ANOVA was performed to evaluate the effect of treatment and time on aphid infestations at 5% significance. The interaction between treatment and time was also tested. When a significant difference was observed, post-hoc comparisons were made using Tukey's test. The significance level was set at 5% for all tests. All graphs were generated using the R package *ggplot2* (Wickham, 2016).

5.3. Results

5.3.1. Lethal effect and endophytic activity of Beauveria bassiana strains under laboratory conditions

Effect of fungal treatments on Brevicoryne brassicae

Kaplan-Meier survival analysis revealed significant differences in survival probability among all groups following direct application of entomopathogenic fungi ($p < 0.001$; 95% confidence level), as shown in Figure 14. All treated groups (GxABT-1, GHA, and KA14) showed a steep decline in survival, with the lethal time 50 (LT_{50}) values of 3 days for GxABT-1 and 4 days for both GHA and KA14. In contrast, the control group showed a much slower decline, with over 75% survival maintained beyond 4 days, confirming that mortality was treatment-induced.

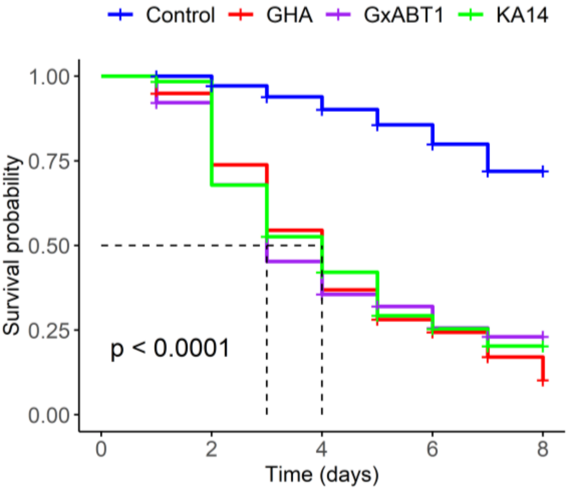

Figure 14. Kaplan-Meier survival probability following direct application of *Beauveria bassiana* to adult *Brevicoryne Brassicae*. The log-rank test was used for significant differences between treatments ($p < 0.05$). Survival rates were calculated based on five replicates for each treatment.

Indirect effects of fungal treatments on Brevicoryne brassicae

Kaplan-Meier survival analysis revealed significant differences in survival probability following exposure to fungus-treated leaves collected at 7 and 14 DAI (Figure 15). Both GHA and KA14 treatments caused rapid mortality, with median survival time dropping sharply within 4 and 5 days of exposure to treated leaves collected at 7 and 14 DAI, respectively. In contrast, the control group maintained high survival (>70% throughout the observation period). The curves for GHA and KA14 overlap closely, suggesting no significant difference in their efficacy.

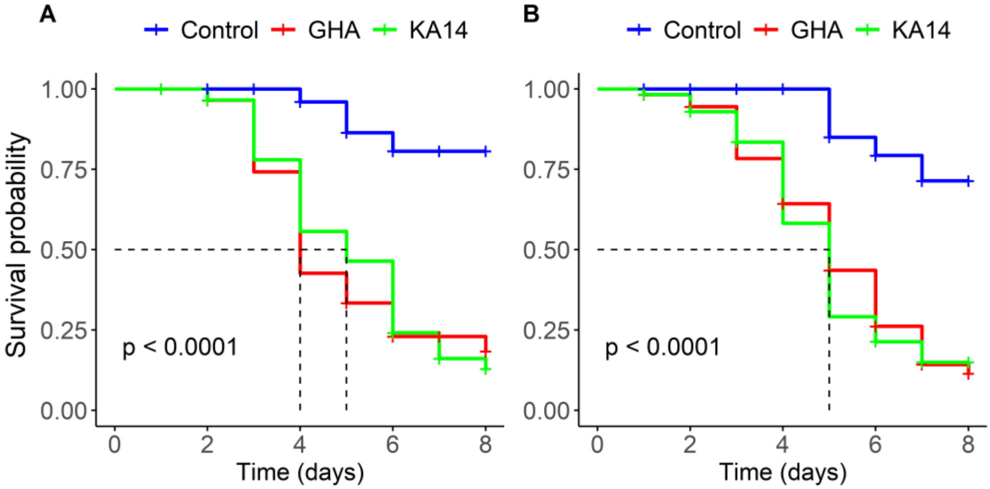

Figure 15. Kaplan-Meier survival probability of adult *Brevicoryne brassicae* exposed to *Beauveria bassiana*. **A:** Survival probability following insect exposure to fungus-treated leaves harvested 7 days after inoculation; **B:** Survival probability following insect exposure to fungus-treated leaves harvested 14 days after inoculation. The log-rank test was used for significant differences between treatments ($p < 0.05$). Survival rates were calculated based on five replicates for each treatment.

Effects of fungal treatments on cabbage plant growth parameters

The effects of the endophytic fungal treatments and the time after inoculation on the Net Height Increase (NHI) are shown in Table 20. No significant differences between treatments were observed for the NHI recorded 7 days after inoculation (DAI). The treatments had no measurable effect on plant height under the conditions tested ($F_{(2,12)} = 0.12$, $p = 0.886$). However, a significant difference exists between treatments in NHI calculated at 14 DAI. Seedlings treated with endophytic fungi solutions show higher values than controls ($F_{(2,12)} = 5.73$, $p = 0.017$).

Table 20. Effect of endophytic *Beauveria bassiana* isolates on the plant height

Days after inoculation	Treatment	Plant height before inoculation (cm)	Plant height after inoculation (cm)	Net Height Increase	*p*-value
7	*B. bassiana* GHA	17.6 ± 1.51	19.2 ± 1.68	1.6 ± 0.22 ^{a}	0.886
	B. bassiana KA14	17.6 ± 1.38	19.1 ± 2.24	1.5 ± 0.93 ^{a}	
	Control	17.7 ± 1.39	19.1 ± 1.71	1.4 ± 0.54 ^{a}	
14	*B. bassiana* GHA	18.4 ± 1.08	21.6 ± 1.24	3.2 ± 0.27 ^{a}	0.017*
	B. bassiana KA14	16.9 ± 2.30	20.3 ± 2.41	3.4 ± 0.83 ^{a}	
	Control	16.8 ± 0.90	18.8 ± 1.03	2.0 ± 0.50 ^{b}	

Means ± standard deviation followed by the same letter do not differ statistically at 5% significance according to the Tukey post hoc test, and an asterisk shows significant differences between treatments: * $p < 0.05$

The potential of the different fungal strains to colonize the different plant parts was registered at 7 and 14 DAI (Figure 16). A Kruskal-Wallis test indicated significant differences in colonization across treatments ($H = 43.3$, df = 2, $p < 0.001$) and among plant segments ($H = 6.7$, df = 2, $p = 0.033$). Beta regression revealed significant variations in endophytic colonization depending on the plant segment and the fungal treatment (Table 21). Stems exhibited a notably higher likelihood of colonization than leaves (GLM: $\chi^2 = 2.68$, $p = 0.007$), suggesting a twofold increase in the likelihood of colonization. The fungal treatments had a significant effect: GHA significantly increased colonization compared with the Control (GLM: $\chi^2 = 4.02$, $p < 0.001$), while KA14 was even more effective (GLM: $\chi^2 = 6.34$, $p < 0.001$), i.e., around two times more effective than GHA. However, no significant difference was observed between roots and leaves (GLM: $\chi^2 = -0.47$, $p = 0.632$) or between days 7 and 14 after inoculation (GLM: $\chi^2 = 0.43$, $p = 0.664$), suggesting temporal stability of colonization.

Table 21. Outputs of beta regression generalized linear model (GLM) summarizing the inoculation of entomopathogenic fungi in cabbage

	Estimate	Std. Error	Z value	Pr(>|z|)
(Intercept)	-2.382	0.309	-7.701	< 0.001***
Root	-0.136	0.284	-0.479	0.632
Stem	0.780	0.290	2.687	0.007**
B. bassiana GHA	1.159	0.288	4.023	< 0.001***
B. bassiana KA14	1.917	0.302	6.344	< 0.001***
7 Days after inoculation	0.101	0.233	0.434	0.664

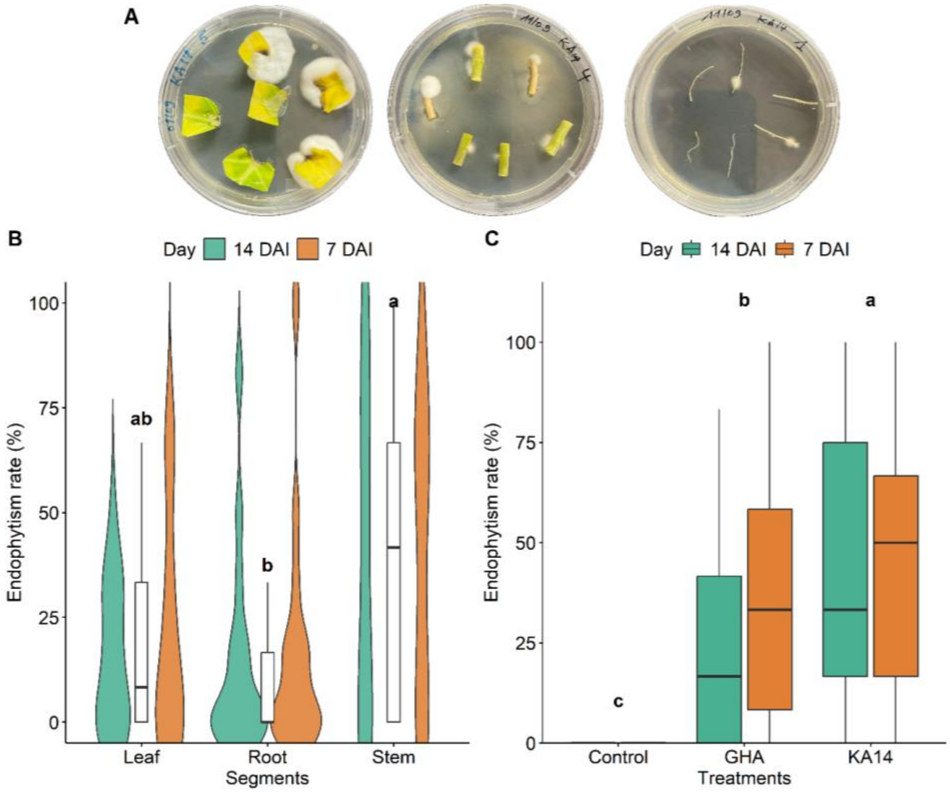

Figure 16. Endophytic potential of *Beauveria bassiana* isolates in cabbage plant. **A:** *Beauveria bassiana* growing from cabbage tissues. From left to right, leaf, stem, and root segments are shown; **B:** Violin plot representing the endophytism rate of fungal isolates from plant segments collected at 7- and 14 days after inoculation (DAI); **C:** Box plot of endophytism rate according to treatments at 7- and 14 DAI. The significance letters (a, b) indicate statistically different groups determined by a post-hoc Dunn's test.

Choice test assays

For the choice test performed with leaves harvested at 7 DAI, the tested aphids were more attracted to leaves from inoculated plants than to those of the controls. More aphids were observed on the discs treated with the *B. bassiana* GHA (GLMM: $\chi^2_{(1, N=50)} = 15.13, p < 0.001$) and *B. bassiana* KA14 (GLMM: $\chi^2_{(1, N=50)} = 20.23, p < 0.001$) than on the control discs. Interestingly, when the experiments were performed on leaves harvested 14 DAI, aphids showed no preference between fungal inoculated leaves (*B. bassiana* GHA or KA14) and Control plants. Aphids' choice among treatments was not influenced by the time factor ($p > 0.05$) (Figure 17).

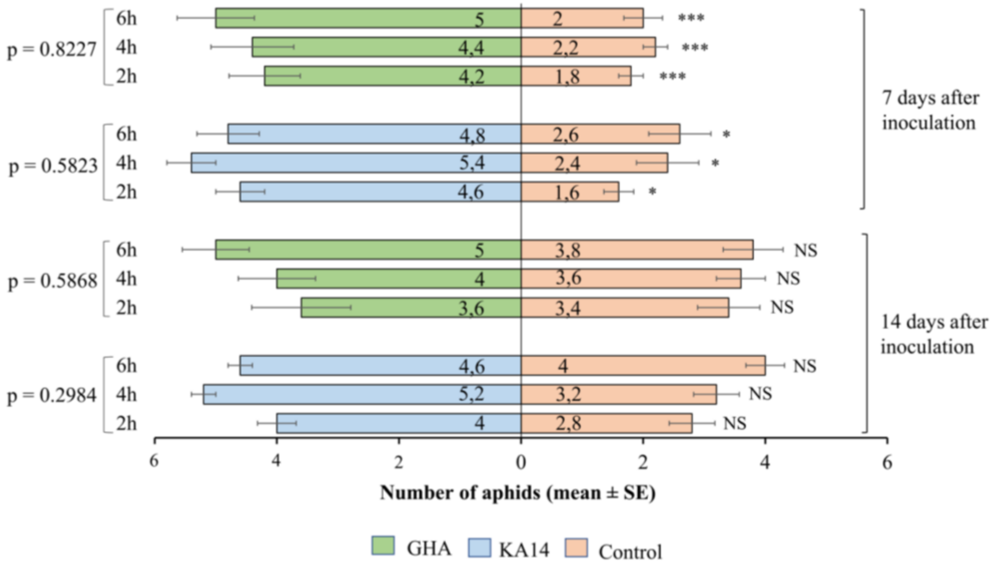

Figure 17. Selection of plants by aphids between Control and fungal inoculated plants (*Beauveria bassiana* GHA and KA14) in two-choice preference tests. For each test, 10 aphids were released and observed after 2, 4, and 6 hours. On the left, the p-values represent tests comparing aphid distribution over time. On the right, there are leaves harvested 7 days and 14 days after inoculation of cabbage seedlings. Error bars are standard errors (SE).

5.3.2. Efficacy of *Beauveria bassiana* GHA treatment against *Brevicoryne brassicae* under field conditions

Significant differences were reported between the treatments in the long rainy season (LRS) experiment ($F_{(2,54)} = 64.33, p < 0.001$) and the short rainy season (SRS) experiment ($F_{(2,54)} = 49.42$, $p < 0.001$) for the infestation rate (Figure 18A and B). Infestation rates were similar for all treatments in the first few days after transplanting (DAT). This was followed by a significantly higher number of aphids for the control plots until the end of the experiments (79.1 ± 4.1% and 83.3 ± 4.1% at 63 DAT in the LRS and SRS experiments, respectively). In contrast, the Cyperscope 5EC and *B. bassiana* GHA treatments effectively reduced aphid infestation, and Post hoc comparisons using Tukey’s test indicated no significant difference among the two treatments.

The aphids’ infestation on *B. brassicae* started at 14 and 7 DAT in the LRS and SRS experiments, respectively. About aphid number, significant differences were observed among the treatments in the LRS ($F_{(2,54)} = 48.49$, $p < 0.001$) and SRS ($F_{(2,54)} = 75.67$, $p < 0.001$) experiments. Over time, aphid numbers were stable in the plots treated with the fungus and chemical insecticide, compared with the control plots, where infestations continued to increase, reaching a maximum of 123.6 ± 23.1 aphids at 63 DAT and 146.6 ± 17.6 aphids at 56 DAT in LRS and SRS experiments, respectively. Post hoc comparisons using Tukey's test indicated that the EPF *B. bassiana* and the Cyperscope 5EC plots showed similar data compared to the control plots, which had the highest aphid number (Figure 18C and D).

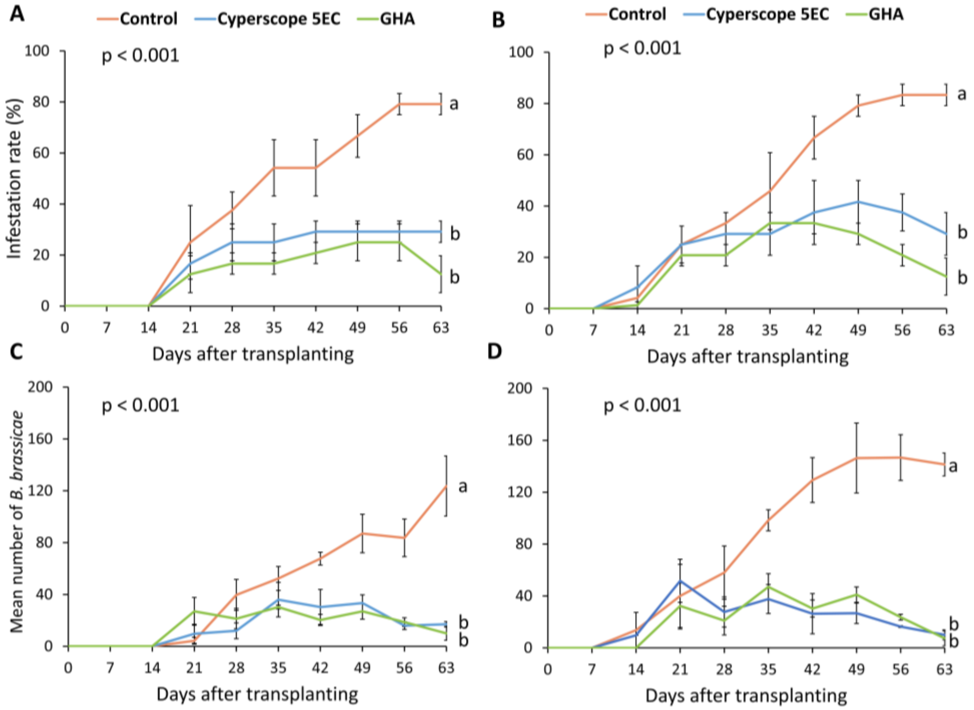

Figure 18. Effects of *Beauveria bassiana* GHA, Cyperscope 5EC, and Control on the population of *Brevicoryne brassicae*. **A:** Aphid infestation rate per plot during the long rainy season (LRS) experiment; **B:** Aphid infestation rate per plot during the short rainy season (SRS) experiment; **C:** Mean number of *B. brassicae* per plant during the LRS experiment; **D:** Mean number of *B. brassicae* during the SRS experiment. Error bars are standard errors (SE). Treatments followed by the same letter do not differ statistically at 5% significance according to the Tukey post hoc test.

5.4. Discussion

5.4.1. Biocontrol potential of *Beauveria bassiana* under laboratory assays

Aphid mortality and survival probability

Survival probability revealed a significant effect of *B. bassiana* treatments on *B. brassicae* aphid mortality, whether by direct application or exposure to inoculated leaves. These results are consistent with previous studies demonstrating the pathogenicity of EPF *B. bassiana* against a wide range of insect pests. For example, Lugendo et al. (2025) evaluated the lethal and sublethal effects of *B. bassiana* (GHA) and *Metarhizium brunneum* (USDA 4556 and V275), both adjusted to 1×10^8 conidia mL^{-1}, on the bean weevil *Bruchus rufimanus* (Boheman, 1833). They reported that *B. bassiana* GHA was the most effective strain, achieving an LT_{50} of 4 days and 96.6% mortality at 10 days. Similarly, Dessauvages et al. (2024) compared two *B. bassiana* strains (GHA and GxABT-1; 1×10^8 conidia mL^{-1}) in a direct spray experiment against the green peach aphid (*M. persicae*) reporting LT_{50} values of 5 and 6 days for GxABT-1 and GHA, respectively. The comparative pathogenicity of the commercial strain GHA and the indigenous strain KA14 highlights subtle but important differences in their speed of action. These differences may be due to genetic differences, the isolate virulence, the ability of spores to adhere to insect cuticles, or interactions with environmental factors such as humidity and temperature (Oberti et al., 2025; Slowik et al., 2024). These findings highlight the importance of selecting appropriate fungal strains to maximize efficacy.

Furthermore, the LT_{50} values observed with KA14 and GHA following exposure to treated leaves at both 7DAI and 14DAI support their efficacy against target pests over time. This finding suggests that *B. bassiana* remains effective after plant application, making it a viable biocontrol tool (Francis et al., 2022). In addition, these results indicate sustained fungal activity and potential compatibility with cabbage as a host plant for endophytic colonization. In a related study, Qayyum et al. (2015) evaluated the pathogenicity of three *B. bassiana* strains against the tomato fruit worm (*H. armigera*) using inoculated tomato leaves. They found that *B. bassiana* WG-40 was the most effective strain, with leaf feeding resulting in the highest mortality one week after inoculation. However, this effect decreased significantly at three and five weeks after inoculation. Their findings suggest that the pathogenicity of *B. bassiana* is strongly influenced by the time elapsed since application. Further research is warranted to explore the mechanisms underlying isolate performance, particularly in plant-mediated applications, and to assess their long-term impact on pest populations and crop health.

Endophytic potential of Beauveria bassiana

Our results suggest that *B. bassiana* could affect plant height, with differences observed at 14 DAI but not at 7 DAI. The lack of a significant effect at 7 DAI may be due to an insufficient incubation period for the endophyte to stimulate plant growth (Q. Wang et al., 2024). Fungi require time to colonize plant tissues and modulate physiological processes such as nutrient uptake and phytohormone synthesis (Grabka et al., 2022). At 14 DAI, inoculated plants exhibited significantly higher normalized height increase values, likely due to improved colonization and increased metabolic activity of the fungal isolates. Endophytic EPF, including *B. bassiana*, are known to produce phytohormones such as indole-3-acetic acid (IAA), enhance nutrient uptake, and induce stress tolerance (Ahsan et al., 2024; Dara et al., 2017; Ownley et al., 2010). These findings highlight the importance of time as a critical factor in plant studies and emphasize the need for long-term monitoring to accurately evaluate the impact of these treatments on plant developmental trends. This is in accordance with Barra-Bucarei et al. (2020), who evaluated the effects of five *B. bassiana* strains on tomato growth parameters, including plant height, at 30 DAI. In their study, all strains resulted in numerically higher plant height than the Control, with the two best-performing strains, RGM-557 and RGM-731, achieving increases of 21% and 18%, respectively.

Our study highlights the performance of the endemic *B. bassiana* KA14, showing the highest and most consistent endophytism rates, particularly at 14 DAI, in comparison to the commercial strain GHA. A temporal decrease in colonization rates was observed for both treatments, which might be explained by the dynamic interactions between the endophytes and the host plant over time (de Carvalho et al., 2021). Notably, colonization was specific to tissues, with leaf and stem segments consistently showing higher endophytism rates than roots, irrespective of the treatment. These findings are consistent with those of Yogananda et al. (2023), who evaluated the colonization of cabbage tissues using different methods of inoculation (seed treatment, seedling root dip, soil drenching, and foliar spray). They reported that fungal colonization was higher in the inoculated cabbage tissues with limited movement to other plant tissues. In our study, foliar application may explain the higher emergence of the fungus in leaf tissues. These results suggest that tissue-specific factors and time-dependent dynamics are critical in shaping microbial colonization patterns (Yogananda et al., 2023). The observed colonization rates of *B. bassiana* strains in plant tissues indicate the compatibility of the fungus, particularly the indigenous isolate KA14, with cabbage plants, which holds promise for pest management. Further research is needed to elucidate the mechanisms driving these colonization patterns and to optimize the use of high-performing strains in agricultural applications.

Aphid preference tests revealed a clear preference for *B. bassiana*-colonized leaves harvested at 7 DAI compared to non-fungal treated leaves. However, no preference was observed at 14 DAI, suggesting a temporal change in the characteristics of the inoculated plants. The increased attractiveness of treated leaves at 7 DAI may be related to physiological or biochemical changes following fungal treatment, such as the production of volatile compounds induced by fungal infection. While these volatile compounds could play a role, this study did not measure them, and their role remains a putative perspective. Previous studies have demonstrated that plant- and fungus-derived volatile compounds, including aldehydes, terpenes, and other small molecules, can mediate insect behavior (Fingu-Mabola et al., 2020; Geedi et al., 2023; González-Mas et al., 2019). For exemple, Fingu-Mabola et al. (2020) reported that tobacco plants treated with *B. bassiana* were more attractive to *M. persicae* than uninoculated plants, possibly due to the high aldehyde production by fungus-inoculated plants. A study by Geedi et al. (2023) found that *M. persicae* was attracted to volatile compounds emitted by *B. bassiana* GHA, and this attraction was observed in both Petri dish and Y-tube olfactometer bioassays, indicating a significant behavioral response to fungal emissions. These results highlight the role of temporal dynamics in plant-insect-fungus interactions. In contrast, other studies have shown that fungus-inoculated plants deter phytophagous insects. For example, Dessauvages et al. (2024) found that sugar beet plants inoculated with *B. bassiana* GxABT-1 and GHA were less attractive to the *M. persicae* than control plants. Future research should aim to investigate whether these preferences change under different environmental conditions or across different insect or host species, and to quantify the plant- and fungus-derived volatile cues underlying these interactions.

5.4.2. In field experiments using *Beauveria bassiana* GHA

Both *B. bassiana* GHA and the chemical insecticide Cyperscope 5EC significantly reduced *B. brassicae* infestation compared to the control plots. The observed effectiveness of *B. bassiana* is consistent with previous studies demonstrating the efficacy of this EPF in controlling a wide range of pest populations under field conditions, including *B. rufimanus* (Lugendo et al., 2025), fall armyworm *Spodoptera frugiperda* (J.E. Smith, 1797) (Kuzhuppillymyal-Prabhakarankutty et al., 2021), cotton aphid *Aphis gossypii* (Glover, 1877), strawberry aphid *Chaetosiphon fragaefolii* (Cockerell, 1901) and western flower thrip *Frankliniella occidentalis*

(Pergande, 1895) (Mantzoukas et al., 2022a). In another cabbage field experiment, Nouh et al. (2022) compared the efficacy of a commercial *B. bassiana* formulation with an insecticide treatment. After weekly monitoring until harvest, they reported low *B. brassicae* infestations with aphids in the *B. bassiana*-treated plots, followed by chemically treated plots, while the highest infestations were observed in the control plots. The high-altitude region of Kivu where we carried out the experiments, is known for its mild and cool climate, which may be favorable for fungal activity. The comparable efficacy of the fungal and chemical treatments suggests that *B. bassiana* could serve as a viable alternative or complement to chemical insecticides in IPM programs.

In terms of aphid number dynamics, the stability of *B. brassicae* infestations in treated plots suggests that both *B. bassiana* and Cyperscope 5EC were effective in suppressing early aphids' establishment. While Cyperscope 5EC, a synthetic pyrethroid, provides an immediate knockdown effect by inducing nerve paralysis in insect pests (Singh et al., 2022), *B. bassiana* acts more gradually by infecting and propagating within aphid populations, potentially reducing the risk of insect pest resurgence (Baverstock et al., 2010). In contrast, the rapid increase in aphid populations in control plots highlights the high reproductive potential of the pest and emphasizes the need for timely intervention (Munthali and Tshegofatso, 2014). In this study, the greater efficacy of *B. bassiana* in the LRS experiment, conducted during the long rainy season, compared to the SRS experiment, conducted during the short rainy season, may be due to prevailing environmental conditions. Overall, the field efficacy of EPF, such as *B. bassiana*, is strongly influenced by abiotic factors such as rainfall, temperature, humidity, and exposure to sunlight (Jaronski, 2010; Mweke et al., 2018). Overall, these findings support the great potential of *B. bassiana* as a biological control agent in different agroecosystems and under different environmental conditions, providing a sustainable alternative with a lower environmental impact than chemical insecticides.

5.5. Conclusion

Exploring the potential of introduced or native insect pathogenic fungi in different agricultural and environmental contexts is of utmost interest to select novel and more eco-friendly biocontrol agents that can support modern agriculture. Laboratory results showed that the endemic strain *B. bassiana* KA14 has promising entomopathogenic and endophytic potential compared to the commercial strain *B. bassiana* GHA. These results support the integration of *B. bassiana* into sustainable pest management strategies that reduce reliance on chemical insecticides. However, more in-depth investigation should be conducted to explore the long-term sustainability of *B. bassiana* applications and its potential synergistic effects when combined with other IPM strategies.

Chapter 6

General discussion and conclusion

Setting up a yellow pantrap in cabbage crops. © P.N. Gakuru

Chapitre 6. General discussion and conclusion

6.1. Cabbage insect diversity and current control methods

The insect communities associated with cabbage crops in eastern Democratic Republic of the Congo (DRC) reflect the ecological diversity of tropical vegetable production agroecosystems, where pest pressure results from interactions between climate, cropping practices, and landscape structure. Although a relatively high diversity of phytophagous insects was recorded, the results indicate that pest management priorities should focus on the dominant species responsible for most economic damage.

The cabbage aphid was consistently recorded as one of the dominant and damaging insect pests in cabbage crops across the surveyed areas, particularly owing to its rapid reproduction through both sexual mating and parthenogenesis (Gill et al., 2013). Asexual reproduction is more common in tropical climates, allowing year-round proliferation in DRC (Javed et al., 2022). This is consistent with research by Shonga and Getu (2021), who found that high temperatures had a significant positive influence on aphid populations. In addition, ecological adaptation enables cabbage aphids to thrive in both cold and warm climates, making them suited to the cooler highland regions of Kivu (Gill et al., 2013). Agricultural practices in the study region also favor aphid proliferation. The high prevalence may be linked to the dominant cabbage monoculture systems in eastern DRC and to continuous cabbage cultivation, which provide near-permanent host availability, thereby facilitating inter-field migration of aphid populations (Gakuru et al., 2025b). Additionally, nitrogen content could contribute to the proliferation of cabbage aphids, although it is a crucial component of fertilization for leafy vegetables, including cabbage. Nutrient-rich plant tissues, often resulting from nitrogen-intensive fertilization, enhance phloem quality, increasing aphid fecundity and survival (Zarghami et al., 2010).

Lepidopteran pests, including *Plutella xylostella* (L., 1758), *Hellula undalis* (Fabricius, 1781), and *Tricoplusia ni* (Hübner, 1803), were also recorded on *Brassica* crops and remain important constraints, particularly during early growth stages (Mpumi et al., 2020; Roy et al., 2023). These findings reinforce our hypothesis that aphids and lepidopteran larvae are the main pest groups in eastern DRC. Although trapping records suggested low adult abundance of *P. xylostella*, field trapping often underestimates population pressure. Female moths of *P. xylostella* exhibit strong dispersal behavior, frequently leaving heavily colonized or resource-poor fields in search of suitable oviposition sites that will promote the development of their offspring (Chapman et al., 2002; Mason, 2022). Consequently, low adult captures do not necessarily reflect low infestation risk, as even a few mated migrant females can establish damaging larval populations (Machekano et al., 2017). This was confirmed by plant inspections, which revealed the significant threat posed by *P. xylostella* in eastern DRC. The substantial presence of chrysomelids (Coleoptera: Chrysomelidae) also reflects common patterns in African *Brassica* fields. Chrysomelids, especially flea beetles (*Phyllotreta* spp.), are recognized as key pests in cabbage crops in the Afrotropical region (Gikonyo et al., 2019). Their prevalence can be explained by several factors, including their host specificity for *Brassica* crops, their high mobility to colonize new cabbage fields, and the near-permanent agroecosystems that favor successive generations (Edde, 2022).

Despite the high pest pressure, cabbage fields supported a diverse community of natural enemies, including predatory beetles (Coccinellidae), hoverflies (Syrphidae), lacewings (Chrysopidae), and parasitoid wasps from the families Braconidae and Ichneumonidae. These groups are widely recognized as central components of biological control of aphids in *Brassica* crops worldwide (Gu et al., 2007; Mamoon-ur-Rashid et al., 2022). Both adult and larval stages of many coccinellid species can consume large numbers of aphids and moth larvae per day, contributing significantly to reductions in population growth rates (Xue et al., 2009). Kulkarni and Evenden (2024) illustrated that both larvae and adults of *Coccinella septempunctata* (L., 1758) were effective at feeding on high numbers of both eggs and larvae of *P. xylostella*, with higher consumption rates observed at warmer temperatures. Lacewings also play a significant role in natural pest regulation with their effectiveness against various hemipteran pests, including bugs (Berteloot et al., 2024), whiteflies (Alghamdi et al., 2018), and aphids (Rana et al., 2017). For example, Mamoon-ur-Rashid et al. (2022) reported the lowest infestation of cabbage aphids in canola plots with the highest number of *Chrysoperla carnea* (Stephens, 1836) egg cards, confirming their effectiveness in regulating aphids. The presence of hoverflies among beneficial insects further supports evidence of natural regulation within agroecosystems in DRC. Syrphid larvae are specialized aphid predators that can consume hundreds of aphids during their development (Fidelis et al., 2018). As adults, hoverflies visit flowers for nectar and pollen, ensuring pollination. Hence, the benefit of promoting hoverflies in agroecosystems lies in their dual role as predators in the larval stage and pollinators in the adult stage (Rodríguez-Gasol et al., 2020).

Parasitoids, although less abundant in the sampling, represent a key component of natural pest suppression. For example, *Diaeretiella rapae* (McIntosh, 1855) (Hymenoptera: Braconidae) was found effective in controlling *B. brassicae* in cauliflower crops (Verma et al., 2019). Ichneumonids such as *Diadegma semiclausum*

(Hellén, 1949) also provide highly effective control in highland ecosystems, where their performance is enhanced by cooler temperatures (Upanisakorn and Lim, 2011). For instance, a few hundred *D. semiclausum* were introduced from Asia to control *P. xylostella* in the highlands of Kenya, Tanzania, and Uganda. A survey conducted two years after the release indicated that the parasitoid had spread over 20–30 km from the release area and was effective against *P. xylostella*. However, parasitism decreased with increasing distance from the release area (Gichini et al., 2008). Parasitoids are more effective when introduced immediately after pest infestation. Neuville et al. (2016) demonstrated that the earliest introduction of *D. rapae* allowed for efficient control of *B. brassicae* compared to later multiple introductions. Predators and parasitoids often act synergistically rather than independently. Predators typically exert density-dependent suppression, removing large numbers of prey, while parasitoids provide density-independent regulation by attacking specific developmental stages with high precision (Gontijo et al., 2015). Such complementary regulation is crucial for suppressing aphids, whose high reproductive capacity would otherwise harm cabbage production.

However, this potential for natural regulation is currently constrained by prevailing pest management practices (Samanta et al., 2023). Farmer surveys revealed frequent applications of broad-spectrum synthetic insecticides, often from moderately to highly hazardous classes according to the WHO classification. Many insecticides are associated with substantial numbers of acute poisoning cases, particularly in developing countries where personal protective equipment and training in pesticide use are limited (Kushwaha et al., 2016; Desye et al., 2024). In addition, broad-spectrum insecticides such as organophosphates and pyrethroids are known to affect beneficial insects, leading to lower reproductive rates and longer life cycles, reducing their abundance and impairing their ability to suppress pest outbreaks (Desneux et al., 2007; Sanchez-Bayo and Goka, 2014; Ayoub et al., 2024). Studies in *Brassica* fields have shown that organophosphates, such as dichlorvos, significantly affect the survival of the parasitoids *C. plutellae* and *D. rapae*, leading to pest resurgence and secondary outbreaks (Lin et al., 2007). Exposure of predators to synthetic insecticides results in both lethal and sublethal effects (Bartling et al., 2024), particularly pronounced in the immature stage, as seen with the pyrethroid lambda-cyhalothrin, which caused high mortality in *Harmonia axyridis* (Pallas, 1773) (Provost et al., 2003). Neonicotinoids are of particular concern for beneficial insects, particularly pollinators, given colony decline reported in many countries (Henriques Martins et al., 2024). Although acetamiprid, the neonicotinoid recorded in the study area, is considered one of the less toxic neonicotinoids, studies have demonstrated sub-lethal effects on bee behavior and reproduction, as well as negative effects on natural enemies (Yang et al., 2020). For instance, *C. septempunctata* exhibited altered aphid consumption and inhibited larval development when exposed to sublethal concentrations of acetamiprid (You et al., 2022). The combination of different hazardous products recorded in the study area may amplify these environmental impacts by simultaneously exposing non-target organisms to multiple modes of action and increasing the overall toxic load in the ecosystem. There is evidence that such mixtures can have additive or even synergistic toxicity for aquatic invertebrates and beneficial arthropods (Atwood et al., 2018).

Another concern is the development of insecticide resistance, which has been extensively documented in *P. xylostella*, *B. brassicae*, and other *Brassica* economic pests in regions where chemical insecticides are commonly used. A field-based study by Pu et al. (2010) highlighted a high resistance observed in *P. xylostella* to abamectin, likely due to enhanced oxidative metabolism. The widespread use of similar products in the study area raises concerns about the development of resistance, which could prompt producers to increase application frequency or dose, creating a cycle of chemical dependence. Promoting beneficial organisms through habitat management and selective biopesticides is a sustainable, cost-effective strategy for Congolese farmers to strengthen natural pest control and enhance system resilience.

6.2. Potential of eco-friendly alternative methods to control cabbage pests

Contribution of agroecological and plant-based strategies

Plant-based regulation emerged from this research as a central component of Integrated Pest Management for cabbage crops in eastern DRC. Both companion planting and plant-derived essential oil influenced the population dynamics of *B. brassicae* through complementary mechanisms, including repellency, toxicity, and disruption of host selection behavior. This aligns with the broader evidence suggesting that companion plants reduce phytophagous pressure by masking host cues and disrupting orientation (Ben-Issa et al., 2017b). Volatile organic compounds (VOCs) play a key role in plant ecosystems by mediating interactions among plants and also with other organisms, including insects (Niu et al., 2024; Wang et al., 2026).

Among the tested species, *Cymbopogon citratus* emerged as the most effective plant in both companion planting and essential oil (EO) applications. Aphids consistently dispersed away from cabbage plants grown near lemongrass, accumulating instead on more distant plants, indicating a strong repellent effect. This observation aligns with previous research demonstrating that lemongrass VOCs, dominated by citral, geraniol, and related monoterpenes, interfere with insect host recognition and survival (Calumpang et al., 2013; Plata-Rueda et al., 2020). Several bioassays have also demonstrated that oils and extracts from *C. citratus* cause strong repellence and mortality in phytophagous insects, supporting our results (Moustafa et al., 2023). For example, Zhang et al. (2016) reported that *C. citratus* EO significantly reduced populations of *Aphis citricola* (v.d. Goot, 1912). *C. citratus* has an advantage because it is already grown in DRC for medicinal and dietary uses, particularly as herbal tea (Avoseh et al., 2015). It could therefore be easily integrated into horticultural areas, particularly for small-scale production. Beyond pest control, *C. citratus* offers additional agronomic benefits, including improvements in soil biological and chemical properties. A study by Pankaj et al. (2017) observed increased mycorrhizal root colonization and enhanced soil organic carbon, total nitrogen, and enzyme activity, especially in the third year after lemongrass planting. However, they found a progressive decrease in soil pH. This would require corrective amendments such as lime, manure, and straw (Zhang et al., 2023).

Lantana camara showed great promise for managing cabbage aphids. The repellent effect observed with *L. camara* EO aligns with previous studies reporting its pesticidal properties (Ogendo et al., 2003; Zandi-Sohani et al., 2012). Recent studies in Sub-Saharan Africa have used leaf extracts of *L. camara* on *Brassica* crops and reported significant reductions in aphid populations (Baryakabona and Mwine, 2017; Mvumi and Maunga, 2018). For example, a study by Baryakabona and Mwine (2017) in Uganda found that *L. camara* extracts alone, or in combination with *Tagetes minuta* L., significantly reduced *B. brassicae* infestations on cabbage, suggesting both pesticidal and repellent properties. Similarly, Mvumi and Maunga (2018) showed that sprays of *L. camara* extracts on rape (*Brassica napus* L.) reduced *B. brassicae* populations compared to untreated plots. Our findings and earlier studies support that *L. camara* can serve as a practical plant-based repellent component in IPM (Kumar et al., 2024). It offers a locally available biopesticide in DRC that can be used in smallholder vegetable farming. When grown near or within vegetable plots, it could reduce aphid settlement. *L. camara* leaves and flowers release a persistent volatile mixture that can mask or counteract the olfactory signals of neighboring crops, thereby disrupting pests' search for hosts (Kumar et al., 2024). In addition, *L. camara* has been shown to affect the recruitment of beneficial insects. Its flowers provide nectar and pollen that attract a wide diversity of insects. Amelia and Irsan (2023) documented a high diversity of insect visitors on *L. camara* intercropped in oil palm plantations, suggesting its potential role in supporting biological control agents. However, the use of *L. camara* presents important limitations for field deployment.

The plant contains toxic compounds that pose risks to livestock, exhibits invasive tendencies, and may exert allelopathic effects on neighboring crops (Ntalo et al., 2022; Kumar et al., 2024). Therefore, *L. camara* should be used with caution, ideally in confined or managed settings, with regular pruning and monitoring to mitigate negative ecological impacts.

Lippia alba exhibited intermediate characteristics, with moderate insecticidal activity and repellent effects against *B. brassicae*. Its essential oil showed lower phytotoxicity in cabbage seedlings, indicating a more balanced efficacy–safety profile. The pesticidal activity of *L. alba* is generally attributed to its chemical composition, dominated by citral, geraniol, caryophyllene, and linalool (Sá Filho et al., 2022). Previous studies have similarly reported the bioactivity of *L. alba* EO against phytophagous insects, such as the potato aphid *Aulacorthum solani* (Kaltenbach, 1843) (Tacaliti et al., 2024) and the fall armyworm *Spodoptera frugiperda* (J.E. Smith, 1797) (Biswas et al., 2024), as well as insect vectors of human diseases, such as mosquitoes (Coulibaly et al., 2024). These findings consistently demonstrate that *L. alba* constituents can significantly impact insect feeding behavior (Shukla et al., 2011). Furthermore, *L. alba* is a plant with medicinal and therapeutic properties widely used in infusions in South America and South Asia (Hennebelle et al., 2008; Teles et al., 2012). However, its potential remains unexploited in DRC. Associated with cabbage, *L. alba* altered aphid dispersal patterns to a lesser extent, suggesting that its volatile compounds may deter *B. brassicae*. Therefore, *L. alba* may be useful for pest control, as a source of botanical biopesticides, and as a repellent plant when grown near cultivated crops. However, *L. alba* grows as a bushy shrub, suggesting its use in patches where regular monitoring is possible.

Overall, our results provide further support for the hypothesis that aromatic plants contribute significantly to aphid suppression by emitting behavior-modifying volatile compounds. Investigations worldwide have documented the repellent and insecticidal properties of aromatic plant extracts and essential oils in pest management. However, the use of essential oils is limited in DRC in the absence of local production facilities. Only a few extracts are occasionally formulated locally by a handful of farmers (Korangi et al., 2021). In contrast, companion planting with aromatic species is a low-cost, preventive strategy that can be easily integrated into smallholder systems. The plant-based interference with pest orientation mirrors the mechanisms reported for other aromatic species. Finch et al. (2003) demonstrated that aromatic plants grown near *Brassica* crops disrupted host-location by cabbage root flies, while Ben-Issa et al. (2017b) reported significant reductions in aphid infestation when vegetables were intercropped with herbs such as basil, coriander, and sage. Therefore, companion plants can enhance pest management strategies, contributing to sustainable cabbage production. However, variability in VOC emissions due to plant age, environmental conditions, and spatial arrangement remains a critical factor influencing field performance (Deletre et al., 2016b). Future studies should therefore focus on optimizing planting configurations and management practices to improve the consistency and sustainability of plant-mediated pest regulation.

Contribution of microbial biopesticides

Microbial biopesticides, especially entomopathogenic fungi (EPF), represent a critical complement to plant-based approaches when preventive measures are insufficient. The efficacy of *Beauveria bassiana* (Bals.-Criv.) Vuill. against cabbage aphids under both laboratory and field conditions demonstrates that microbial biocontrol is technically achievable in eastern DRC. The indigenous isolate (*B. bassiana* KA14) showed promise as an endophyte and in promoting plant growth. Our findings align with previous studies in Africa, where *B. bassiana* applications reduced aphids, diamondback moth, and other cabbage pests (Agboyi et al., 2020; Gebreyohans et al., 2022; Nouh et al., 2022). EPF dominate the commercial microbial biopesticide market and show promise for *Brassica* crops. EPF are particularly attractive because they can infect through the insect cuticle and act synergistically with botanical biopesticides (Gebreyohans et al., 2022). In open-field crops, they can be applied easily with conventional spray equipment (Lugendo et al., 2025). To control cabbage aphids, chemically mediated defenses may complicate fungal infection, as aphids sequester glucosinolates, which they can activate into toxic isothiocyanates. These isothiocyanates could limit the success of pathogens. However, *B. bassiana* has evolved to circumvent the cabbage aphid's chemical defense system by detoxifying its toxins (Sun et al., 2023), making the fungus effective against this aphid.

Although some sub-Saharan African countries are developing microbial pesticides on a local scale, many remain import-dependent, and others, such as DRC, import virtually none (Srinivasan et al., 2019). In West and East Africa, *B. bassiana*- and *Metarhizium*-based products already protect cabbage and other vegetables from key pests, often matching the performance of synthetic insecticides while reducing environmental and health risks (Godonou et al., 2009). In East Africa, extensive work by ICIPE (International Centre of Insect Physiology and Ecology) has built a collection of entomopathogens and developed EPF-based products against several pests, including *S. frugiperda* (Akutse et al., 2020). Similarly, in West Africa, IITA (International Institute of Tropical Agriculture) and partners have developed *B.*

bassiana-based biopesticides targeting the diamondback moth *P. xylostella* (Godonou et al., 2009; Agboyi et al., 2020). Generally, EPF products derived from locally selected strains show better adaptation to field conditions and pests than those derived from generic strains from other regions (Akutse et al., 2020). The indigenous strain *B. bassiana* KA14, isolated from collections in DRC, could be an important component of local, sustainable plant protection. The introduction of microbial biocontrol agents in Congolese farming should reduce dependence on synthetic insecticides while maintaining effective control of economic pests.

6.3. Implementation of IPM strategies in Congolese vegetable farming

Integrated Pest Management (IPM) provides a relevant and timely framework for sustainable vegetable pest management in DRC, where reliance on synthetic pesticides remains widespread. In this context, priority should be given to preventive, cultivation-based measures that reduce pest pressure before curative interventions become necessary.

Cultural and agroecological practices form the cornerstone of preventive pest management. These include varietal selection, crop rotation, intercropping, and optimized planting dates (Vincent et al., 2003; Angon et al., 2023). *Brassica* crops are vulnerable to pest attacks from early growth stages, notably by cutworms (*Agrotis* spp.) (Moustafa et al., 2021), highlighting the importance of selecting tolerant varieties and avoiding synchrony between pest activity and crop susceptibility through appropriate planting dates (Sidoine and Xavier, 2022). In addition, preventive strategies such as crop rotation and companion planting are essential. Intercropping cabbage with repellent plants adapted to eastern DRC conditions, including lemongrass, basil, rosemary, garlic, onion, mint, and celery, represents a promising agroecological option (Ben-Issa et al., 2017b; Mayanglambam et al., 2021). The integration of non-competitive flowering plants can further enhance the conservation of natural enemies and associated ecosystem services (da Silva et al., 2022; Hithesh et al., 2024).

Although not explored in this research, additional preventive approaches, such as trap cropping strategies (e.g. push–pull or attract-and-kill systems), have shown potential against key *Brassica* pests in other production contexts and could be adapted to local cabbage systems (Zhu et al., 2021; Boni et al., 2024; Mutua et al., 2024). For example, Boni et al. (2024) reported that a push-pull arrangement, with cabbage as the main crop, onion as the push crop, and French bean treated with kale extract as the pull component, was effective against both diamondback moth and aphids. In line with our experimental results, *C. citratus* appears particularly promising as a repellent component within such integrated cropping designs. Passive physical methods were not evaluated in our research but have also proven effective in reducing pest infestations and may complement cabbage protection strategies as a cost-effective option, particularly in small-scale farming (Kiptoo et al., 2015).

When preventive measures are ineffective, biological control constitutes a key component of IPM. In this research, essential oils (EOs) of *C. citratus*, *L. camara*, and *L. alba* showed strong insecticidal and repellent activity against *B. brassicae*, highlighting their potential as botanical biopesticides in IPM programs. EOs may also be applied to insect-proof nets, as demonstrated with *C. citratus* EO, which was active against *Bemisia tabaci* (Gennadius, 1889) (Deletre et al., 2016a). Despite their efficacy, the practical use of EOs in DRC remains constrained by limited local production capacity and restricted market availability. Beyond EOs, eastern DRC hosts a wide diversity of pesticidal plants whose crude extracts may contribute to reducing reliance on synthetic pesticides. Several studies conducted in the region have confirmed their effectiveness against vegetable pests (Mbelo and Luhata, 2017; Gakuru et al., 2019; Byamungu et al., 2024; Tshibamba et al., 2025). However, their broader adoption is constrained by limited access to standardized formulations. Practical guidelines for the formulation and use of plant-based biopesticides applicable to the Congolese context are provided by Anjarwalla et al. (2020).

Biological control should also integrate natural enemies through conservation and, where possible, augmentative approaches, particularly in protected cropping systems (Walgenbach, 2018). Microbial biopesticides, especially entomopathogenic fungi, represent an important complementary tool. Field applications of *B. bassiana* GHA have significantly reduced cabbage aphid infestations in eastern DRC, demonstrating the potential of microbial biocontrol under local conditions (Gakuru et al., 2025a). In addition to pest suppression, endophytic microbial agents such as *B. bassiana* may enhance plant tolerance to abiotic stresses, contributing to climate-smart agriculture (Guo et al., 2024). However, their adoption in the DRC is limited due to the absence of commercial formulations and insufficient institutional support (Gakuru et al., 2025b; Korangi et al., 2021).

In the short term, sustainable cabbage pest management in eastern DRC should prioritize low-cost, easily implementable strategies, including resistant varieties, repellent companion plants, physical barriers such as available insect-proof netting, and increased awareness of the preparation and use of botanical extracts. Essential oils may represent effective curative tools within IPM, depending on product availability. When chemical control is required, selective pesticides and rotation of modes of action should be preferred, with targeted applications used to minimize environmental contamination and non-target effects (Walgenbach, 2018).

6.4. General conclusion

This thesis addressed sustainable cabbage pest management in eastern DRC, with a focus on the cabbage aphid, *Brevicoryne brassicae*, one of the dominant and economically damaging pests in *Brassica* crops in the region. Findings confirm heavy reliance broad-spectrum synthetic insecticides, highlighting the need for alternative management approaches. The results indicate that effective aphid management can be achieved through an IPM framework based on prevention, ecological regulation, and rational intervention. Varietal selection, habitat management, and crop monitoring emerged as essential preventive components, while the conservation of natural enemies represents a cost-effective strategy for smallholder farmers. Curative measures should prioritize plant-based biopesticides, then microbial biopesticides. Significant challenges remain for the adoption of bio-based strategies in DRC, including the limited access to standardized formulations and weak extension services. Addressing these challenges requires a strategic transition toward innovative pathways co-developed with farmers, reinforced through training and knowledge transfer, and embedded within supportive policy frameworks to ensure the sustainability of vegetable farming in the region.

Future studies should focus on field-based validation of plant-derived products and microbial biopesticides under the diverse agroecological conditions of eastern DRC. Under controlled conditions, *L. camara* essential oil was the most repellent at 10 µL/mL (77.22%), while *C. citratus* oil was the most toxic, causing 100% mortality at 7.5 µL/mL after 48 hours of aphid exposure. Further research is needed to examine their performance under variable abiotic factors, such as rain and solar radiation, which may affect their persistence and efficacy. Research on formulation strategies that enhance stability and reduce phytotoxicity will be critical to improving their practical use. The indigenous strain of *B. bassiana* was found to be endophytic in cabbage plants and virulent against cabbage aphid, with an LT_{50} of four days following direct application. Therefore, it is important to expand studies on its potential across agroecosystems in DRC and to develop more indigenous strains, as they are known to better adapt to their native environments.

Scientific communication

Scientific communication

1. Scientific publications related to the thesis

Published articles

Gakuru, P. N., Muhashy Habiyaremye, F., Noël, G., Caparros Megido, R., Francis, F. (2025). Assessment of Cabbage (*Brassica oleracea* L.) insect pests and management strategies in eastern Democratic Republic of Congo. *Agriculture* 15(21), 2203. https://doi.org/10.3390/agriculture15212203

Gakuru, P.N., Cokola, M.C., Dessauvages, Mate Mweru, JP., Muhashy Habiyaremye, F., Caparros Megido, R., Francis, F., Ben Fekih, I. (2025). Dual impact assessments of *Beauveria bassiana* (Ascomycota: Hypocreales) against *Brevicoryne brassicae* (Hemiptera: Aphididae). *Neotropical Entomology* 54, 111. https://doi.org/10.1007/s13744-025-01327-w

Gakuru, P.N., Noël, G., Muhashy Habiyaremye, F., Francis, F., 2024. Monitoring entomofauna associated with cabbage, *Brassica oleracea* L. var *capitata* in eastern DR Congo. *Journal of Insect Biodiversity and Systematics* 10(3), 453-466. https://doi.org/10.61186/jibs.10.3.453

Articles in preparation

Gakuru, P.N., Segers, A., Fauconnier, M-L., Francis, F. Bioactivity of essential oils from *Cymbopogon citratus*, *Lantana camara*, and *Lippia alba* against *Brevicoryne brassicae* (Hemiptera: Aphididae).

Gakuru, P.N., Francis, F. Dispersal response of cabbage aphid, *Brevicoryne brassicae*, to aromatic companion plants.

2. Scientific conferences and symposiums

Gakuru, P.N., Noël, G., Muhashy Habiyaremye, F., Caparros Megido, R., Francis, F. (13 November 2025). Pesticide use and risk exposure in Congolese vegetable farming: A case study of *Brassica* growers around Goma city [Poster presentation]. Second PhD Symposium Gembloux Agro Bio Tech, Gembloux, Belgium. https://hdl.handle.net/2268/337644

Gakuru, P.N., Segers, A., Fauconnier, M.-L., Francis, F. (22 October 2025). Valorisation des plantes aromatiques dans la gestion intégrée du puceron du chou *Brevicoryne brassicae* (Hemiptera : Aphididae) [Poster presentation]. Colloque Scientifique Régional « One Health »: recherche synergique pour la santé, l'alimentation et la nutrition en Afrique de l'Ouest, Benin. https://hdl.handle.net/2268/337790

Gakuru, P.N., Cokola Cuma, M., Dessauvages, K., Mate Mweru, J.-P., Muhashy Habiyaremye, F., Caparros Megido, R., Francis, F., Ben Fekih, I. (22 May 2025). Efficacy and endophytic potential of *Beauveria bassiana* (Ascomycota: Hypocreales) against *Brevicoryne brassicae* (Hemiptera: Aphididae) [Poster presentation]. 4th Agrinatura Conference 2025, Gent, Belgium. https://hdl.handle.net/2268/332121

Pierquin, F., Segers, A., Burgeon, C., Glacet, L., **Gakuru, P.N.**, Jacques, P., Jijakli, H., Fauconnier, M.-L., Francis, F. (20 May 2025). Developing essential oil based bioinsecticides against *Macrosiphum euphorbiae* (Thomas, C., 1878) (Hempitera: Aphididae) in tomato crops [Poster presentation]. 76th International Symposium on Crop Protection 2025, Gent, Belgium. https://hdl.handle.net/2268/333583

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Kokah, E. U., Long, Y., Nguyen Ngoc, T.T., **Gakuru, P.N.**, Shu, H., Touré, F. (2023). Agriculture and Animal husbandry in Belgium: A case of the dairy farm "Le Bailli" [Paper presentation]. AGreenSmart Erasmus + KA2 2023, Lille, France. https://hdl.handle.net/2268/308553

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Appendices

Appendices

Figure 1. Setting up a yellow pantrap and collecting insects on plants.

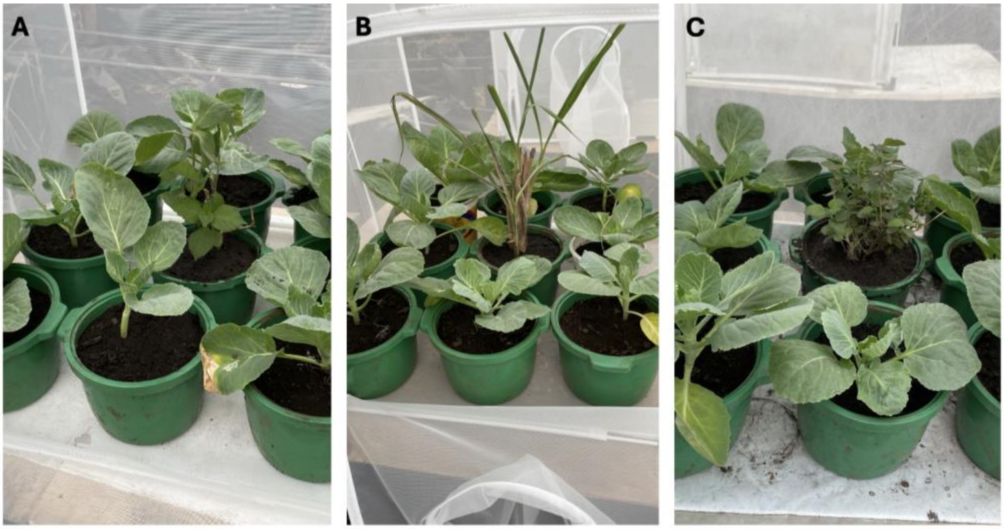

Figure 2. Cabbage plants associated with aromatic plants: (A) *Lantana camara*, (B) *Cymbopogon citratus*, and (C) *Lippia alba*.

Figure 3. Selected pictures of winged and unwinged aphids infected by *Beauveria bassiana*, showing external fungal sporulation.

Figure 4. Setting up and monitoring field trials to assess the efficacy of *Beauveria bassiana* in eastern DRCongo.